DOCTORAL (PhD) DISSERTATION

HARUNA GADO YAKUBU

HUNGARIAN UNIVERSITY OF AGRICULTURE AND

LIFE SCIENCES

KAPOSVÁR CAMPUS

DOCTORAL SCHOOL OF ANIMAL SCIENCE

2023

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

INSTITUTE OF PHYSIOLOGY AND ANIMAL NUTRITION

The Head of Doctoral (PhD) School:

PROF. DR. ANDRÁS SZABÓ, DSc

Supervisor:

DR. GYÖRGY BÁZÁR, PhD

DEVELOPMENT OF RAPID ANALYTICAL METHODS BASED ON ELECTRONIC NOSE TECHNOLOGY AND NEAR INFRARED SPECTROSCOPY TO ADVANCE DAIRY PRODUCTION

DOI: 10.54598/002660

Written by:

HARUNA GADO YAKUBU

Kaposvár

2023

ABBREVIATIONS

ADF	acid detergent fiber
ANN	artificial neural network
ANN-SOM	artificial neural network self-organizing map
ANOVA	analysis of variance
CA	crude ash
CF	crude fiber
C-H	carbon-hydrogen bond
CP	crude protein
CTR	control
C-UHT	commercial ultra-high temperature
DFA	discriminant factor analysis
DFA	discriminant function analysis including
DHA	docosahexaenoic acid
DM	dry matter
EE	ether extract
ELM	extreme learning machine
e-nose	electronic nose
EPA	eicosapentaenoic acid
EU	European Union
E-UHT	experimental ultra-high temperature
EXP	experiment
EXP-1	experiment one
EXP-2	experiment two
EXP-3	experiment three

EXP-4	experiment four
FA	fatty acid
FDA	Fisher discriminant analysis
FID	flame ionization detector
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GDP	gross domestic product
IC	intact casein
ICA	independent component analysis
InGaAs	Indium Gallium Arsenide
IRG	Italian ryegrass
K-NN	k-nearest neighbor
LDA	linear discriminant analysis
LV	latent variables
LVF	linear variable filter
MANOVA	multivariate analysis of variance
ME	metabolizable energy
MEMS	micro-electro-mechanical system
Mix A	mixture A
Mix B	mixture B
Mix C	mixture C
Mix D	mixture D
MLP	multilayer perceptron
MOS	metal oxide semiconductor sensors
MOSFET	metal-oxide-semiconductor field-effect transistor
MPLSR	modified partial least square regression
MS	mass spectrometry
MSC	multiplicative scatter correction

MUFAs	monounsaturated fatty acids
MUN	milk urea nitrogen
n3	omega-3
NDF	neutral detergent fiber
N-H	nitrogen-hydrogen bond
NIR	near-infrared
NPN	non-protein-nitrogen
O-H	oxygen-hydrogen bond
PbS	lead Sulphide
PC	principal component
PCA	principal component analysis
PLS	partial least square
PLS-DA	partial least square discriminant analysis
PLSR	partial least square regression
PNN	probabilistic neural network
PUFAs	polyunsaturated fatty acids
QMB	quartz microbalance
R^2	coefficient of determination
R^2_{CV}	determination coefficient of cross-validation
RF	random forest
RMSEC	root mean square error calibration
RMSECV	root mean square error cross-validation
RPDs	ratios of performance to deviation
SCC	somatic cell count
SCP	soluble crude protein
SE_{CV}	standard error of cross-validation
SEPs	standard errors of prediction

SEPs	standard errors of prediction
SG	Savitzky-Golay derivatives
S-H	sulphur hydrogen bond
Si	Silicon
SIMCA	soft independent modelling by class analogy
SNF	solid-non-fat
SNV	standard normal variate
SVM	support vector machine
TMR	total mixed ratio
UHT	ultra-high temperature
VCs	volatile compounds
WC	winter cereals
WC+IRG	winter cereals plus Italian ryegrass
WSC	water-soluble carbohydrates

TABLE OF CONTENTS

Contents

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The production and consumption of dairy products contribute significantly to the growth of world economies. For instance, the production of raw milk on the farms of the European Union (EU) alone was 161.1 million tons in 2021, with apparent milk yield per cow estimated at 7, 682 kg, which form significant part of the agricultural gross domestic product (GDP) of the EU (Eurostat, 2021). Aside from the economic importance, the nutritional benefits of milk and its derivatives, to consumers make the product a world delicacy. Milk is a good source of fat soluble vitamins, such as vitamins A, D, and E, and water soluble vitamins, such as vitamin B complex and vitamin C. Milk is also a rich source of minerals such as calcium (Ca), phosphorus(P), potassium(K), magnesium(Mg) and zinc(Zn), protein, fat and energy (Pereira, 2014). According to available nutritional guidelines, milk and its derivatives are beneficial to all age groups, except those with specific metabolic conditions such as lactose intolerance or milk protein allergy (Marangoni et al., 2018).

Worldwide, dairy production continues to follow a trend towards increased intensification of small, medium and larger farms, into more specialized dairy production units (Douphrate et al., 2013). In intensive dairy farms, the importance of efficient feeding or nutrition cannot be underestimated, since feeding influences the overall growth and development of the animals, and also accounts for almost 70% of dairy production cost (Salinas-Martínez et al., 2020). Efficient feeding plays a huge role in the building of cows' body reserves for the next lactation cycle, which success largely depends on the quality and quantity of feed supplied (Butler, 2000). Also, various chemical and physical dietary factors such as neutral detergent fiber (NDF) concentration and particle size can affect rumen fermentation,

and consequently milk production and composition (Leonardi and Armentano, 2003). It is important to state that livestock farmers are aware of the importance of feed quality, but until the introduction of rapid analytical technologies such as near infrared (NIR) spectroscopy, it was not easy to obtain real time quantitative information at an affordable price.

Physiologically, the nutritional composition of milk or dairy products may be influenced by the transfer of specific chemical compounds in the form of nutrients from the feed to the milk through metabolism (Jenkins & McGuire, 2006a). Therefore, quality testing and control of milk during and after production is important.

Ideally, dairy products quality is not only about the nutritional composition, but also includes organoleptic or sensory properties. Oxidation is an important chemical process which influences the organoleptic properties such as the aroma or odor of dairy products. Off-flavor or odor in dairy products originate mostly from bacterial metabolism, enzymatic activity, photo-oxidation, heat, and oxidation catalysed by chemicals such as pro-oxidants (Marsili, 2000).

For example, headspace of milk typically presents a complex mixture of organic volatiles (e.g. acetone at overwhelming concentration, hexanal, 2-butanone, toluene, limonene, heptanal, styrene, chloroform, etc.) at varying concentrations and with a high percentage of relative humidity. The aforementioned volatile organic compounds make milk vulnerable to oxidation, and may likely produce off-flavor or odor in milk. This may likely affect the consumer preference for milk, and further reduce the quality of processed milk products.

Through supplementary feeding, ie., the addition of other nutritive sources to the base feed, milk nutritive value can be improved (Cronjé, 1990). In a situation where that is not successful, fortification or enrichment is mostly used to improve the product quality. Dairy product fortification

involves the addition of specific micronutrients which are evidently deficient in the dairy product, in order to improve its nutritive value (Olson et al., 2021). Fortification of dairy products has been widely used in the dairy industry to achieve desirable nutritional level, flavour and aroma, to increase product acceptability by consumers (Abbasi and Azari, 2011).

Thus, the composition of feed and dairy products qualities (nutritive and organoleptic) have to be objectively determined, using fast and reproducible analytical methods in order to ensure acceptable final product quality to consumers (Falchero et al., 2009). Emerging rapid analytical methods such as near infrared (NIR) spectroscopy have significantly addressed the issue of cost and time, since the technique can provide reliable estimates of feed and dairy product quality at an affordable cost in real time. Similarly, the electronic nose (e-nose) technology or machine olfaction has made it easier to detect odors in milks and dairy products, in order to guarantee quality.

This doctoral work focused on the possible applications of correlative analytical methods (i.e., NIR and e-nose) in feeds and dairy products quality assessment. In the below listed sections (1.2.1, 1.2.2, and 1.2.3) literature on some feed factors and their relationship with dairy product composition, supplementary feeding in dairy cow management, and dairy product fortification are elaborated. In section 1.2.4, two comprehensive reviews published recently are incorporated that discuss the application of NIR spectroscopy and e-nose in the analysis of animal feed and dairy products, such as, milk, cheese, butter, and yogurt. The limitations and future development of the two technologies have also been discussed.

1.2. Literature Review

1.2.1. Feeding factors and dairy product constituent changes

Feeding has been the main tool used by nutritionists to improve or alter the quality of bovine milk (Capuano et al., 2014). The composition of milk fat or lipids is the easiest amongst the composition of milk to alter with nutrition, especially, with the use of high quality forages or pasture and oil supplemented diets (Capuano et al., 2014; Elgersma et al., 2006). An improvement or modification of milk fat or fatty acids can also influence the processed products (cheese, yoghurt etc.) nutritional and organoleptic quality as well, since lipids or fatty acids are odor precursors (Seguel et al., 2020). Milk nitrogen or protein alterations in some cases are achievable when high protein containing feed materials such as urea are used. (Jenkins and McGuire, 2006), but generally, dietary protein tends to have a small effect on milk protein. Amongst all the constituents, lactose is the least constituent of milk to be altered through feeding, because it is the most active osmotic constituent of milk, and will always remain remarkably constant (Sutton, 1989). And if changes in lactose composition occur, mostly it is of no significant or practical value (Sutton, 1989).Therefore feeding generally is used to target improving milk fat, and to a lesser extent protein, in order to improve the overall quality of dairy products.

When discussing the influence of feed on animal product constituent change, it is very important to consider some factors, mostly referred to as feed factors; amount and composition of dietary fiber, energy intake and dietary protein intake. The explanation of these factors in sections 1.2.1.1, 1.2.1.2 and 1.2.1.3 will be brief, since the same factors discussed also play a significant role in feed supplementation, thus, will form the basis of the discussion on how dairy products can significantly be modified through supplementary feeding in section 1.2.2.

1.2.1.1. Amount and composition of dietary fat

Milk fat of ruminant origin is unique in composition, due to the great diversity of its component FA. The diversity originates from the influence of ruminal biohydrogenation on dietary unsaturated FAs and the range of FAs synthesized *de novo* in the mammary gland (Palmquist, 2009). Amongst the components of milk, milk fat is the most variable component, both in concentration and composition. In bovine feeding, milk fat concentration can be reduced by giving diets that contain significant proportions of readily-fermentable carbohydrates (starch) and unsaturated fat. On the other hand, the percentage of milk fat can be increased by feeding rumen-inert fats. In ruminants, unlike with monogastrics, dietary fat has little influence on milk fat composition, because the microbiota of ruminants may digest fats in the rumen and all supplemented value may be lost. Nevertheless, little changes in composition which may affect dairy product manufacturing functionality can be influenced by feeding different fats (Palmquist, 2009).

The effects of dietary fat on milk fat composition have been widely studied, perhaps wider than any other factor. For instance, Grummer (1991) reported that the proportions of fatty acid (FA) synthesized *de novo* reduced linearly as supplemental dietary fat increased from 1 to 5% of feed dry matter (DM) and that the changes in palmitic ($C_{16}H_{32}O_2$) and stearic ($C_{18}H_{36}O_2$) acids in milk FA were dependent on the ratio of C_{16} to C_{18} in feed. Carroll et al. (2006) reported that addition of yellow grease (45 g/kg of diet fed) as fat source to dairy cows diet resulted in higher proportions of short and medium-chain FAs in milk. Secchiari et al. (2003) reported that an addition of calcium soap of palm oil in diet of dairy cows gave the highest milk content of both saturated and medium chain FAs, whereas the ratio of saturated to unsaturated FAs of milk fat was decreased when cows were fed with toasted full fat soybean in diet.

1.2.1.2. Dietary protein intake

In most cases, dietary protein level and form do not correlate with milk protein content (Ni et al., 2001), making it very difficult to modify the protein composition of milk. Indeed, dietary protein influences milk yields more than it affects milk protein concentration. Generally, feeding more energy, more protein, or less fiber causes significant increase in the concentration of protein in milk (Emery, 1978). There are three main sources of protein to animal metabolism. Mostly, dietary protein, rumen microbial protein, and protein from tissue depletion, which supply the necessary amino acids for animal body maintenance and milk protein synthesis (Edwards et al., 1980). Another important protein component of milk is the nitrogen fraction. The nitrogen fractions of milk can be categorized into casein, whey, and non-protein-nitrogen (NPN). Amongst these, casein forms about 78% nitrogen in milk, 17% whey and NPN (5%). These protein fractions may greatly affect the production of some dairy products such as cheese. During cheese production, the curd structure, firmness, and cheese yield are directly related to casein content. Feed factors which are likely to influence milk protein content are forage-to-concentrate ratio, the amount and source of dietary protein, and the amount and source of dietary fat. The most significant method of milk protein alteration showed an increase of milk protein from 2.85 to 3.27%, as dietary rumen-protected amino acids varied from 15.0 to 19.5% (Jenkins & McGuire, 2006c).

1.2.1.3. Energy intake and source

The influence of dietary energy intake on milk fat composition largely depends on the energy balance of the animal. When the animal is in a positive energy balance, FA of milk will be influenced by high carbohydrate and fat in diets (Palmquist et al., 1993). As energy balance reduces and becomes negative, dietary supply of acetate and glucose reduces, initiating

lower synthesis of short-chain FA by mammary tissue which increases the mobilization of adipose tissue FAs (Palmquist et al., 1993). Most if not all metabolic disorders or diseases are related to negative energy balance, this suggests an important role than energy balance plays in the dietary supply of lipogenic and glucogenic nutrients (Knegsel et al., 2007). Knegsel et al. (2007) reported that dairy cows fed a lipogenic diet mostly partitioned more energy to milk than cows fed the glucogenic diet and also showed a higher milk fat production. During late gestation and early lactation, an increase in the energy requirements superimposed on an animal by these phases, may lead to a significant drop in dry matter intake just before calving, which make the dairy cow highly susceptible to ketosis and hepatic lipidosis, which may affect the quality and quantity of milk produced (Gerloff, 2000). Dietary carbohydrate fermentation, especially the soluble carbohydrates, catalyzed by the microbiota in the rumen can be the primary source of energy for ruminants. However, ruminants provided with continuous soluble carbohydrate-containing feed can suffer a metabolic disorder called "acidosis", which may lead to reduction in milk production and quality (Darwin et al., 2018).

1.2.2. Supplementary feeding in dairy cows

Mostly, forage based diets alone are not able to meet the dietary needs of high producing dairy cows (Auldist et al., 2013). Reduced forage quality may demand an addition of feed supplements to improve the overall feed quality (Auldist et al., 2013). In the design of feed supplement, the initial step is to determine nutrients that limit utilization of forage based diets (Kunkle et al., 2000). Feed supplementation is very important in dairy nutrition in order to maintain high yielding cows and quality milk products (Kunkle et al., 2000). In times of negative energy balance, feed supplementation is used to improve the nutritional status of the dairy cow. Feed supplementation is

mostly done using fat sources in the form of oils and oilseeds, micro nutrients supplementation in the form of antioxidants and microelement additions, protein source such as fish meal, legumes, bone meal and grain supplementation.

However, the feeding strategy that has the most influence on the yield and composition of milk fat is the use of fat sources in the diet (Sampelayo et al., 2007). And, that also remains the most common feed supplementation done in dairy feed (Schroeder et al., 2004). Fat supplementation enables the control of the content and composition of milk fat. Mostly, the supplementation effect depends on the nature, amount of the supplemented fat, and the existence of interactions between the basal diet, such as forage type, starchy concentrate, and dietary lipids (Sampelayo et al., 2007).

It has been reported by Schroeder et al. (2004) that feeds supplemented with unsaturated fats generally increased the concentration of long-chain unsaturated fatty acids in milk, while milk protein concentration reduced marginally. The use of linseed, sunflower oil and fish oils have also proven to increase the fatty acids composition of milk over the years (AbuGhazaleh and Holmes, 2007; Kennelly, 1996). Gomez-Cortes et al. (2008) studied the effect of olive oil supplementation on milk fat composition and protein content. The results showed that the supplemented diet decreased the milk protein content but increased the milk fat and total solids yield. The medium-chain saturated FA (C10:0 to C12:0) content was also reduced, whereas C18:0 and cis-9 C18:1 content increased. Lipid-encapsulated algae oil supplementation of dairy feed was also found to have increased n3 content in milk fat without adversely affecting milk fat yield. Póti et al. (2015) also reported that micro-algae supplemented feed significantly increased n3 fatty acid concentration in milk which also produced a favorable n6/n3 ratio (3.36) and high rumenic or bovinic acid concentration.

With microelement supplementation, the addition of selenium (Se) and Vitamins E, to animal feed are the most common (Castillo et al., 2013). Weiss and Wyatt (2003) reported that the α-tocopherol (Vitamin E) concentrations in supplemented dietary feed were linearly related to concentrations in bovine milk. Again, the addition of either sodium selenite, a selenium Metasolate or a selenium yeast at three inclusion levels of 0.38, 0.76 and 1.14 mg kg^{-1} DM for each, all the three sources markedly increased milk selenium concentration with increasing inclusion level in the diet, however, selenium yeast gave a much greater response than the other two sources of selenium.

With respect to protein supplementation, the biological value of some high-protein feed supplements such as urea, legumes, and animal and plant protein sources must be considered. In dairy feeding, urea, a non-protein-nitrogen, is one of the most popular sources of nitrogen for protein synthesis in ruminants. Urea is not protein. It has no energy value, but can be effectively degraded within the rumen. It is usually used as a substitute for the actual protein sources in total mixed rations (TMR) and pelleted. Urea is mostly effective when added in a mixture with energy bases such as corn silage (Hassen et al., 2022). A high supplementation of urea in an animal diet may hugely influence the urea concentration in milk, which may affect milk quality (Kertz, 2010).

In sole pasture grazing, grain or concentrate supplementation was reported to have positive responses to milk yield, fat and protein (Auldist et al., 2013). Tufarelli et al. (2012) reported that grain peas or soybean as supplementary protein source in dairy cows diet yielded milk with desirable quality.

1.2.3. Fortification of dairy products

The concept of functional dairy foods has received enormous attention in the dairy industry. Fortifying milk and dairy foods with bioactive compounds means adding those essential compounds into the final dairy product, to improve its biological value. It is a well-accepted method by food manufacturers and consumers of the dairy food chain (Adinepour et al., 2022). Milk and dairy products are among the most consumed foods in the world, and, therefore remain the suitable food option to deliver compounds of health value into the body (Adinepour et al., 2022). Fortification also improves the nutritional value of milk and other developed dairy products to meet the dietary requirement, improve acceptability and preference of consumers.

Again, dairy food fortification can be a very good cost effective public health intervention, since the consumption of fortified dairy products also effectively reduce or prevent diseases associated with nutritional deficiencies (Hashemi et al., 2015). In order to improve the levels of micro and macro elements in dairy products, the fortification process utilizes minerals, mostly those with antioxidant properties, such as iron (Fe) and zinc (Zn) (Torrejo et al., 2004), Selenium (Se) (Pfrimer et al., 2018), calcium (Ca) (Kruger et al., 2006), fat soluble vitamins such as vitamins A and D (Zahedirad et al., 2019), vitamin E (Pfrimer et al., 2018), and lipid or fatty acids sources such as fish oil or microalgae oil, to improve omega-3 (n3) fatty acid levels of the final product (Ekin et al., 2021). It is important to state that the fortification method should enrich the product within the dietary level recommendation for consumers of such products, any excess enrichment process may likely result in the production of dairy products of undesirable organoleptic properties (Adinepour et al., 2022).

The fortification method may differ with each additive and dairy product. In the case of vitamin D3 for example, addition of the vitamin in the

form of emulsions is reported to be one of the best options (Kazmi et al., 2007). Leskauskaite & Jasutiene (2016) studied the effectiveness of fortifying yoghurt with vitamin D_3 emulsion, and reported an effective enrichment value of 2.0 μg/g. With regards to n3, emulsions can be applied in the case of yogurt while micro-encapsulation can be done for milk powders (Gumus & Gharibzahedi, 2021). The fortification of semi-solid or solid dairy products such as cheese can be done by either fortifying the milk or brim solution meant for cheese ripping to ensure an effective fortification process (Gulbas & Saldamli, 2005). In order to confirm the success of the fortification process, it is important to always determine the recovery content of the fortifying agent in the final product, especially with Se and Zn (Gulbas & Saldamli, 2005).

With microelements such as Fe, the fortification process is not an easy process because it may produce undesirable metallic taste and odor as a result of the oxidation or rancidity of fats, unpleasant colour changes emanating from the likely interaction with compounds such as anthocyanins and flavonoids. It must be noted that high Fe content may also lead to the degradation of vitamins and minerals (Mehanso, 2006). It is therefore recommended that iron salts should be microencapsulated to limit these negative effects (Xia & Xu, 2005).

The totality of available scientific literature on dairy product fortification suggests a great opportunity to improve the intake of bioactive enriched dairy products in order to meet the dietary nutrient requirements of consumers around the world.

1.2.4. The recent advances of near-infrared spectroscopy in dairy production– a review

Title	The recent advances of near-infrared spectroscopy in dairy production– a review
Authors	Yakubu Haruna Gado, Kovacs Zoltan, Toth Tamas, Bazar George
Journal	Critical Reviews in Food Science and Nutrition
Year Volume Issue	2022 62 3
Pages Quartile IF	810-831 D1 11.208
Doi	10.1080/10408398.2020.1829540

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1829540

Taylor & Francis
Taylor & Francis Group

REVIEW

The recent advances of near-infrared spectroscopy in dairy production—a review

Haruna Gado Yakubu[a], Zoltan Kovacs[b], Tamas Toth[c,d], and George Bazar[a,d]

[a]Department of Nutritional Science and Production Technology, Faculty of Agricultural and Environmental Sciences, Szent István University, Kaposvár, Hungary; [b]Department of Physics and Control, Faculty of Food Science, Szent István University, Budapest, Hungary; [c]Agricultural and Food Research Centre, Széchenyi István University, Győr, Hungary; [d]Adexgo Kft, Balatonfüred, Hungary

ABSTRACT

One of the major issues confronting the dairy industry is the efficient evaluation of the quality of feed, milk and dairy products. Over the years, the use of rapid analytical methods in the dairy industry has become imperative. This is because of the documented evidence of adulteration, microbial contamination and the influence of feed on the quality of milk and dairy products. Because of the delays involved in the use of wet chemistry methods during the evaluation of these products, rapid analytical techniques such as near-infrared spectroscopy (NIRS) has gained prominence and proven to be an efficient tool, providing instant results. The technique is rapid, nondestructive, precise and cost-effective, compared with other laboratory techniques. Handheld NIRS devices are easily used on the farm to perform quality control measures on an incoming feed from suppliers, during feed preparation, milking and processing of cheese, butter and yoghurt. This ensures that quality feed, milk and other dairy products are obtained. This review considers research articles published in reputable journals which explored the possible application of NIRS in the dairy industry. Emphasis was on what quality parameters were easily measured with NIRS, and the limitations in some instances.

KEYWORDS

Butter; cheese; forage; milk; rapid evaluation; total mixed ration; yoghurt

Introduction

The crucial goal of every dairy farm is to achieve maximum or optimum milk production with the accepted market quality, to maximize profit. Over the years, issues of microbial contamination and deliberate adulteration of milk and milk products have been a major concern to stakeholders in the dairy industry. For example, whey protein has been reported by Bilge et al. (2016) to be a major adulterant used by some farmers to improve on the protein content of milk and other dairy products. This and other fraud issues are a major concern to players in the industry.

Feedstuff suppliers and buyers are also concerned about the quality issues, such as mycotoxin contamination (Pettersson 2012), while milk producers are concerned about the influence of feeding on milk quality, as well. Based on gas chromatography measurements, Tóth et al. (2019) reported that supplementation of linseed and fish oil in dairy feed improved the fatty acid (FA) composition of the milk fat by increasing the concentrations of polyunsaturated FAs and beneficially decreasing the n-6/n-3 FA-ratio, without adversely affecting the sensory properties of the milk, but had no significant influence on the quantity of milk fat and protein.

The consumers of milk, cheese, butter and yoghurt are conscious of the quality. Over the years, rapid analytical methods have come to complement or replace existing methods in the evaluation of feed, milk and dairy products. One of such advances is near-infrared spectroscopy (NIRS), the benefits and drawbacks of which are summarized in Figure 1.

The application of NIRS in the dairy industry has brought significant improvement in the analysis and nutritional evaluation of animal feeds and products by providing a rapid examination (Karoui and De Baerdemaeker 2007). NIRS is a widely used spectroscopic method in the dairy industry, utilizing the near-infrared (NIR) region (800–2500 nm wavelength interval, 12,500–4000 cm^{-1} wavenumber interval) of the electromagnetic spectrum by shining NIR light of known intensity on the investigated substance and measuring the intensity of the reflected or transmitted radiation. Most diffuse transmittance measurements utilize the short-wavelength NIR region (800–1800 nm) representing higher energy that is capable to get through the scanned layers. It is widely used for liquid samples such as milk (Coppa et al. 2014), for particulate samples like grains and forages (Modroño et al. 2017), semi-transparent thin layers of solid samples and slurries such as cheese, butter or yoghurt (Coppa et al. 2014; González-Martín, Hernández-Hierro, et al. 2011; González-Martín, Severiano-Pérez, et al. 2011). Diffuse reflectance measurements may include also the longer wavelengths and cover the whole NIR region.

CONTACT George Bazar bazar@agrilab.hu Department of Nutritional Science and Production Technology, Faculty of Agricultural and Environmental Sciences, Szent István University, Kaposvár Campus, 40 Guba Sándor Str., 7400 Kaposvár, Hungary.

Figure 1. Advantages and disadvantages of near-infrared spectroscopy.

These measurements are generally used for thick layers of solids and slurries like feeds (Karayilanli et al. 2016), forages (Hetta et al. 2017), cheese (Ma, Babu, and Amamcharla 2019), butter (Mabood et al. 2018) or yoghurt (Dvořák, Lužová, and Šustová 2016).

Both measurement modes allow a wide range of sample presentation, even lacking thorough physical or chemical preparation before scanning. Figure 2 shows the basic setup of a light source, detectors and sample, marking the various ways of light during scanning a sample (Williams, Antoniszyn, and Manley 2019).

Biological products, such as feeds and dairy, when exposed to NIR light, have a characteristic absorbance spectrum in the NIR region, which is a result of the various chemical constituents and physical structures within the product (Karoui and De Baerdemaeker 2007). Typical raw and 2nd derivative spectra of fermented forage, cereal grains, milk, cheese, butter and yoghurt are shown in Figure 3.

The NIR light absorbance is mostly caused by the hydrogen-related chemical bonds (such as C-H, O-H, N-H, S-H in biological samples), but because of many influencing factors (e.g., different molecular conformations, overlapping absorbance peaks, light scattering), there is no direct interpretation of a recorded NIR spectrum based on the wavelength (or wavenumber) and the relating absorbance (Williams and Norris 2001). The spectral information other than sole absorbance of light, such as light scattering, may also be important for some applications and may be avoidable in other cases (Bogomolov, Melenteva, and Dahm 2013). Because the exact composition of samples of any substance, be it feed ingredients or forages, milk or dairy products, vary from one another, there exists a range of typical spectra for these substances.

Therefore, a collection of representative spectra is required, to which the spectrum of a test material may be compared to establish its quality or authenticity. Accordingly, NIRS is a correlative, indirect measurement, which in most applications needs to be calibrated against a reference method. The usual approach is to take both spectral data and reference measurements for many samples (training set), use this training set to establish a formula that predicts reference from spectral data, validate the formula on further samples, and finally apply the validated model on independent samples and predict the variables trained for (Figure 4).

A set of samples having both reference data and spectral data may be used for training multivariate models and building up quantitative calibrations (regression analyses) or qualitative supervised classifications (discriminant analyses). Samples similar in nature and also having both reference and spectral data may be used as independent subjects being predicted by the trained model in a validation, while the comparison of the predicted and reference values may give feedback about the prediction performance of the trained model. The carefully trained and properly tested models

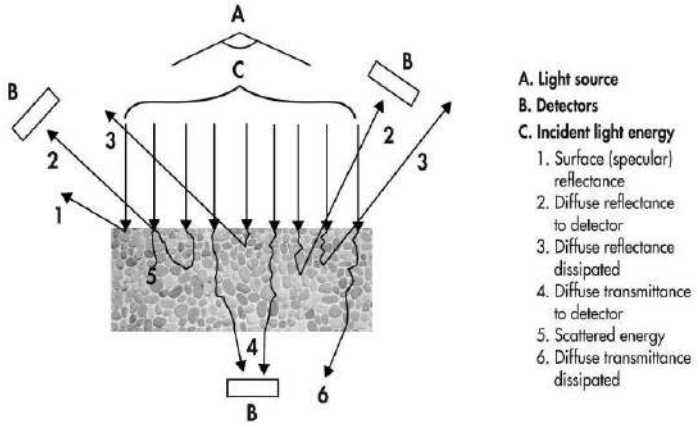


Figure 2. Pathways of light during near-infrared spectroscopic measurement of a sample (adapted from Williams, Antoniszyn, and Manley 2019, p. 33, with permission from AFRICAN SUN MeDIA and Authors).

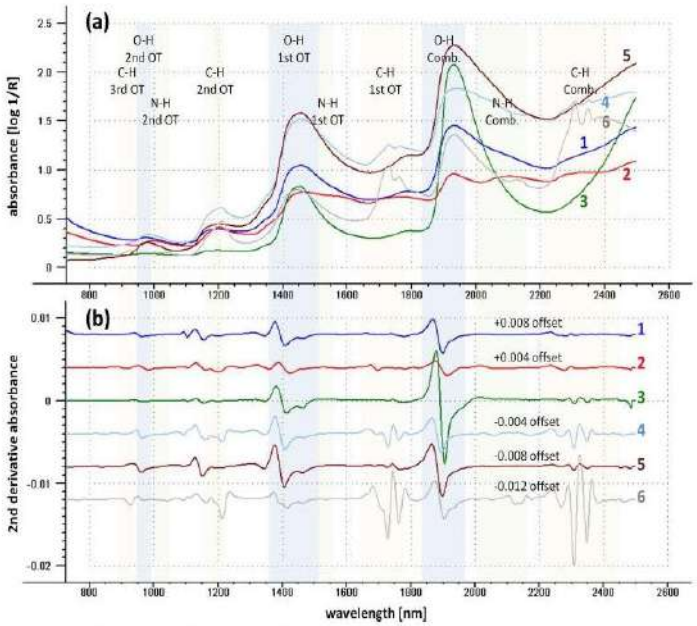


Figure 3. Raw (a) and 2nd derivative (b) reflectance NIR spectra of a (1) fermented forage (fresh corn silage), (2) cereal grains (harvested wheat), (3) liquid bovine milk*, (4) gouda cheese, (5) yoghurt, and (6) butter, with the indication of the major absorption bands (Created by using authors' unpublished data). OT: overtone band, Comb.: combination band of various types of C-H, O-H, N-H bonds characteristic for fat or carbohydrates, water, and protein, respectively. Band assignments are based on Williams and Norris (2001); Workman and Weyer (2012). Offsets are applied in the 2nd derivative spectra for better visualization. *NIR spectrum of a thin layer of milk was recorded in reflectance mode, using a transflectance cup mounted with gold reflector.

may be used for predicting qualitative or quantitative parameters of samples having solely spectral data, thus, may contribute to the decision making protocols.

Multivariate data analysis (MVDA) allows evaluation of collinear spectral data containing hundreds or thousands of variables by mathematically converting them to precise quantitative or qualitative information of chemical and physical nature of the investigated samples. MVDA describing the relation between spectral and reference data makes NIRS analysis useful for a variety of difficult sample types (powders, slurries, chopped or ground materials), more or less without any sample preparation (Martens and Stark 1991). Spectral pretreatments are generally used for reducing instrumental noise, signal distorting effects of sample preparation and presentation or any other effects arisen from the spectrometer, the sample, and the environment (e.g., stray light, light scattering, mechanical vibrations). Besides being used for improving the performance of the MVDA, some

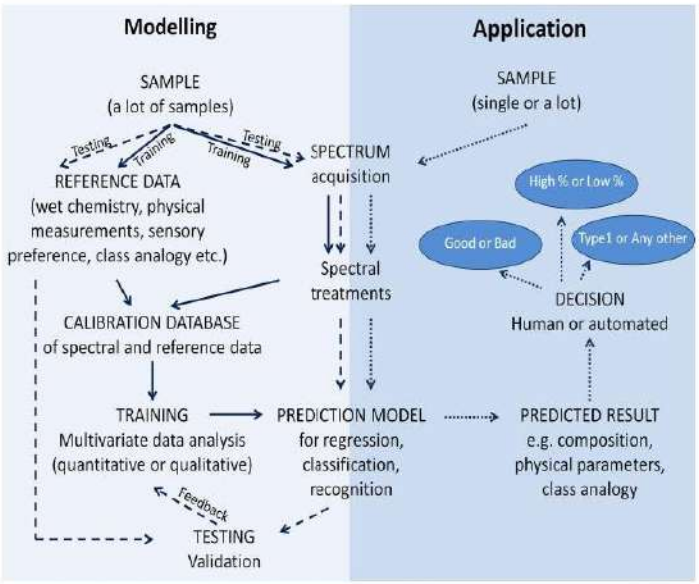


Figure 4. Generalized flowsheet of near-infrared spectroscopic modeling and application. Continuous arrows: training process of multivariate calibration and classification; dashed arrows: testing of the trained models with any kind of validation; dotted arrows: application of the trained and validated multivariate models.

pretreatments, like derivatives (Figure 3), are also used for unfolding covered spectral signals for visual interpretation or band assignment. During the data analysis process, the nature of the datasets and the objectives of the study will determine the form of data pre-processing and the multivariate chemometric techniques required (Karoui and De Baerdemaeker 2007; Fagan and O'Donnell 2011).

After a calibration equation is computed, it is essential to determine its ability to predict the concerning variables for new, unknown samples, and this is the case when choosing between alternative calibration methods and deciding what exact settings of data pretreatment and model training to use (Fearn 2002). The most often used testing methods are grouped cross-validation, full (or leave-one-out) cross-validation, and independent validation. Cross-validations are based on the calibration data only, while independent validation is aimed to represent the real-life testing, however, a calibration equation will show different performance for different test sets, thus, independent test samples must cover the relevant range of samples as well as possible. The predictive precision and accuracy of calibration models are mostly demonstrated with the determination coefficient (R^2) and standard error (SE), respectively, and each may be calculated for the calibration itself, and the validation. As a rule of thumb, the 95% confidence interval for an estimated value is $\pm 2 \times SE$ of prediction calculated in the validation test (Fearn 2002). Figure 5 summarizes the generally used spectral pretreatment protocols, while the most widely used MVDA methods are gathered in Figure 6. Spectral pretreatments, MVDA methods and chemometrics, including the various validation methods and performance measures, are discussed in details by Naes et al. (2002), Williams and Norris (2001) and Ozaki, Morita, and Du (2007).

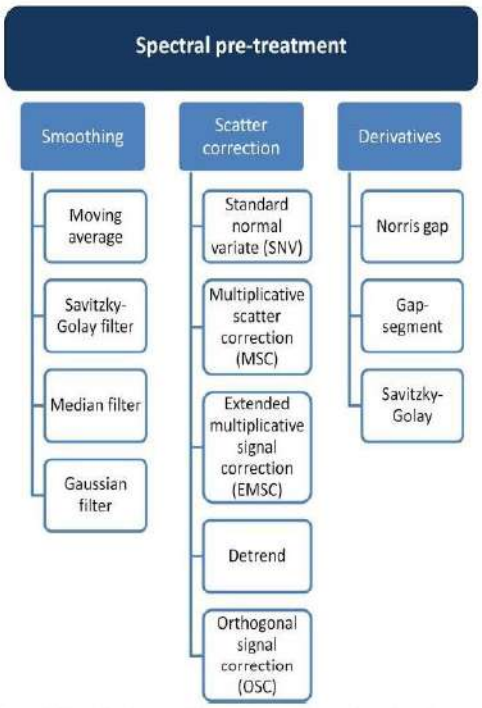


Figure 5. Spectral pretreatments frequently used in near-infrared spectroscopy.

The NIRS method gives fast and reliable information for product analysis, while it is cost-effective, considering the long term benefit to initial costs of the NIRS devices (Wüst and Rudzik 2003). Recently, not only did the originally

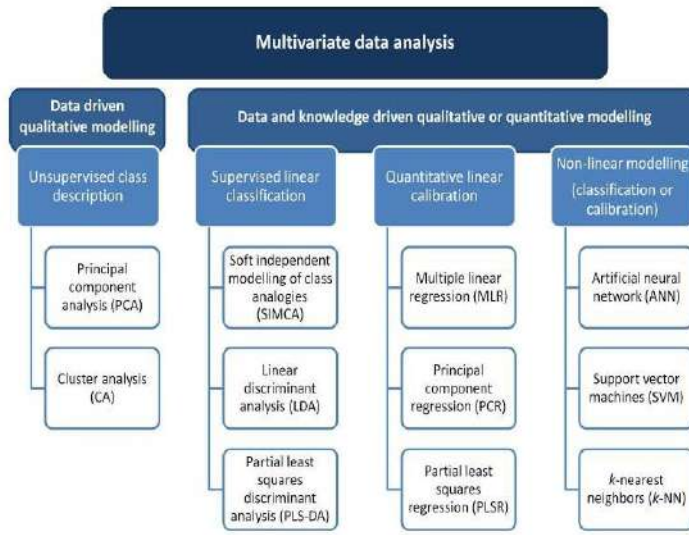


Figure 6. Multivariate data analysis techniques frequently used in near-infrared spectroscopy.

designed benchtop instruments meant for laboratory measurements have become more precise while getting more compact, but the miniaturization has opened the possibility to move spectrometers out of the laboratories or measurement rooms to the field of production and processing. This kind of technological development has boomed in the past decade (Bec et al. 2020) and novelties in instrumentation, like powerful chip-sized spectrometers (Dale 2018) or smartphone accessories (McGonigle et al. 2018), are expected every day. Nowadays, NIR spectrometers may be in the form of benchtop, portable, handheld, and more often used as automated process analytical technology (PAT) (Wetzel 2001; Yan and Siesler 2018; Dickens 2010). Latter ones are at-line, on-line or in-line applications. At-line refers to a technology not being integrated into the industrial process, but being in closer distance than off-line (in-lab) solutions, giving the chance to rapid feedbacks; on-line refers to a technology being integrated into a special sampling line of the process; while in-line refers to a technology fully integrated into the undisturbed process. Benchtop instruments are used mostly in laboratories or in an environment where fixed and robust applications are possible or required, even as PAT. Generally, these spectrometers are equipped with scanning grating monochromator or with interferometer in Fourier-transform systems (FT-NIR), or with fixed grating and diode array (DA) detectors. Portable and handheld devices are out-of-lab applications, often used as PAT, ranging from the gun-sized spectrometers to the palm sized scanners (Bec et al. 2020).

Typical technologies for portable and handheld spectrometers are micro-electro-mechanical systems (MEMSs) with single element detectors and linear variable filters (LVFs) with photodiode arrays. The in-line instruments are fitted to the requirements of process analytics to provide immediate results in the production lines (Bec et al. 2020; Dickens 2010). Based on the type of detector, the NIR spectrometers can be classified into two categories: detector array and single-detector instruments (Yan and Siesler 2018). Further division of the NIRS instrumentation is summarized in Figure 7 and widely discussed by Williams and Norris (2001) and (Ozaki, Morita, and Du 2007).

The availability of accurate, rapid and cheap methods of evaluating feed is becoming increasingly important to meet the nutritional requirements of dairy animals for profitable milk production. This is important to achieve an efficient and economic animal production system, to maintain or improve animal health and to minimize environmental impact as a result of waste pollution. Precise and accurate evaluation methods are needed concerning national and international legislation that regulates the circulation, trade and inspection of feeds and animal products. This will aid the effective functioning of the market by way of reducing feed and food fraud, and will also guard the safety of animals and humans (Givens, De Boever, and Deaville 1997).

This review aims to critically discuss the wide application of the near-infrared spectroscopic technique in dairy production systems. The recent trends of its application, the devices and limitations of the application shall be considered.

Near-infrared spectroscopy to evaluate animal feed, feedstuff or forage

In dairy nutrition, the most important component of fresh or ensiled forages is the fiber composition, especially, the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) fractions. The reduction in fiber content of the ration, as a result of high-grain, is associated with changes in milk fat and metabolic problems, such as acidosis, hoof problems, displaced abomasum, liver abscesses, and a general decline in health. Adequate fiber and/or quality forage

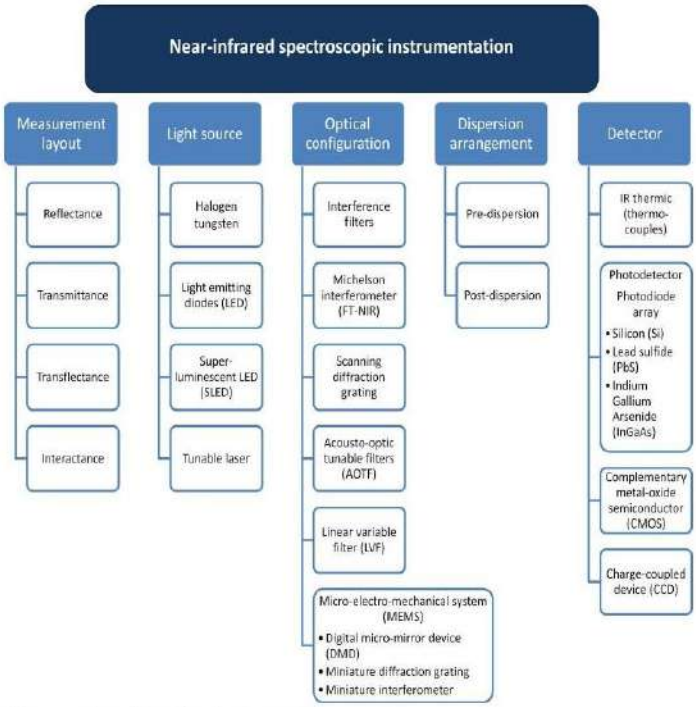


Figure 7. Basic division of the commonly used near-infrared spectroscopic instrumentation.

promotes good health and better performance (Zebeli et al. 2012). Other components of feed, such as dry matter (DM) influences feed intake, which is a key determinant of dairy animal performance because dry matter intake is correlated to milk production (Oba and Allen 1999; Dillon 2007).

Various studies involving dairy nutrition in recent years as presented in Table 1 explored the application of NIRS technique to evaluate animal feed and forage quality. Feed components such as ADF, NDF, crude protein (CP), crude ash (CA), acid detergent lignin (ADL) etc. have been evaluated using NIRS. The ratio of forage components needed for efficient mix silage formulation and preparation was proven to be reliable when NIRS was used (Karayilanli et al. 2016). Arzani et al. (2015) estimated the nitrogen (N) and ADF content of grass species using NIRS. In that study, a total of 171 samples of grasses (*Poaceae*) were evaluated and calibration models were developed between chemical and NIRS data, using partial least squares regression (PLSR) with low prediction errors between the chemical analyses and NIR prediction. In experiments to investigate the feasibility of NIRS to predict fresh forage quality, Alomar et al. (2009) reported good NIR prediction results of forage DM and several chemical constituents, such as CP, NDF, ADF, soluble crude protein (SCP), neutral detergent insoluble N (NDIN), and metabolizable energy (ME). However, ADF, DM, CP were best predicted compared to ME and NDIN. In a study to evaluate and quantify urea, biuret and poultry litter in alfalfa-based forage by NIRS with a fiber-optic probe, González-Martín and Hernández-Hierro (2008) reported accurate prediction results. The study permitted the instantaneous and simultaneous prediction and determination of the mentioned components in the forage, applying the fiber-optic probe directly on the ground dried samples. To assess the effect of two drying methods (oven drying at 65 °C verses freeze-drying) on silage quality by means on NIRS, Alomar, Fuchslocher, and Stockebrand (1999) reported that a principal component analysis (PCA) of the NIRS spectra for the two drying methods showed a 60% variation in PC 1. The spectral information obtained for this variation showed significant changes in CP, CF, NDF and ADF when silage samples were oven-dried at 65 °C. These changes were further confirmed with a pair comparison analysis with the chemical data. The study concluded that silage samples that were oven-dried at 65 °C affected the silage quality compared with freeze-dried treatment. In a similar experiment, García and Cozzolino (2006) investigated the chemical composition of forages in broad-based calibration models when forage samples were dried in an air forced oven at 60 °C to constant weight for 48 h. The results suggested that air forced oven-dried at 60 °C did not reduce the performance or accuracy of calibration models. Overall, the broad-based NIR calibrations for DM, CP, ADF, ash and IVOMD resulted in relatively high determination coefficients and low SECV.

In an experiment to predict of the chemical composition of pasture silage (mainly derived from perennial ryegrass, *Lolium perenne*, L.) by NIR spectroscopy, Ibáñez and Alomar (2008), also reported an accurate performance of

Table 1. A comparative summary of representative studies investigating the prediction capacity of NIRS on quantitative components of feeds and forages.

Product	N	Parameter	R^2_{cv}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
Lolium spp. + clover + Oats mixtures	107	DM (g kg^{-1} DM)	0.98	7.50	SNV, Detrend, 2nd deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Alomar et al. (2009)
		CP (g kg^{-1} DM)	0.93	16.70	SNV, Detrend, 1st deriv.			
		NDF (g kg^{-1} DM)	0.80	33.50	SNV, Detrend, 2nd deriv.			
		ADF (g kg^{-1} DM)	0.90	13.90	2nd deriv.			
		NDIN (g kg^{-1} DM)	0.61	1.89	2nd deriv.			
		SP (g kg^{-1} DM)	0.85	14.40	SNV, Detrend, 1st deriv.			
		ME (MJ kg^{-1} DM)	0.80	0.36	SNV, Detrend, 2nd deriv.			
Broad based herbage species	105	DM (%)	0.95	0.98	MSC	PLSR	Benchtop, FT-NIR, InGaAs detector, 833–2630 nm, reflectance	Parrini et al. (2018)
		CP (%)	0.97	1.21				
		CF (%)	0.93	1.96				
		NDF (%)	0.95	2.90				
		ADF (%)	0.94	2.49				
		ADL (%)	0.92	0.91				
		Ash (%)	0.75	0.85				
Alfalfa	75	Urea (%)	0.99	0.28	SNV, 2nd deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	González-Martín and Hernández-Hierro (2008)
		Biuret (%)	0.99	0.29	2nd deriv.			
		Poultry litter (%)	0.93	2.08	Detrend, 1st deriv.			
Maize	132	CP (%)	0.94	0.04	MSC	PLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Hetta et al. (2017)
		ADF (%)	0.88	0.22				
		WSC (%)	0.96	0.10				
		Starch (%)	0.93	0.13				
Poaceae spp.	171	Nitrogen (g kg^{-1})	0.90	0.30	SNV	PLSR	Benchtop, 256 pixel InGaAs photodiode array, 950–1650 nm, reflectance	Arzani et al. (2015)
Fresh Grasses	1322	ASH (%)	0.90	0.94	SNV, Detrend, 2nd deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Tran et al. (2010)
		Protein (%)	0.98	0.80				
		NDF (%)	0.96	2.27				
		ADF (%)	0.95	1.54				
Legume	332	Na (g kg^{-1} DM)	0.83	0.80	SNV, Detrend, 2nd deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Cozzolino and Moron (2004)

(*continued*)

Table 1. Continued.

Product	N	Parameter	R^2_{CV}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
		S (g kg^{-1} DM)	0.86	2.50				
		B (mg kg^{-1} DM)	0.80	4.40				
		Zn (mg kg^{-1} DM)	0.80	10.60				
		Mn (mg kg^{-1} DM)	0.78	22.90				
		Cu (mg kg^{-1} DM)	0.76	0.83				
		Fe (mg kg^{-1} DM)	0.57	25.70				
Compound feed	100	CP (%)	0.88	0.88	SNV, 2nd deriv.-SG	PLSR	Handheld, LVF, 128 pixels InGaAs photodiode array, 950–1650 nm, reflectance	Modroño et al. (2017)
		CF (%)	0.91	1.36				
		Starch (%)	0.91	2.62				
Broad based forage mixture	600	DM (%)	0.95	0.70	SNV, Detrend	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	García and Cozzolino (2006)
	610	CP (%)	0.98	3.60				
	581	Ash (%)	0.90	0.99				
	542	IVOMD (%)	0.90	3.60				
	151	NDF (%)	0.86	2.00				
	200	ADF (%)	0.95	5.40				
Ryegrass based silage	65	Moisture (%)	0.95*	2.83**	SNV, Detrend	MPLSR	Handheld, MEMS, InGaAs detector, 900–1700 nm, reflectance	Park et al. (2011)
	66	CP (%)	0.28*	1.31**				
	66	ADF (%)	0.59*	1.89**				
	66	NDF (%)	0.56*	2.58**				

N, sample number; R^2_{CV}, determination coefficient of cross-validation; SECV, standard error of cross-validation; SNV, standard normal variate; MSC, multiplicative scatter correction; deriv., gap-segment derivatives; deriv.-SG, Savitzky-Golay derivatives; PLSR, partial least squares regression; MPLSR, modified PLSR; InGaAs, Indium Gallium Arsenide; PbS, Lead Sulfide; Si, Silicon; LVF, linear variable filter; MEMS, micro-electro-mechanical system; DM, dry matter; CP, crude protein; CF, crude fiber; ADF, acid detergent fiber; NDF, neutral detergent fiber; CP, crude protein; ME, metabolizable energy; NDIN, neutral detergent insoluble nitrogen; SP, soluble crude protein; WSC, water-soluble carbohydrates; Na, sodium; S, selenium; B, boron; Zn, zinc; Mn, manganese; Cu, copper; Fe, iron, IVOMD, *in vitro* organic matter digestibility.

*Determination coefficient (R^2) of calibration.

**Standard error of calibration (SEC).

the models to predict CF, CP, ADF and NDF. Hetta et al. (2017) studied the possibility to evaluate nutritive, morphological and agronomic characteristics of maize forage, predicted by using a high-quality NIR spectrometer and the development of PLSR models for NDF, starch, water-soluble carbohydrates (WSC) and organic matter digestibility. Local calibration is a form of PLS regression when the calibration set is selected from a pool of samples and the selection is made based on the PCA projected similarity to the samples being predicted (Fearn 2001; Berzaghi, Shenk, and Westerhaus 2000). Contrary to the generally used global calibrations, where the entire calibration set is used, the local calibration technique allows evaluation of independent samples based on a certain number of samples of a database that are most similar to them. Tran et al. (2010) in a study to develop global and local prediction equations (Fearn 2001; Berzaghi, Shenk, and Westerhaus 2000) for dairy diet nutritional quality, reported the ability of NIRS to successfully and more accurately predict dairy cow diet properties (intake, quality, and digestibility) based on NDF, ADF, CA, and CP composition with local calibration techniques compared with classic global techniques, specifically, on an averaged data set. Authors noted that local calibration techniques represent a promising method and potentially a decision support tool to decide whether diets meet dairy cattle requirements or need to be modified. Parrini et al. (2018) investigated the use of FT-NIRS to estimate the nutritional value and the chemical composition of natural pastures and reported good accuracy and precision. The best estimates were obtained for CP and DM followed by ADL, while the lower estimation capacity is shown for the ash components.

Modroño et al. (2017) applied a handheld NIRS spectrometer with linear variable filter technology for routine compound feed quality control at real-time analysis and field monitoring and reported that calibration models displayed the greatest predictive capacity for CP, crude fiber (CF) and starch. A similar handheld NIRS gave a good accuracy of performance in the measurement of moisture (%), moderately good models for ADF, NDF and very poor performance for CP in Italian Ryegrass based silage (Park et al. 2011). In the evaluation of total dietary fiber in the homogenized meal (animal protein, vegetable protein and carbohydrate-based) employing NIRS, Kim, Singh, and Kays (2006) reported good performance of the calibration model and an accurate validation test. Cozzolino and Moron (2004) also predicted trace elements in legume forage and reported good prediction results.

Again, Pérez-Marín et al. (2004) examined the application of NIR technique to label compound feedstuffs based on the chemical composition. The results showed that the coefficient of determination (R^2_{CV}) and the standard error of cross-validation (SECV) values ranged from good to very good, depending on the parameter: moisture (0.87 and 4.9 g kg^{-1}), CP (0.97 and 5.5 g kg^{-1}), crude fat (ether extract, EE) (0.94 and 6.1 g kg^{-1}), CF (0.98 and 5.2 g kg^{-1}) and CA (0.85 and 6.9 g kg^{-1}). Lovett et al. (2004) used NIRS to predict the biological parameters of maize silage, and reported that NIRS demonstrated only a moderate ability (R^2 > 0.60–0.80) to predict in vitro digestibility, modeled kinetics of gas production and the modeled ruminally soluble DM fraction. In a study to identify how mathematical transformation could influence the accuracy of NIRS calibrations for the prediction of chemical composition and fermentation parameters in corn silage, Park et al. (2016) reported prediction coefficients for moisture and CP as R^2_{CV} 0.83–0.91 and 0.65–0.75, respectively), whereas for NDF and ADF (R^2_{CV} 0.60–0.86 and 0.78–0.86, respectively). The results also showed that calibration models for fermentation parameters had lower predictive accuracy than chemical constituents. However, some fermentation parameters such as pH, butyric and lactic acids were predicted with considerable accuracy (R^2_{CV} 0.74–0.77), whereas acetic acid was predicted with lower accuracy (R^2_{CV} 0.10–0.70). To predict the amount of grass which made a good alfalfa-grass silage mixture using NIRS results from Karayilanli et al. (2016) showed that the ratio of grass was predicted with good precision and accuracy giving biases of 2.49 and standard errors of prediction (SEP) of 5.06%, with R^2 of 0.972. NIR, therefore, could be a precise tool to estimate the ratio of formulation for grass mix silages (Karayilanli et al. 2016).

A study by Thomson et al. (2018) which assessed the accuracy of NIRS analysis for grass-clover mixture silages showed that, of the 15 chemical components that were tested for prediction accuracy, only volatile-corrected dry matter and nitrogen were well predicted (R^2 0.98 and 0.86). NDF and digestible organic matter in total dry matter (DOMD) showed R^2 values (0.56 and 0.64, respectively). However, the predicted and observed datasets had no significant bias between them and were therefore also considered as fit for purpose.

From the above-reviewed studies, it could be observed that the easiest predictable chemical components from fresh and ensiled forages are ADF, NDF, CP, DM, CA, moisture, starch, and minerals. The prediction coefficients were good, whereas the prediction errors obtained were also low. This reinforces the potential of NIRS to be used as a rapid analytical tool in the evaluation or determination of such constituent in forage or compound feeds. However, less accurate models were observed in ADL, ME, NDIN, Fe, and some fermentation parameters such as acetic and propionic acids, because of the low prediction coefficients and high prediction errors obtained during cross-validation tests.

The NIRS devices mostly used in the reviewed studies were dispersive grating system benchtop devises and portable devices. The portable device gave a similar performance in most of the cases when compared with the benchtop devices, this suggest that NIRS can be a very effective feed analytical tool on the farm to promote precision feeding of cows especially in periods when the metabolic balance is highly compromised (e.g., after calving, at peak lactation, metabolic disease, ketosis, acidosis).

Near-infrared spectroscopy in the evaluation of the quality of milk and dairy products

The quality of milk and dairy products largely depends on the composition and sensory properties. The most important

parameters of milk, either from the cow, goat or ewe, are the milk fat, proteins, lactose and fatty acid (FA) composition. The variations in milk fat and protein, especially, may influence the fat and protein contents of the final dairy product, whiles the FAs may influence the sensory properties (Hadjipanayiotou 1995).

The application of NIRS in the evaluation of milk protein, lactose and fat, has been documented by many authors. The recent advances have also looked at the possibility of using NIRS to detect adulterants in milk from different species and dairy products. The next phase of this review will focus on the application of NIRS to evaluate milk and some dairy products. A wide selection of results of the reviewed studies on milk, cheese and butter are presented in Tables 2–4, respectively, allowing comparison of analysis subjects, instrumentation and multivariate methods.

Milk quality analysis

Over the years, NIRS techniques have been developed to further provide accurate results to compliment other laboratory methods in the evaluation of bovine milk quality (Kawasaki et al. 2008). A recent study by de la Roza-Delgado et al. (2017) on matching portable NIRS instruments for in situ monitoring indicators of bovine milk composition also confirmed an accurate calibration for fat, protein and poor calibration for solid-non-fat (SNF). Similar performance was observed when cross-validation was done. Llano Suárez et al. (2018) studied the potential of a portable NIRS instrument in on-site monitoring of the fatty acid profile of bovine milk. The authors reported that calibration models displayed good predictive capacity for total saturated, monounsaturated (MUFAs) and polyunsaturated FAs (PUFAs) with high coefficients of determination of cross validation ($R^2_{CV} > 0.8$). In a study, Purnomoadi et al. (1999) investigated the influence of feed source on the determination of fat and protein in milk by NIRS, and reported that, NIR prediction of milk fat content was not influenced by the supplementary feed of animals, however, the accuracy of protein prediction was significantly affected by the kind of feedstuff used in the ration. This may be because the fat in the rations used in the study were similar and small to cause changes in milk fat content. Whereas dietary protein content and intake influences milk protein, and that different protein sources in diet may cause different proportions of nitrogen in milk.

In another study to evaluate the effectiveness of NIRS to predict FA composition of milk, Coppa et al. (2014) found that monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), n-6 FAs content and n-6/n-3 ratio were with high accuracy when milk was oven-dried. Authors concluded that the high prediction performance exhibited by NIR allows its use for routine milk FAs composition evaluation. In an experiment to determine the feasibility of measuring tetracycline at the ppb levels in bovine milk, Sivakesava and Irudayaraj (2002) used Fourier transform near-infrared (FT-NIR) spectroscopic technique. It was observed that prediction errors of milk samples spiked with different concentrations of tetracycline were high when the calibration model was developed using a wide range of tetracycline concentrations (4 to 2000 ppb). The maximum correlation coefficient (R^2) value of 0.87 was obtained for the validation models developed using different tetracycline concentration ranges as the adulterants. Kalinin et al. (2013) determined the composition of proteins and fat in bovine milk using a self-developed portable near-infrared spectrometer, and they reported strong cross-validation results for fat, casein and whey proteins in drinking milk. Laporte, Martel, and Paquin (1998) used a NIR reflectance fiber-optic probe and a coagulometer (thermal probe) for monitoring rennet coagulation of milk from individual cows. The study showed that NIR reflectance is a reliable method for monitoring and predicting milk coagulation. Furthermore, NIR technology provides a better estimate of the coagulation profile than the coagulometer (thermal probe).

Laporte and Paquin (1999) investigated how fat, crude protein, true protein, and casein could be determined in bovine milk by NIR transmission spectroscopy. Partial and overall PLS calibrations were performed on two sets of samples: partial calibration included 76 unhomogenized samples, whereas overall calibration used 96 homogenized and unhomogenized samples. They reported that except for fat, all of the statistical parameters were better with overall than with partial calibrations, which indicates that homogenization affects NIR-based fat determination. This confirms, that homogenization has an effect on fat globules, which causes scatter (Bogomolov, Melenteva, and Dahm 2013) and the scattering effect influences the prediction of fat.

Núñez-Sánchez et al. (2016) also employed NIR to evaluate the quality of dairy goat milk and reported that transflectance analysis gave better or similar cross-validation results than reflectance mode. The authors interestingly concluded that NIRS analysis allowed direct prediction of the atherogenicity (AI) and thrombogenicity indices (TI), which are useful for the interpretation of the nutritional value of goat milk. Kawasaki et al. (2008) also investigated the NIRS sensing system for on-line bovine milk quality assessment in a milking robot. Calibration models for determining three major milk constituents (fat, protein and lactose), somatic cell count (SCC) and milk urea nitrogen (MUN) of unhomogenized milk were developed, and the precision and accuracy of the models were validated. The coefficient of calibration and standard error of prediction of the validation set for fat were very good. The results of the study suggested that the NIR spectroscopic system can be used to assess milk quality in real-time in an automatic milking system. They added that such a system could provide dairy farmers with information on milk quality and physiological or health condition of an individual cow which can give a feedback control for optimizing dairy farm management. By using the system, dairy farmers will be able to produce high-quality milk and precision dairy farming will be realized.

Tsenkova et al. (2001) studied the influence of SCC of bovine milk on the accuracy of NIR spectroscopic determination of fat, protein and lactose content of non-homogenized milk. They reported that the best accuracy for fat,

Table 2. A comparative summary of representative studies investigating the prediction capacity of NIRS on the quality parameters of milk.

Product	N	Parameter	R^2_{cv}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
Bovine milk	258	Fat (%)	0.99*	0.25**	1st deriv.-SG	PLSR, 10 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, transmission	Tsenkova et al. (2001)
		Protein (%)	0.67*	0.17**				
		Lactose (%)	0.85*	0.09**				
	250	n-6 (g/100 g)	0.58	0.38	SNV, 2nd deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, transmission	Coppa et al. (2014)
		n-3 (g/100 g)	0.84	0.18				
		PUFAs (g/100 g)	0.84	0.46				
		MUFAs (g/100 g)	0.87	1.06				
		Total CLAs (g/100 g)	0.86	0.21				
	60	Tetracycline (ppb)	0.87	110.00	1st deriv.	PLSR, 4 factors	Benchtop, Scanning grating monochromator, DTGS detector, 1250–2857 nm, transmission	Sivakesava and Irudayaraj (2002)
	52	Coagulation (%)	0.87	0.36	None	MPLSR, 11 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, transmission	Laporte, Martel, and Paquin (1998)
	on-line NIRS	Fat (%)	0.95	0.25	None	PLSR, 10 factors	In-line NIRS sensing system, scanning in every 10 s during milking, linear array CCD, 2048 pixels, 600–1050 nm, transmission	Kawasaki et al. (2008)
		Lactose (%)	0.83	0.26		PLSR, 10 factors		
		Protein (%)	0.72	0.15		PLSR, 12 factos		
		SCC (logSCC/mL)	0.68	0.28		PLSR, 13 factors		
		MUN (mg/dL)	0.53	1.50		PLSR, 8 factors		
	81	Total Fat (%)	0.90	0.07	None	PLSR on combined spectra	Handheld, Modified shortwave NIRS analyzer, 800–1060 nm, transmission and backscattering	Kalinin et al. (2013)
		Casein (%)	0.88	0.12				
		Whey protein (%)	0.94	0.06				
	542	Fat (%)	0.96	0.10	SNV, Detrend, 2nd deriv.		Handheld, MEMS, PbS detector, 1600–2400 nm, reflectance	de la Roza-Delgado et al. (2017)
		Protein (%)	0.68	0.13		MPLSR		
		SNF (%)	0.48	0.22				
	108	MUFAs (g/100 g)	0.86	3.56	SNV, 2nd deriv.	PLSR, 7 factors	Handheld, MEMS, PbS detector, 1600–2400 nm, reflectance	Llano Suárez et al. (2018)
		PUFAs (g/100 g)	0.82	0.72		PLSR, 5 factors		
		SFAs (g/100 g)	0.82	3.86	1st deriv.	PLSR, 4 factors		
Unhomogenized bovine milk	96	Fat (%)	1.00	0.05	SNV, 2nd deriv.	MPLSR, 4 factors	Benchtop, Scanning grating monochromator, Si + PbS	Laporte and Paquin (1999)

(*continued*)

Table 2. Continued.

Product	N	Parameter	R^2_{CV}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
							detectors, 400–2500 nm, transmission	
		CP (%)	0.95	0.09		MPLSR, 7 factors		
		TP (%)	0.91	0.12				
		Casein (%)	0.96	0.07				
Homogenized bovine milk		Fat (%)	1.00	0.07	SNV, 2nd deriv.	MPLSR, 4 factors		
		CP (%)	0.99	0.06		MPLSR, 8 factors		
		TP (%)	0.99	0.05				
		Casein (%)	0.98	0.06				
Goat milk	805	MUFAs (g/100 g)	0.76	1.47	SNV, Detrend, 1st deriv.	MPLSR on combined spectra	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance and transmission	Núñez-Sánchez et al. (2016)
		PUFAs (g/100 g)	0.68	1.47				
		AI	0.77	0.28				
		TI	0.74	0.30				
		n-3 (g/100 g)	0.70	0.07				
		n-6 (g/100 g)	0.65	0.35				

N, sample number; R^2_{CV}, determination coefficient of cross-validation; SECV, standard error of cross-validation; SNV, standard normal variate; deriv., gap-segment derivatives; deriv.-SG, Savitzky-Golay derivatives; PLSR, partial least squares regression; MPLSR, modified PLSR; DTGS, deuterated triglycine sulfate; PbS, lead sulfide; Si, silicon; MEMS, micro-electro-mechanical system; CCD, charge-coupled device; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-3, omega-3 PUFA; n-6, omega-6 PUFA; AI, atherogenicity index; TI, thrombogenicity index; CP, crude protein; TP, true protein; CLA, conjugated linoleic acid; TP, true protein; SCC, somatic cell count; MUN, milk urea nitrogen.

*Correlation coefficient (r) of calibration.

**Standard error of calibration (SEC).

Table 3. A comparative summary of representative studies investigating the prediction capacity of NIRS on the quality parameters of cheese.

N	Parameter	R^2_{CV}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
60	Moisture (%)	0.92	0.02	None	PLSR	Benchtop, Bandpass filter NIR analyzer, PbS detector, reflectance	Adamopoulos, Goula, and Petropakis (2001)
	Protein (%)	0.89	0.03				
	Fat (%)	0.92	0.03				
131	Moisture (%)	0.96	1.64	MSC, 1st deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	González-Martín et al. (2008)
	Fat (%)	0.97	0.99	1st deriv.			
	Protein (%)	0.78	0.76				
	Chlorides (%)	0.89	0.11				
64	Fat (%)	0.98	0.45	SNV, Detrend, 2nd deriv.	MPLSR, 5 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Blazquez et al. (2004)
	Moisture (%)	0.99	0.50	SNV, Detrend, 2nd deriv.	MPLSR, 4 factors		
	Inorganic salts (%)	0.90	0.26	SNV, Detrend	MPLSR, 7 factors		
250	Total solids (%)	0.99	0.50	None	PLSR, 9 factors	Benchtop, FT-IR, DTGS detector, 833–5000 nm, reflectance	Sultaneh and Rohm (2007)
	Protein (%)	0.98	0.54		PLSR, 10 factors		
50	Hardiness	0.89	0.30	Detrend, 2nd deriv.	MPLSR, 7 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	González-Martín, Severiano-Pérez, et al. (2011)
	Creamy	0.53	0.50	MSC	MPLSR, 5 factors		
	Holes	0.79	0.80	2nd deriv.	MPLSR, 7 factors		
	Chewiness	0.83	0.30		MPLSR, 7 factors		
	Salty	0.56	0.50		MPLSR, 4 factors		
	Buttery flavor	0.72	0.50		MPLSR, 7 factors		
	Rancid flavor	0.76	0.50		MPLSR, 10 factos		
	Pungent intensity	0.82	0.50		MPLSR, 7 factors		
	Retro-nasal	0.82	0.70		MPLSR, 7 factos		
112	Hydrophilic peptides (%)	0.87	0.30	MSC	MPLSR, 9 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	González-Martín et al. (2009)
	Hydrophobic peptides (%)	0.87	1.83				
170	Calcium (g/kg)	0.74	0.82	2nd deriv.	MPLSR, 8 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	González-Martín, Hernández-Hierro, et al. (2011)
	Phosphorus (g/kg)	0.69	0.33	MSC, 1st deriv.	MPLSR, 8 factors		
	Potassium (g/kg)	0.86	0.16	MSC, 2nd deriv.	MPLSR, 8 factors		
	Sodium (g/kg)	0.92	0.80	MSC, 2nd deriv.	MPLSR, 10 factors		
	Magnesium (ppm)	0.72	40.20	Detrend	MPLSR, 10 factors		
445	Caprylic acid (g/100 g)	0.84	0.52	Detrend, 1st deriv.	MPLSR	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Lucas et al. (2008)

(continued)

Table 3. Continued.

N	Parameter	R^2_{CV}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
	Caprlc acid (g/100 g)	0.93	1.54	SNV, Detrend, 1st deriv.			
	linoleic acid (g/100 g)	0.60	0.62	SNV, Detrend, 1st deriv.			
46	Moisture (%)	0.97	0.94	SNV, 1st deriv.	PLSR, 4 factors	Handheld; Diode array sensor, 740–1070 nm, reflectance	Wiedemair et al. (2019)
	Fat (%)	0.98	1.11	SNV, 1st deriv.	PLSR, 4 factors		
49	Intact casein (g/kg)	0.91	0.58	SNV, 2nd deriv.-SG	PLSR, 14 factors	Handheld; Diode array sensor, 740–1070 nm, reflectance	Ma, Babu, and Amamcharla (2019)
	Total Protein (%)	0.87	0.69	SNV	PLSR, 8 factors		

N, sample number; R^2_{CV}, determination coefficient of cross-validation; SECV, standard error of cross-validation; SNV, standard normal variate; MSC, multiplicative scatter correction; deriv., gap-segment derivatives; deriv.-SG, Savitzky-Golay derivatives; PLSR, partial least squares regression; MPLSR, modified PLSR; DTGS, deuterated triglycine sulfate; PbS, lead sulfide; Si, silicon.

protein and lactose content estimation was found for calibration equations, derived from samples with low SCC. However, the standard error of prediction was reported to have increased and the coefficient of determination decreased significantly when equations derived from samples with low SCC milk were used to predict the content of the examined components in samples with high SCC, and when equations, obtained for samples with high SCC were used to predict the composition of samples with low SCC. Therefore, milk samples with high SCC in a calibration data set highly influenced the accuracy of fat, protein and lactose determination.

Maraboli, Cattaneo, and Giangiacomo (2002) reported that NIR spectroscopy proved an effective method in determining vegetable proteins from soy, pea and wheat isolates in milk powder. Wu, Feng, and He (2007) also investigated the potential of the NIR technique for rapid and nondestructive measurement of fat content in milk powder and reported good cross-validation results. Chen et al. (2018) also reported good results for classification based on principal component analysis (PCA) and quantization of milk powder by NIRS and mutual information-based variable selection and partial least squares regression or PLSR. In a study to evaluate the possible use of visible (VIS) and NIR spectroscopic analysis of raw milk for cow health monitoring, VIS-NIR reflectance measurements allowed for very accurate monitoring of the fat and crude protein ($R^2 > 0.95$) content in raw milk (Aernouts et al. 2011; Melenteva et al. 2016; Woo et al. 2002) but resulted in weaker lactose prediction ($R^2 < 0.75$). However, VIS-NIR transmittance spectra of the milk samples gave accurate fat and crude protein predictions ($R^2 > 0.90$) and useful lactose ($R^2 = 0.88$) predictions (Aernouts et al. 2011). Unfortunately, neither VIS-NIR reflectance nor transmittance spectroscopy led to an acceptable prediction of the milk urea content.

Numthuam et al. (2017) studied the possible use of NIR spectroscopy in the region of 700–2500 nm o determine total bacterial count (TBC) in raw bovine milk. They showed that bacterial cultivation had an impact on TBC prediction accuracy. Their results showed that the best calibration was obtained from 90 minutes cultivation samples, with the correlation coefficient (R) of 0.90 and root mean square error of prediction of 0.32 $\log_{10}$ CFU/ml. The study concluded that the NIR method combined with bacterial cultivation was satisfactory for the rapid analysis of TBC in raw milk.

NIRS has been used to also determine milk adulteration or authentication. In a study, Balabin and Smirnov (2011) reported that melamine (2, 4, 6-tri amino-1, 3, 5-triazine) detection by NIR spectroscopy achieved a good prediction. It was found that near-infrared spectroscopy is an effective tool to detect melamine in dairy products, such as infant formula, milk powder, or liquid milk. They further added that the relationship between NIR spectrum of milk products and melamine content is nonlinear. Thus, nonlinear regression methods are needed to correctly predict the triazine-derivative content of milk products. This agrees with the results of Scholl et al. (2017) which stated that the prominent absorbance band at 1468 nm of melamine was

Table 4. A comparative summary of representative studies investigating the prediction capacity of NIRS on the quality parameters of butter.

N	Parameter	R^2_{cv}	SECV	Spectral pretreatment	Multivariate model	Spectrometer technology, spectral range, scanning mode	Reference
102	Moisture (%)	0.83	0.25	SNV, Detrend, 1st deriv.	MPLSR, 6 factors	Benchtop, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Hermida et al. (2001)
	Solids-non-fat (%)	0.94	0.07		MPLSR, 8 factors		
	Fat (%)	0.72	0.37		MPLSR, 8 factors		
99	Tallow adulteration (%)	0.95	1.53	1st deriv.	PLS-DA	Benchtop, FT-NIR, DTGS detector, 1000–2500 nm, reflectance	Mabood et al. (2018)
26	Fat (%)	0.90	1.16	None	PLSR	Benchtop, FT-NIR, InGaAs detector, 833–2630 nm, reflectance	Dvořák, Lužová, and Šustová (2016)
	Moisture (%)	0.90	2.08				
	Acid number ($mg\ g^{-1}$)	0.27	0.29				
76	Solid Fat (%)	0.92–0.97	0.39–0.76	SNV, Detrend, 1st deriv.	PLSR	At-line NIRS, Scanning grating monochromator, Si + PbS detectors, 400–2500 nm, reflectance	Meagher et al. (2007)

N, sample number; R^2_{cv}, determination coefficient of cross-validation; SECV, standard error of cross-validation; SNV, standard normal variate; deriv., gap-segment derivatives; PLSR, partial least squares regression; MPLSR, modified PLSR; PLS-DA, partial least-squares discriminant analysis; DTGS, deuterated triglycine sulfate; InGaAs, indium gallium arsenide; PbS, lead sulfide; Si, silicon.

retained when it was dry-blended with skim milk powder but disappeared in wet-blended mixtures, where spray dried milk powder samples were prepared from solution. Liu et al. (2018) worked on the evaluation of portable and benchtop NIRS for organic milk authentication and reported that both instrumentations were successful to distinguish organic milk from conventional milk. In a similar study, Kasemsumran, Thanapase, and Kiatsoonthon (2007) confirmed the feasibility of NIRS to detect and to quantify whey adulterants in bovine milk. The study concluded that, for the detection of whey adulterant, a suitable wavelength region of 1100–2500 nm and pretreated spectra with MSC and 2nd-derivative method were needed.

From the above discussions and reviewed information in Table 2, the most predicted milk quality parameters were milk protein, lactose, total fat and fatty acid composition, mainly PUFAs and MUFAs. When compared with milk protein, high prediction coefficients were observed for milk fat and lactose. The n-3 fatty acid in milk had averagely, higher prediction coefficients compared with n-6. Other studies also considered the atherogenicity and thrombogenicity indices, somatic cell count and milk urea nitrogen, which are important for milk quality, as well. Somatic cell count has proven to influence the shelf life of milk and therefore has become an important parameter to consider in fresh milk storage. It is therefore important to have a tool like NIRS for the rapid determination of these milk parameters, especially, the portable NIRS devices to ensure real-time or 'on the spot' analysis. Milk urea nitrogen was less accurately predicted. In milk quality analysis using NIRS, most of the reviewed studies used, portable, in-line and benchtop devices, mostly with dispersive grating monochromators.

Cheese quality analysis

In related studies which examined the possibility of using NIRS for the determination of total solids and protein content in cheese curd, calibration models built using PLSR showed high coefficients of determination for total solids and protein content (Sultaneh and Rohm 2007; Frank and Birth 1982; Fagan et al. 2009). Ma, Babu, and Amamcharla (2019) in a study to predict total protein and intact casein (IC) in cheddar cheese using a low-cost handheld short-wave near-infrared spectrometer reported accurate performance of calibration and validation models to predict total protein and intact casein in cheese. The authors concluded that handheld NIRS can provide useful quantification tool for a rapid prediction of IC and total protein in cheddar cheese. Wiedemair et al. (2019) in a similar study compared the performance of a novel pocket-sized and benchtop NIRS for grated cheese analysis and reported that the PLS-R yielded coefficients of determination (R^2) for moisture and fat above 0.9. The results further showed that ratios of performance to deviation (RPDs) and standard errors of prediction (SEPs) suggest that the performance of the pocket-sized spectrometer is comparable to the benchtop device.

Adamopoulos, Goula, and Petropakis (2001) also studied the application of NIRS method to evaluate the quality

during the processing of traditional Greek feta cheese. The prediction models obtained for moisture, fat and protein were reported to be accurate. From the study, it was concluded that the NIR technique can be applied successfully for the on-line quality control of the feta cheese production. Using VIS-NIR spectroscopy on cheese to authenticate cow feeding regimes, Andueza et al. (2013) found that cheeses prepared from cow milk obtained from cows fed with 63% fresh grass, 12% hay and 23% concentrate were correctly determined by NIRS using PCA, and concluded that NIRS was able to classify cheese samples from different feeding regimes or ration.

González-Martín, Severiano-Pérez, et al. (2011) evaluated the visual (presence of holes), taste (salty, buttery rancid flavor), texture (hardness, chewiness, creamy) attributes, and other sensations (pungency, retronasal sensation) of cheeses prepared from milk collected from various species (cow, ewe, goat) in winter and summer, and subjected to ripening times of 4 and 6 months. Authors reported that the robustness of the NIR method was successfully tested on 14 unknown cheese samples in the case of physical factors, such as hardness. González-Martín et al. (2009) studied the application of NIRS technology and a remote reflectance fiber-optic probe for the determination of hydrophilic and hydrophobic peptides in cheeses (cow's, ewe's and goat's) with different ripening times. It was found that a good prediction of hydrophobic and hydrophilic peptides in cheese was possible and the method allows immediate control because of the direct application of the fiber-optic probe to the cheese without prior sample treatment or destruction.

González-Martín, Hernández-Hierro, et al. (2011) studied the application of NIRS to determine the mineral content (Ca, P, Mg, K, and Na) in cheeses (made from cow, ewes and goat milk) with different ripening times. Their results showed that it is possible to rapidly quantify the major minerals in unknown cheeses elaborated with 0%–100% percentages of milk from different species (cow, ewe, and goat) by direct measurements with fiber-optic probe. The study further indicated that the developed models allow the determination of Ca, P, K, Na and Mg in unknown samples of cheeses of varying compositions up to 6 months of ripening.

In a similar experiment, Manuelian et al. (2017) investigated the possible prediction of minerals, fatty acids (FAs) composition and cholesterol content of commercial cheeses by NIR transmittance spectroscopy. The study reported satisfactory models ($R^2 > 0.85$) developed for Ca, P, S, Mg and Zn, and for FAs groups (saturated, unsaturated, monounsaturated and polyunsaturated FAs), major FAs (myristic, palmitic and oleic acids) and some minor FAs, whereas cholesterol content could not be predicted with adequate accuracy). Lucas et al. (2008) studied the fatty acid composition of fresh and freeze-dried cheeses by VIS-NIR reflectance spectroscopy. The results showed that the coefficient of determination in external validation (R^2) and residual predictive deviation in the validation set (RPDV) were good for total saturated (0.89, 2.84), monounsaturated (0.90, 2.90), polyunsaturated (0.88, 2.54), trans (0.92, 3.24) FAs in fresh cheeses. Approximate or poor predictions were obtained for caproic, caprylic, lauric, stearic, oleic, linoleic, and linolenic acids ($R^2 \leq 0.80$; RPDV ≤ 2.08). The quantification was significantly more accurate for caprylic, capric, and linoleic acids with freeze-dried cheeses compared with fresh cheeses (Table 3). The study concluded that freeze-drying is needed to properly obtain good calibration for caprilic, capric and linoleic acids. In a similar study to authenticate the cow feeding regime (pasture versus preserved forage) together with the cheese sample preparation methods (fresh versus freeze dried), Andueza et al. (2013) used NIRS and PCA. The results of the study showed that the proportion of cheeses correctly classified by NIRS and visible spectra was respectively 96 and 91% for pasture samples, and 96 and 79% for preserved-forage samples. No significant differences were found when fresh and freeze-dried cheeses were compared. They concluded that NIRS can classify cheese samples from different regimes (pasture versus preserved forage), and further added that removing water from the cheese before NIRS analysis by freeze-drying did not improve the accuracy of discrimination models obtained with fresh cheeses, thus, the freeze-drying of cheese before NIRS analysis seemed to be unnecessary. This contradicts the results or observations by Lucas et al. (2008).

In a work to determine the potential of NIRS together with a remote reflectance fiber-optic probe for the analysis of fat, moisture, protein and chlorides contents of commercial cheeses produced with mixtures of cow, ewe and goat's milk and with different curing times, González-Martín et al. (2008) reported that the calibrations developed for the cheese samples allowed to predict fat, moisture, protein, and chloride contents accurately ($R^2 = 0.78–0.97$).

Madalozzo, Sauer, and Nagata (2015) in a study to estimate the fat, protein and moisture contents in ricotta cheese employed NIRS and multivariate calibration techniques. The results showed that the multivariate models with six latent variables (LVs) allowed good prediction capability for fat and protein determinations, with average relative errors of 6.37% and 5.95%, respectively. And for the moisture content, a more robust model was obtained with 4 LVs, showing better prediction capacity and error of 1.91%. Blazquez et al. (2004) also studied the prediction of moisture, fat and inorganic salts in processed cheese by NIRS and multivariate data analysis, and reported that results were sufficiently accurate and, further recommended NIR reflectance spectroscopy for off-line quality assessment of processed cheese. Similar results were documented in a study which involved the nondestructive determination of components in processed cheese slice wrapped with a polyethylene film (Pi et al. 2009).

Nicolau et al. (2015) studied how NIR light backscatter fiber optic sensor could be implemented to monitor coagulation of sheep milk and assess the possibility of estimating both clotting and cutting times during the manufacture of sheep's cheese. The results of the study suggested that optical parameters generated from the NIR light backscatter profile adequately responded to the kinetic changes induced by varying the concentration of enzyme and that prediction of rheologically determined cutting time using NIR light

backscatter is more precise than the visually determined cutting time, i.e., 50% lower standard error of prediction (SEP) than the standard error observed in the visually determined reference values. It is important to note that the optical parameters generated during the sheep milk coagulation were obtained before the rheological ones or the first flocks of casein were visible to the human eye. The prediction equations allowed the subjective clotting and cutting time established by the cheese-maker to be predicted with a high correlation coefficient, thus, the method could assist sheep cheese manufacturers in achieving a better homogeneity in the end product.

Karoui et al. (2006) also studied a comparison and joint use of NIR and MIR spectroscopic methods for the determination of some parameters in European Emmental cheese. The parameters studied were fat, sodium chloride (NaCl), pH, non-protein nitrogen (NPN), total nitrogen (TN) and water-soluble nitrogen (WSN). The results showed accurate determination of fat and TN contents, when NIR was applied, while MIR gave accurate results for NaCl, NPN contents, and pH. Results from the combined spectra of both NIR and MIR showed an improvement in the results while providing comparable results to those obtained from either the NIR or MIR spectroscopy. Currò et al. (2017) studied the feasibility of NIR transmittance spectroscopy to predict cheese ripeness using the ratio of WSN to TN as an index of cheese maturity (WSN/TN) and reported that the coefficients of determination for WSN and TN were above 0.85 both in cross-validation and independent validation. The study concluded that the high accuracy of the prediction equations for WSN and TN could facilitate the implementation of NIR transmittance spectroscopy in the dairy industry to objectively, rapidly, and accurately monitor the ripeness of cheese through the use of WSN/TN ratio.

Priyashantha et al. (2020) also reported that near-infrared hyperspectral (NIR-HS) imaging was able to visualize and model the maturity of long-ripening hard cheeses. Blazquez et al. (2006) in a study investigated the application of NIR reflectance spectroscopy to the measurement of texture (sensory and instrumental) in experimental processed cheese samples. The results showed that sensory attributes and instrumental texture measurements were modeled with sufficient accuracy to recommend the use of NIR reflectance spectroscopy for routine quality assessment and control of processed cheese.

In the evaluation of cheese quality using NIRS, the moisture, protein, fat, inorganic salts, total solids or mineral composition and some other quality attributes, such as hardiness and presence of holes were considered. Generally, high prediction coefficients above 0.90 were observed for fat and moisture constituents of cheese, whereas protein ranged from 0.78 to 0.98. This variation in protein prediction could be due to the composition of the milk used in the preparation of the cheese or to the difference in the NIRS devices used. Again, inorganic salts or mineral composition, such as P, Ca, K, Mn and Na had fairly good prediction results. Creaminess, saltiness as a sensation, and presence of holes in cheese had less accuracy of prediction. These parameters are influenced by many factors and could show large differences among cheese types, thus, may be poorly followed by NIR spectroscopy. Holes, for instance, may indicate the damage of certain types of cheese, while being an essential indicator of good ripening in others, like Emmental. Type-specific studies may provide better results for such parameters.

The portable NIRS devices performed accurately just like the benchtops. Furthermore, the NIRS devices mostly used in the reviewed quality evaluations of cheese were dispersive grating systems.

Butter quality analysis

Meagher et al. (2007) employed an at-line NIRS technique for the prediction of the solid fat content of milk fat from New Zealand butter in a temperature range of 0 °C to 35 °C in 5 °C increment. Accurate predictions for the solid fat content were developed by PCA and PLS regression models to relate the NIR spectra to the corresponding nuclear magnetic resonance (NMR) spectroscopy values. Excellent predictive ability of NIRS was confirmed by validation obtained in the 0 °C to 30 °C range. Hermida et al. (2001) studied the analysis of moisture, solids-non-fat and fat in butter without any previous sample treatment. The study reported that NIR was a useful tool in predicting moisture, solid-non-fat and fat. Authors added that no significant differences were found in the validation set when the results obtained by NIR spectroscopy were compared with those obtained by the reference methods, using linear regression and paired t-test ($p = 0.05$).

Dvořák, Lužová, and Šustová (2016) evaluated and compared the butter quality parameters available on the Czech market with the use of FT-NIR technology. They demonstrated that FT-NIR could be used to divide the measured samples of butter into two classes according to their origin, i.e., Czech and foreign. The statistical progressing of the results did not confirm conclusive differences in the amount of the measured components (fat, dry matter and acid number) between Czech and foreign butter. They concluded that functionality of the calibration models for the fat content and dry matter was demonstrated, while the calibration model for the assessment of the acid number was unreliable. Mabood et al. (2018) applied NIR spectroscopy coupled with multivariate methods for the detection and quantification of tallow adulteration in clarified butter samples. There was an excellent performance of the model which was proved by the low root mean square error of prediction (RMSEP) value and the high determination coefficient. It was concluded that the newly developed method was robust, non- destructive, highly sensitive, and economical with very small sample preparation and good ability to quantify less than 1.5% of tallow adulteration in clarified butter samples.

To obtain a rapid method that could detect adulteration of butter fats with cheaper vegetable fats, Heussen et al. (2007) explored NIR spectroscopy and multivariate modeling technique and reported that prediction models had

RMSEPs for butterfat and C4:0 fatty acid level in the range of 4.3–8.2 and 0.33–0.38% (w/w), respectively.

Similar to cheese and milk quality analyses, the use of NIRS to evaluate butter quality also emphasized on the moisture content, the fat, solids-non-fat and adulteration. Moisture, fat, and solids-non-fat, had good prediction coefficients with low prediction errors. The acid number which is significant in the determination of butter rancidity obtained a poor prediction. The possibility of detecting tallow adulterants in butter using NIRS was also noted with high accuracy of prediction and low prediction errors.

Yoghurt quality analysis

The application of NIR spectroscopy in the analysis and authentication of yoghurt in the dairy industry is also gaining attention. Few studies have employed this technique to either authenticate or predict the constituents present in yoghurt samples during the processing and after the process to ensure quality and safety of the product. He et al. (2006) reported that NIRS was able to classify five types of yoghurt (MengNiu from Neimenggu, China), JunYao (from Shanghai, China), GuangMing (from Shanghai, China), YiLi (from Neimenggu, China) and Shuang-Feng (from Hangzhou, China) using PCA. Xu et al. (2013) reported that adulterations of yoghurt with 1% (w/w) edible gelatin, 2% (w/w) industrial gelatin, and 2% (w/w) soy protein powder can be safely detected by the NIR method. The results showed orthogonal projection (OP) with a sensitivity of 0.900 and specificity of 0.949.

Shao and He (2009) in a study to measure soluble solids content (SSC) and pH of yoghurt using VIS-NIR spectroscopy and chemometrics, reported that VIS-NIR spectroscopy combined with least squares support vector machine (LS-SVM) models could predict both values. He et al. (2006b) also reported a correlation coefficient between NIR-predicted sugar content and laboratory-measured sugar content of yoghurt samples to be more than 0.93. Fluvià Sabio (2015) documented a good prediction of fat in yoghurt using a handheld NIRS device for the detection of fraud in the fat content of natural yoghurts with a precision of 0.92% in a range of 0 to 9.9% using a PLS model ($R^2 = 0.975$, RMSEP = 0.46). Jarén et al. (2012) reported that the correlation coefficient between NIR-predicted and laboratory determined fat content of fresh yoghurt was 0.965, the standard error of calibration (SEC) was 0.587, and the standard error of prediction (SEP) was 0.642, the study further affirmed the usefulness of NIRS in analyzing yoghurt quality.

In a study to determine the acidity of five brands of yoghurts bought from the Chinese market, He et al. (2006a) based on PLS modeling, found a correlation coefficient of 0.89 for pH, and SEC of 0.037, SEP of 0.043, and concluded that NIRS is a useful tool to predict yoghurt acidity. In an in-line control of yoghurt fermentation process using a NIR light backscatter sensor, results demonstrated that NIR spectroscopy was a promising method for the inline control of pH and fermentation process in industrial yoghurt production (Aljaafreh 2015; Arango, Castillo, and Castillo 2020). According to Slavchev et al. (2015), NIRS combined with aquaphotomics data evaluation protocol (Tsenkova 2009) was able to classify yoghurts by the probiotic activity of the cultivated *Lactobacillus* strains. Again, the spectra information provided by water conformation reveals the important differences between probiotic and non-probiotic *Lactobacillus* strain.

Conclusions

The application of NIRS in the dairy industry has evolved over the years. Its usefulness in the evaluation of animal feed, compared with wet chemistry methods has proven to be significant. More importantly, the rapid determination of ADF, NDF, EE, CP, moisture, ash, and ADL of forages or TMR, using NIRS could give a fair understanding of the nutritional composition of the feed and could support feeding specialist aiming precision feeding, especially in highly sensitive periods of cows when the metabolic balance is compromised.

Considering studies where different drying methods were applied on forages, oven drying at 65 °C results a significant spectral change in the absorption regions of CP, CF, NDF and ADF compared with freeze-dried samples, and, there is no reduction in the accuracy of DM, CP, ADF, and IVOMD calibrations when air forced oven drying at 60 °C for 48 h with freeze-drying is compared. The quantification of cheese FAs composition shows that significantly more accurate predictions can be achieved for some FAs with freeze-dried cheeses compared with fresh cheeses.

In the evaluation of the performance of some of the NIRS devices, contradicting results were obtained, especially for the self-modified NIRS devices. This may be a limitation that needs further consideration. The above review has also shown the possibility of NIRS as a rapid method to determine milk, cheese, butter and yoghurt quality. The fat and protein contents were the major components which were studied when considering, milk, cheese, butter and yoghurt. The mineral compositions were also considered in some of the studies. The results obtained for these milk components ranged from moderately good to accurate calibration models and cross-validation test. Dairy product authentication or qualification was also demonstrated to be possible employing NIRS.

Overall, it can be concluded that NIRS is a rapid and noninvasive method for providing the nutritional and quality attributes of animal feed and dairy products. Handheld and in-line devices gave a similar performance in many investigated attributes as the benchtop devices. However, usage of the handheld devices was low among researches compared with the benchtops. And, with regards to butter and yoghurt analysis, no or very limited research involving handheld NIRS exist. It is therefore important to explore more, the usage of handheld devices, to ease the NIR analysis of feed and dairy products, and to further move the analysis from the laboratory to field for real-time analysis. Handheld technology will not only assist the decision making of feed analysts, animal nutritionists and food technologists, but will also improve the quality of products and

production, and the intensive development of miniature instrumentation might open other fields of NIRS application that are not currently considered.

Acknowledgment

The authors acknowledge Phil Williams, John Antoniszyn and Marena Manley, and the publisher, AFRICAN SUN MeDIA, for granting the permission to use Figure 2.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry for Innovation and Technology under GINOP-2.3.2-15-2016-00046; the Ministry of Human Capacities under EFOP-3.6.3-VEKOP-16-2017-00005; the Hungarian Academy of Sciences under MTA-KE-SZIE 13003; New National Excellence Program of the Ministry for Innovation and Technology under ÚNKP-19-4 (Zoltan Kovacs) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Zoltan Kovacs).

ORCID

Haruna Gado Yakubu http://orcid.org/0000-0002-9606-7140
Zoltan Kovacs http://orcid.org/0000-0003-0641-8830
Tamas Toth http://orcid.org/0000-0003-4457-1473
George Bazar http://orcid.org/0000-0001-9829-4366

References

Adamopoulos, K. G., A. M. Goula, and H. J. Petropakis. 2001. Quality control during processing of feta cheese – NIR application. *Journal of Food Composition and Analysis* 14 (4):431–40. doi: 10.1006/jfca.2000.0976.

Aernouts, B., E. Polshin, J. Lammertyn, and W. Saeys. 2011. Visible and near-infrared spectroscopic analysis of raw milk for cow health monitoring: Reflectance or transmittance? *Journal of Dairy Science* 94 (11):5315–29. doi: 10.3168/jds.2011-4354.

Aljaafreh, A. 2015. Evaluation of using NIR simplified spectroscopy in yogurt fermentation automation. In 8th International Conference on Researches in Engineering, Technology and Sciences (ICRETS), Istanbul (Turkey), 1–3.

Alomar, D., R. Fuchslocher, J. Cuevas, R. Mardones, and E. Cuevas. 2009. Determination of quality parameter of pasture by NIR reflentance spectroscopy. *Chilean Journal of Agricultural Research* 69 (2): 198–206. doi: 10.4067/S0718-58392009000200009.

Alomar, D., R. Fuchslocher, and S. Stockebrand. 1999. Effects of oven- or freeze-drying on chemical composition and NIR spectra of pasture silage. *Animal Feed Science and Technology* 80 (3–4):309–19. doi: 10.1016/S0377-8401(99)00053-X.

Andueza, D., C. Agabriel, I. Constant, A. Lucas, and B. Martin. 2013. Using visible or near infrared spectroscopy (NIRS) on cheese to authenticate cow feeding regimes. *Food Chemistry* 141 (1):209–14. doi: 10.1016/j.foodchem.2013.02.086.

Arango, O., M. Castillo, and M. Castillo. 2020. Inline control of yoghurt fermentation process using a near infrared light backscatter sensor. *Journal of Food Engineering* 277:109885–6. doi: 10.1016/j.jfoodeng.2019.109885.

Arzani, H., A. Sanaei, A. Barker, S. Ghafari, and J. Motamedi. 2015. Contents available at ISC and SID journal homepage: www.rangeland.ir estimating nitrogen and acid detergent fiber contents of grass species using near infrared reflectance spectroscopy (NIRS). *Journal of Rangeland Science* 5 (4):260–8.

Balabin, R. M., and S. V. Smirnov. 2011. Melamine detection by mid- and near-infrared (MIR/NIR) spectroscopy: A qquick and sensitive method for dairy products analysis including liquid milk, infant formula, and milk powder. *Talanta* 85 (1):562–8. doi: 10.1016/j.talanta.2011.04.026.

Bec, K. B., J. Grabska, H. W. Siesler, and C. W. Huck. 2020. Handheld near-infrared spectrometers: Where are we heading? *NIR News* 31 (3–4):28–35. doi: 10.1177/0960336020916815.

Berzaghi, P., J. S. Shenk, and M. O. Westerhaus. 2000. LOCAL prediction with near infrared multi-product databases. *Journal of near Infrared Spectroscopy* 8 (1):1–9. doi: 10.1255/jnirs.258.

Bilge, G., B. Sezer, K. E. Eseller, H. Berberoglu, A. Topcu, and I. H. Boyaci. 2016. Determination of whey adulteration in milk powder by using laser induced breakdown spectroscopy. *Food Chemistry* 212:183–8. doi: 10.1016/j.foodchem.2016.05.169.

Blazquez, C., G. Downey, C. O'Donnell, D. O'Callaghan, and V. Howard. 2004. Prediction of moisture, fat and inorganic salts in processed cheese by near infrared reflectance spectroscopy and multivariate data analysis. *Journal of near Infrared Spectroscopy* 12 (3):149–57. doi: 10.1255/jnirs.420.

Blazquez, C., G. Downey, D. O'Callaghan, V. Howard, C. Delahunty, E. Sheehan, C. Everard, and C. P. O'Donnell. 2006. Modelling of sensory and instrumental texture parameters in processed cheese by near infrared reflectance spectroscopy. *The Journal of Dairy Research* 73 (1):58–69. doi: 10.1017/S0022029905001536.

Bogomolov, A., A. Melenteva, and D. J. Dahm. 2013. Fat globule size effect on visible and shortwave near infrared spectra of milk. *Journal of near Infrared Spectroscopy* 21 (5):435–40. doi: 10.1255/jnirs.1076.

Chen, H., C. Tan, Z. Lin, and T. Wu. 2018. Classification and quantitation of milk powder by near-infrared spectroscopy and mutual information-based variable selection and partial least squares. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy* 189:183–9. doi: 10.1016/j.saa.2017.08.034.

Coppa, M., A. Revello-Chion, D. Giaccone, A. Ferlay, E. Tabacco, and G. Borreani. 2014. Comparison of near and medium infrared spectroscopy to predict fatty acid composition on fresh and thawed milk. *Food Chemistry* 150:49–57. doi: 10.1016/j.foodchem.2013.10.087.

Cozzolino, D., and A. Moron. 2004. Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Animal Feed Science and Technology* 111 (1–4):161–73. doi: 10.1016/j.anifeedsci.2003.08.001.

Currò, S., C. L. Manuelian, M. Penasa, M. Cassandro, and M. De Marchi. 2017. Technical note: Feasibility of near infrared transmittance spectroscopy to predict cheese ripeness. *Journal of Dairy Science* 100 (11):8759–63. doi: 10.3168/jds.2017-13001.

Dale, M. 2018. Scaling spectroscopy: Matthew Dale discovers how scaling spectrometers down to the chip level enables them to be mass-produced in standard MEMS foundries, making spectroscopy more affordable for various new applications. *Electro Optics* 281:20. https://go.gale.com/ps/anonymous?id=GALE%7CA532077120&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=00134589&p=AONE&sw=w.

de la Roza-Delgado, B., A. Garrido-Varo, A. Soldado, A. González Arrojo, M. Cuevas Valdés, F. Maroto, and D. Pérez-Marín. 2017. Matching portable NIRS instruments for in situ monitoring indicators of milk composition. *Food Control* 76:74–81. doi: 10.1016/j.foodcont.2017.01.004.

Dickens, J. E. 2010. Chapter 1: Overview of process analysis and PAT. In *Process analytical technology: Spectroscopic tools and implementation strategies for the chemical and pharmaceutical industries*. 2nd ed., ed. K. A. Bakeev. West Sussex, UK: John Wiley & Sons, Ltd. doi: 10.1002/9780470689592.ch1.

Dillon, P. 2007. Achieving high dry-matter intake from pasture with grazing dairy cows. *Fresh Herbage for Dairy Cattle* 2007:1–26. doi: 10.1007/978-1-4020-5452-5_1.

Dvořák, L., T. Lužová, and K. Šustová. 2016. Comparison of butter quality parameters available on the Czech market with the use of FT

NIR technology. *Mljekarstvo* 66 (1):73–80. doi: 10.15567/mljekarstvo.2016.0108.

Fagan, C. C., M. Castillo, D. J. O'Callaghan, F. A. Payne, and C. P. O'Donnell. 2009. Visible-near infrared spectroscopy sensor for predicting curd and whey composition during cheese processing. *Sensing and Instrumentation for Food Quality and Safety* 3 (1):62–9. doi: 10.1007/s11694-009-9073-5.

Fagan, C. C., and C. P. O'Donnell. 2011. ChemInform abstract: Applications of vibrational spectroscopy to the study of cheese and other fermented, solid and semi-solid dairy products. In *Handbook of Vibrational Spectroscopy*, eds. P. Griffiths and J. M Chalmers. Hoboken, NJ: John Wiley & Sons, Ltd. doi: 10.1002/0470027320.

Fearn, T. 2001. Local or global? *NIR News* 12 (3):10–1. doi: 10.1255/nirn.614.

Fearn, T. 2002. Assessing calibrations: SEP, RPD, RER, R^2. *NIRS News* 13 (6):12–4. doi: 10.1255/nirn.689.

Fluvià Sabio, S. 2015. NIR techniques and chemometric data analysis applied to food adulteration. Master's thesis.

Frank, J. F., and G. S. Birth. 1982. Application of near infrared reflectance spectroscopy to cheese analysis. *Journal of Dairy Science* 65 (7):1110–6. doi: 10.3168/jds.S0022-0302(82)82319-9.

García, J., and D. Cozzolino. 2006. Use of near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration models. *Agricultura Técnica* 66 (1):41–7. doi: 10.4067/S0365-28072006000100005.

Givens, D. I., J. L. De Boever, and E. R. Deaville. 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. *Nutrition Research Reviews* 10 (1):83–114. doi: 10.1079/nrr19970006.

González-Martín, I., C. González-Pérez, J. M. Hernández-Hierro, and J. M. González-Cabrera. 2008. Use of NIRS technology with a remote reflectance fibre-optic probe for predicting major components in cheese. *Talanta* 75 (2):351–5. doi: 10.1016/j.talanta.2007.11.028.

González-Martín, I., and J. M. Hernández-Hierro. 2008. Detection and quantification of additives (urea, biuret and poultry litter) in Alfalfas by NIR spectroscopy with fibre-optic probe. *Talanta* 76 (5):1130–5. doi: 10.1016/j.talanta.2008.05.013.

González-Martín, I., J. M. Hernández-Hierro, I. Revilla, A. Vivar-Quintana, and I. Lobos Ortega. 2011. The mineral composition (Ca, P, Mg, K, Na) in cheeses (cow's, ewe's and goat's) with different ripening times using near infrared spectroscopy with a fibre-optic probe. *Food Chemistry* 127 (1):147–52. doi: 10.1016/j.foodchem.2010.12.114.

González-Martín, I., J. M. Hernández-Hierro, A. Vivar-Quintana, I. Revilla, and C. González-Pérez. 2009. The application of near infrared spectroscopy technology and a remote reflectance fibre-optic probe for the determination of peptides in cheeses (cow's, ewe's and goat's) with different ripening times. *Food Chemistry* 114 (4):1564–9. doi: 10.1016/j.foodchem.2008.11.050.

González-Martín, M. I., P. Severiano-Pérez, I. Revilla, A. M. Vivar-Quintana, J. M. Hernández-Hierro, C. González-Pérez, and I. A. Lobos-Ortega. 2011. Prediction of sensory attributes of cheese by near-infrared spectroscopy. *Food Chemistry* 127 (1):256–63. doi: 10.1016/j.foodchem.2010.12.105.

Hadjipanayiotou, M. 1995. Composition of ewe, goat and cow milk and of colostrum of ewes and goats. *Small Ruminant Research* 18 (3):255–62. doi: 10.1016/0921-4488(95)00697-3.

He, Y., S. Feng, X. Deng, and X. Li. 2006. Study on lossless discrimination of varieties of yogurt using the visible/NIR-spectroscopy. *Food Research International* 39 (6):645–50. doi: 10.1016/j.foodres.2005.12.008.

He, Y., S. Feng, D. Wu, and X. Li. 2006a. Study of vis/NIR spectroscopy measurement on acidity of yogurt. In Fourth International Conference on Photonics and Imaging in Biology and Medicine, *Tianjin* (China), 6047:604722. doi: 10.1117/12.710919.

He, Y., S. Feng, D. Wu, and X. Li. 2006b. Study on fast measurement of sugar content of yogurt using vis/NIR spectroscopy techniques. In Fourth International Conference on Photonics and Imaging in Biology and Medicine, Tianjin (China), 6047:7. doi: 10.1117/12.710922.

Hermida, M., J. M. Gonzalez, M. Sanchez, and J. L. Rodriguez-Otero. 2001. Moisture, solids-non-fat and fat analysis in butter by near infrared spectroscopy. *International Dairy Journal* 11 (1–2):93–8. doi: 10.1016/S0958-6946(01)00039-5.

Hetta, M., Z. Mussadiq, J. Wallsten, M. Halling, C. Swensson, and P. Geladi. 2017. Prediction of nutritive values, morphology and agronomic characteristics in forage maize using two applications of NIRS spectrometry. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science* 67 (4):326–33. doi: 10.1080/09064710.2017.1278782.

Heussen, P. C. M., H. G. Janssen, I. B. M. Samwel, and J. P. M. van Duynhoven. 2007. The use of multivariate modelling of near infrared spectra to predict the butter fat content of spreads. *Analytica Chimica Acta* 595 (1–2):176–81. doi: 10.1016/j.aca.2007.01.048.

Ibáñez, L., and D. Alomar. 2008. Prediction of the chemical composition and fermentation parameters of pasture silage by near infrered reflectance spectroscopy (NIRS). *Chilean Journal of Agricultural Research* 68 (4):352–9. doi: 10.4067/S0718-58392008000400005.

Jarén, C., M. Gago, S. Arazuri, A. López, N. Arias, A. C. Agulheiro-Santos, and P. C. Correa. 2012. The potential of near infrared spectroscopy as a method of determination of the fat content in yogurt. In AgEng International Conference of Agricultural Engineering, Federacion de Gremios de Editores de España, 2–3.

Kalinin, A., V. Krasheninnikov, S. Sadovskiy, and E. Yurova. 2013. Determining the composition of proteins in milk using a portable near infrared spectrometer. *Journal of near Infrared Spectroscopy* 21 (5):409–15. doi: 10.1255/jnirs.1054.

Karayilanli, E., J. H. Cherney, P. Sirois, D. Kubinec, and D. J. R. Cherney. 2016. Botanical composition prediction of alfalfa–grass mixtures using NIRS: Developing a robust calibration. *Crop Science Society of America* 56 (6):3361–6. doi: 10.2135/cropsci2016.04.0232.

Karoui, R., and J. De Baerdemaeker. 2007. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry* 102 (3):621–40. doi: 10.1016/j.foodchem.2006.05.042.

Karoui, R., A. M. Mouazen, É. Dufour, L. Pillonel, E. Schaller, D. Picque, J. D. Baerdemaeker, and J. O. Bosset. 2006. A comparison and joint use of NIR and MIR spectroscopic methods for the determination of some parameters in European emmental cheese. *European Food Research and Technology* 223 (1):44–50. doi: 10.1007/s00217-005-0110-2.

Kasemsumran, S., W. Thanapase, and A. Kiatsoonthon. 2007. Feasibility of near-infrared spectroscopy to detect and to quantify adulterants in cow milk. *Analytical Sciences* 23 (7):907–10. doi: 10.2116/analsci.23.907.

Kawasaki, M., S. Kawamura, M. Tsukahara, S. Morita, M. Komiya, and M. Natsuga. 2008. Near-infrared spectroscopic sensing system for on-line milk quality assessment in a milking robot. *Computers and Electronics in Agriculture* 63 (1):22–7. doi: 10.1016/j.compag.2008.01.006.

Kim, Y., M. Singh, and S. E. Kays. 2006. Near-infrared spectroscopy for measurement of total dietary fiber in homogenized meals. *Journal of Agricultural and Food Chemistry* 54 (2):292–8. doi: 10.1021/jf051975b.

Laporte, M. F., R. Martel, and P. Paquin. 1998. The near-infrared optic probe for monitoring rennet coagulation in cow's milk. *International Dairy Journal* 8 (7):659–66. doi: 10.1016/S0958-6946(98)00101-0.

Laporte, M. F., and P. Paquin. 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. *Journal of Agricultural and Food Chemistry* 47 (7):2600–5. doi: 10.1021/jf980929r.

Liu, N., H. A. Parra, A. Pustjens, K. Hettinga, P. Mongondry, and S. M. van Ruth. 2018. Evaluation of portable near-infrared spectroscopy for organic milk authentication. *Talanta* 184:128–35. doi: 10.1016/j.talanta.2018.02.097.

Llano Suárez, P., A. Soldado, A. González-Arrojo, F. Vicente, and B. de la Roza-Delgado. 2018. Rapid on-site monitoring of fatty acid profile

in raw milk using a handheld near infrared sensor. *Journal of Food Composition and Analysis* 70:1–8. doi: 10.1016/j.jfca.2018.03.003.

Lovett, D. K., E. R. Deaville, F. Mould, D. I. Givens, and E. Owen. 2004. Using near infrared reflectance spectroscopy (NIRS) to predict the biological parameters of maize silage. *Animal Feed Science and Technology* 115 (1–2):179–87. doi: 10.1016/j.anifeedsci.2004.02.007.

Lucas, A., D. Andueza, A. Ferlay, and B. Martin. 2008. Prediction of fatty acid composition of fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. *International Dairy Journal* 18 (6):595–604. doi: 10.1016/j.idairyj.2007.12.001.

Ma, Y. B., K. S. Babu, and J. K. Amamcharla. 2019. Prediction of total protein and intact casein in cheddar cheese using a low-cost hand-held short-wave near-infrared spectrometer. *LWT - Food Science and Technology* 109:319–26. doi: 10.1016/j.lwt.2019.04.039.

Mabood, F., G. Abbas, F. Jabeen, Z. Naureen, A. Al-Harrasi, A. M. Hamaed, J. Hussain, M. Al-Nabhani, M. S. Al Shukaili, A. Khan, et al. 2018. Robust new NIRS coupled with multivariate methods for the detection and quantification of tallow adulteration in clarified butter samples. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 35 (3):404–11. doi: 10.1080/19440049.2017.1418090.

Madalozzo, E. S., E. Sauer, and N. Nagata. 2015. Determination of fat, protein and moisture in ricotta cheese by near infrared spectroscopy and multivariate calibration. *Journal of Food Science and Technology* 52 (3):1649–55. doi: 10.1007/s13197-013-1147-z.

Manuelian, C. L., S. Currò, M. Penasa, M. Cassandro, and M. De Marchi. 2017. Prediction of minerals, fatty acid composition and cholesterol content of commercial cheeses by near infrared transmittance spectroscopy. *International Dairy Journal* 71:107–13. doi: 10.1016/j.idairyj.2017.03.011.

Maraboli, A., T. M. P. Cattaneo, and R. Giangiacomo. 2002. Detection of vegetable proteins from soy, pea and wheat isolates in milk powder by near infrared spectroscopy. *Journal of near Infrared Spectroscopy* 10 (1):63–9. doi: 10.1255/jnirs.322.

Martens, H., and E. Stark. 1991. Extended multiplicative signal correction and spectral interference subtraction: New preprocessing methods for near infrared spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* 9 (8):625–35. doi: 10.1016/0731-7085(91)80188-F.

McGonigle, A. J. S., T. C. Wilkes, T. D. Pering, J. R. Willmott, J. M. Cook, F. M. Mims, and A. V. Parisi. 2018. Smartphone spectrometers. *Sensors (Switzerland)* 18 (2):223–15. doi: 10.3390/s18010223.

Meagher, L. P., S. E. Holroyd, D. Illingworth, F. Van De Ven, and S. Lane. 2007. At-line near-infrared spectroscopy for prediction of the solid fat content of milk fat from New Zealand butter. *Journal of Agricultural and Food Chemistry* 55 (8):2791–6. doi: 10.1021/jf063215m.

Melenteva, A., V. Galyanin, E. Savenkova, and A. Bogomolov. 2016. Building global models for fat and total protein content in raw milk based on historical spectroscopic data in the visible and short-wave near infrared range. *Food Chemistry* 203:190–8. doi: 10.1016/j.foodchem.2016.01.127.

Modroño, S., A. Soldado, A. Martínez-Fernández, and B. Dela Roza-Delgado. 2017. Handheld NIRS sensors for routine compound feed quality control: Real time analysis and field monitoring. *Talanta* 162:597–603. doi: 10.1016/j.talanta.2016.10.075.

Naes, T., T. Isaksson, T. Fearn, and T. Davies. 2002. *A user-friendly guide to multivariate calibration and classification*. Chichester, UK: NIR Publictions.

Nicolau, N., M. Buffa, D. J. O'Callaghan, B. Guamis, and M. Castillo. 2015. Estimation of clotting and cutting times in sheep cheese manufacture using NIR light backscatter. *Dairy Science & Technology* 95 (4):495–507. doi: 10.1007/s13594-015-0232-7.

Numthuam, S., J. Hongpathong, R. Charoensook, and S. Rungchang. 2017. Method development for the analysis of total bacterial count in raw milk using near-infrared spectroscopy. *Journal of Food Safety* 37 (3):e12335. doi: 10.1111/jfs.12335.

Núñez-Sánchez, N., A. L. Martínez-Marín, O. Polvillo, V. M. Fernández-Cabanás, J. Carrizosa, B. Urrutia, and J. M. Serradilla. 2016. Near infrared spectroscopy (NIRS) for the determination of the milk fat fatty acid profile of goats. *Food Chemistry* 190:244–52. doi: 10.1016/j.foodchem.2015.05.083.

Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science* 82 (3):589. doi: 10.3168/jds.S0022-0302(99)75271-9.

Ozaki, Y., S. Morita, and Y. Du. 2007. Spectral analysis. In *Near-infrared spectroscopy in food science and technology*, eds. Y. Ozaki, W. F. McClure, and A. A. Christy. Hoboken, NJ: John Wiley & Sons, Inc.

Park, H. S., J. H. Kim, K. C. Choi, and H. S. Kim. 2016. Mathematical transformation influencing accuracy of near infrared spectroscopy (NIRS) calibrations for the prediction of chemical composition and fermentation parameters in corn silage. *Journal of the Korean Society of Grassland and Forage Science* 36 (1):50–57. doi: 10.5333/KGFS.2016.36.1.50.

Park, H. S., S. H. Lee, J. G. Kim, K. C. Choi, S. Seo, W. H. Kim, H. W. Lee, and Y. C. Lim. 2011. Evaluation of field application of portable near infrared reflectance spectrometer (NIRS) for quality evaluation of Italian ryegrass silages. *Journal of the Korean Society of Grassland and Forage Science* 31 (4):415–22. doi: 10.5333/KGFS.2011.31.4.415.

Parrini, S., A. Acciaioli, A. Crovetti, and R. Bozzi. 2018. Use of FT-NIRS for determination of chemical components and nutritional value of natural pasture. *Italian Journal of Animal Science* 17 (1):87–91. doi: 10.1080/1828051X.2017.1345659.

Pérez-Marín, D. C., A. Garrido-Varo, J. E. Guerrero-Ginel, and A. Gómez-Cabrera. 2004. Near-infrared reflectance spectroscopy (NIRS) for the mandatory labelling of compound feedingstuffs: Chemical composition and open-declaration. *Animal Feed Science and Technology* 116 (3–4):333–49. doi: 10.1016/j.anifeedsci.2004.05.002.

Pettersson, H. 2012. Mycotoxin contamination of animal feed. In *Animal feed contamination: Effects on livestock and food safety*, ed. J. Fink-Gremmels. Cambridge, UK: Woodhead Publishing Ltd. doi: 10.1533/9780857093615.3.233.

Pi, F., H. Shinzawa, Y. Ozaki, and D. Han. 2009. Non-destructive determination of components in processed cheese slice wrapped with a polyethylene film using near-infrared spectroscopy and chemometrics. *International Dairy Journal* 19 (10):624–29. doi: 10.1016/j.idairyj.2009.05.002.

Priyashantha, H., A. Höjer, K. H. Saedén, A. Lundh, M. Johansson, G. Bernes, P. Geladi, and M. Hetta. 2020. Use of near-infrared hyperspectral (NIR-HS) imaging to visualize and model the maturity of long-ripening hard cheeses. *Journal of Food Engineering* 264:109687–9. doi: 10.1016/j.jfoodeng.2019.109687.

Purnomoadi, A., K. K. Batajoo, K. Ueda, and T. Fuminori. 1999. Influence of feed source on determination of fat and protein in milk by near-infrared spectroscopy. *International Dairy Journal* 9 (7):447–52. doi: 10.1016/S0958-6946(99)00050-3.

Scholl, P. F., M. M. Bergana, B. J. Yakes, Z. Xie, S. Zbylut, G. Downey, M. Mossoba, J. Jablonski, R. Magaletta, S. E. Holroyd, et al. 2017. Effects of the aadulteration technique on the near-infrared detection of melamine in milk powder. *Journal of Agricultural and Food Chemistry* 65 (28):5799–5809. doi: 10.1021/acs.jafc.7b02083.

Shao, Y., and Y. He. 2009. Measurement of soluble solids content and pH of yogurt using visible/near infrared spectroscopy and chemometrics. *Food and Bioprocess Technology* 2 (2):229–33. doi: 10.1007/s11947-008-0180-9.

Sivakesava, S., and J. Irudayaraj. 2002. Rapid determination of tetracycline in milk by FT-MIR and FT-NIR spectroscopy. *Journal of Dairy Science* 85 (3):487–93. doi: 10.3168/jds.S0022-0302(02)74099-X.

Slavchev, A., Z. Kovacs, H. Koshiba, A. Nagai, G. Bázár, A. Krastanov, Y. Kubota, and R. Tsenkova. 2015. Monitoring of water spectral pattern reveals differences in probiotics growth when used for rapid bacteria selection. *PLoS One* 10 (7):e0130698. doi: 10.1371/journal.pone.0130698.

Sultaneh, A. L. L., and H. Rohm. 2007. Using near infrared spectroscopy for the determination of total solids and protein content in cheese curd. *International Journal of Dairy Technology* 60 (4):241–44. doi: 10.1111/j.1471-0307.2007.00347.x.

Thomson, A. L., D. J. Humphries, C. Rymer, J. E. Archer, N. W. Grant, and C. K. Reynolds. 2018. Assessing the accuracy of current near infra-red reflectance spectroscopy analysis for fresh grass-clover mixture silages and development of new equations for this purpose. *Animal Feed Science and Technology* 239:94–106. doi: 10.1016/j.anifeedsci.2018.03.009.

Tóth, T., P. J. Mwau, G. Bázár, G. Andrássy-Baka, H. Hingyi, É. Csavajda, and L. Varga. 2019. Effect of feed supplementation based on extruded linseed meal and fish oil on composition and sensory properties of raw milk and ultra-high temperature treated milk. *International Dairy Journal* 99:104552. doi: 10.1016/j.idairyj.2019.104552.

Tran, H., P. Salgado, E. Tillard, P. Dardenne, X. T. Nguyen, and P. Lecomte. 2010. 'Global' and 'local' predictions of dairy diet nutritional quality using near infrared reflectance spectroscopy. *Journal of Dairy Science* 93 (10):4961–75. doi: 10.3168/jds.2008-1893.

Tsenkova, R. 2009. Introduction aquaphotomics: Dynamic spectroscopy of aqueous and biological systems describes peculiarities of water. *Journal of near Infrared Spectroscopy* 17 (6):303–14. doi: 10.1255/jnirs.869.

Tsenkova, R., S. Atanassova, Y. Ozaki, K. Toyoda, and K. Itoh. 2001. Near-infrared spectroscopy for biomonitoring: Influence of somatic cell count on cow's milk composition analysis. *International Dairy Journal* 11 (10):779–83. doi: 10.1016/S0958-6946(01)00110-8.

Wetzel, D. L. 2001. Contemporary near-infrared instrumentation. In *Near-infrared spectroscopy in the agricultural and food industries*, eds. P. C. Williams and K. H. Norris. St. Paul, MN: American Association of Cereal Chemists Inc.

Wiedemair, V., D. Langore, R. Garsleitner, K. Dillinger, and C. Huck. 2019. Investigations into the performance of a novel pocket-sized near-infrared spectrometer for cheese analysis. *Molecules* 24 (3):428–13. doi: 10.3390/molecules24030428.

Williams, P. C., J. Antoniszyn, and M. Manley. 2019. Near Infrared Spectroscopy: Getting the Best out of Light. *African Sun MeDia and Sun PRess*. Stellenbosch, South Africa. doi: 10.18820/9781928480310.

Williams, P. C., and K. H. Norris. 2001. *Near infrared technology in the agricultural and food industries*. St. Paul, MN: American Association of Cereal Chemists Inc.

Woo, Y. A., Y. Terazawa, J. Y. Chen, C. Iyo, F. Terada, and S. Kawano. 2002. Development of a new measurement unit (MilkSpec-1) for rapid determination of fat, lactose, and protein in raw milk using near-infrared transmittance spectroscopy. *Applied Spectroscopy* 56 (5):599–604. doi: 10.1366/0003702021955150.

Workman, J., Jr., and J. Weyer. 2012. *Pratical guide and spectral ATLAS for interpretive near infrared spectroscopy*. 2nd ed. New York, NY: Taylor & Francis.

Wu, D., S. Feng, and Y. He. 2007. Infrared spectroscopy technique for the nondestructive measurement of fat content in milk powder. *Journal of Dairy Science* 90 (8):3613–19. doi: 10.3168/jds.2007-0164.

Wüst, E., and L. Rudzik. 2003. The use of infrared spectroscopy in the dairy industry. *Journal of Molecular Structure* 661–662 (1–3):291–98. doi: 10.1016/S0022-2860(03)00514-3.

Xu, L., S. M. Yan, C. B. Cai, Z. J. Wang, and X. P. Yu. 2013. The feasibility of using near-infrared spectroscopy and chemometrics for untargeted detection of protein adulteration in yogurt: Removing unwanted variations in pure yogurt. *Journal of Analytical Methods in Chemistry* 2013 (9):201873. doi: 10.1155/2013/201873.

Yan, H., and H. W. Siesler. 2018. Hand-held near-infrared spectrometers: State-of-the-art instrumentation and practical applications. *NIR News* 29 (7):8–12. doi: 10.1177/0960336018796391.

Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boguhn, B. N. Ametaj, and W. Drochner. 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *Journal of Dairy Science* 95 (3):1041–56. doi: 10.3168/jds.2011-4421.

1.2.5. Trends in artificial aroma sensing by means of electronic nose technologies to advance dairy production – a review

Title	Trends in artificial aroma sensing by means of electronic nose technologies to advance dairy production – a review
Authors	Yakubu Haruna Gado, Kovacs Zoltan, Toth Tamas, Bazar George
Journal	Critical Reviews in Food Science and Nutrition
Year Volume Issue	2022 63 2
Pages Quartile IF	234-248 D1 11.208
Doi	10.1080/10408398.2021.1945533

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1945533

Taylor & Francis
Taylor & Francis Group

REVIEW

Trends in artificial aroma sensing by means of electronic nose technologies to advance dairy production – a review

Haruna Gado Yakubu[a], Zoltan Kovacs[b], Tamas Toth[c,d], and George Bazar[a,d]

[a]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvár, Hungary; [b]Department of Measurements and Process Control, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary; [c]Agricultural and Food Research Centre, Széchenyi István University, Győr, Hungary; [d]Adexgo Kft, Balatonfüred, Hungary

ABSTRACT

Controversies surrounding the name and how the electronics nose (e-nose) works have been at the center stage since the advent of the technology. Notwithstanding the controversies, the technology has gained popularity in the sensory analysis of dairy foods, because of its rapid results delivery on product aroma profile or pattern, which can be used to assess quality. This review critically evaluated the advances made in the application of the e-nose or artificial sensory system in the dairy industry, focusing on the evaluation of milk, yoghurt and cheese properties, and the trends and prospects of the technology. Most of the e-nose devices applied in the available scientific publications used sensors such as metal oxide semiconductor sensors (MOS), metal-oxide-semiconductor field-effect transistor (MOSFET), conducting polymers composites and quartz microbalance (QMB), and flame ionization detector FID, in a recent study. Though known for aroma sensing, the technology has been applied to evaluate the shelf life or microbial spoilage and to discriminate dairy products based on the volatile profile composition, as determined by the sensors. In most cases, the limitation of the technology is the inability of it to provide information on the nature of constituting compounds, except in gas chromatography and mass spectrometry-based e-nose systems.

KEYWORDS

E-nose; smell; odor; volatile; quality; milk; yoghurt; cheese

Highlights

- Electronic noses provide rapid delivery of aroma profile of dairy products
- Different characteristics of sensors enable efficient evaluation of product quality
- Effective evaluation of shelf life, spoilage or quality levels of dairy products
- Limitations in providing information on the nature of constituting compounds
- GC and MS based e-nose systems to overcome the challenge of compound identification

Introduction

Food flavor is defined as the combined sensation of a portion of food in the mouth, detected through the sense of taste, smell, and chemical irritation (Mottram and Elmore 2003), while the aroma of a food can be considered as a specific sensory characteristic detected when volatile compounds (VCs) enter the nasal part of the human olfactory system, which is detected by receptors known as the olfactory detectors (Santonico et al. 2008; Gómez et al. 2006). Conventionally, flavor and aroma are among the main properties of foods analyzed using various sensory tests, such as triangle test, ranking test, scoring, acceptance testing and ratio-scaling test described by Larmond (1997) and quantitative approaches such as aroma extract dilution analysis (AEDA) and odor activity value (OAV) (Pu et al. 2020). These tests or evaluations are done to improve product quality and acceptance on the market (Biolatto et al. 2007).

Over the years, there has been an increasing awareness of consumers on the sensory properties of dairy products, leading to the increasing change in the tastes and preferences of the consumers (Biolatto et al. 2007). The most important sensory property of foods that consumers most consider is the aroma (Biolatto et al. 2007; Gómez et al. 2006). The flavor or aroma of milk, cheese and yoghurt mostly influence the consumers' preference, and the electronic nose (e-nose), in recent times, has served as a fast method to determine the flavor or aroma of these dairy products, in order to match the consumers' preference in the market (Korel and Balaban 2002). The e-nose as an instrument was first described by Persaud and Dodd (1982) which according to Gardner and Bartlett (1994) consist of an array of heterogeneous electrochemical gas sensors with partial specificity and a pattern recognition system capable of recognizing simple or complex odors based on the electronic signals of the sensors.

CONTACT George Bazar bazar@agrilab.hu

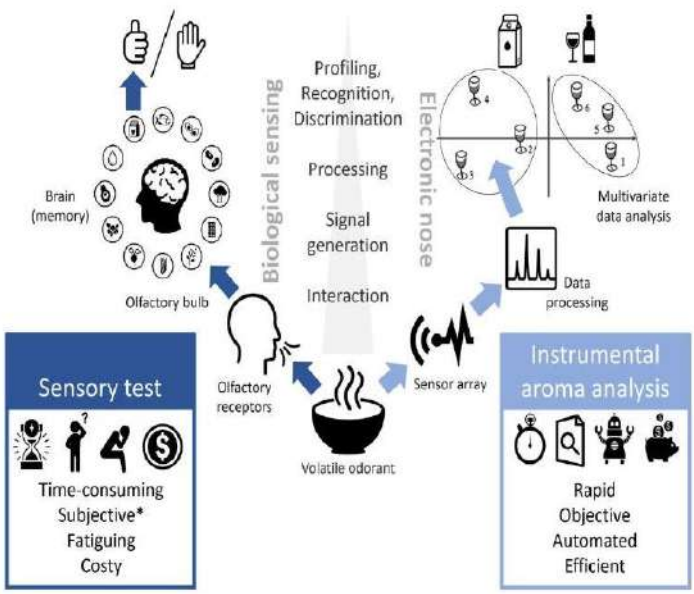


Figure 1. Analogies and differences in the odor evaluation using biological or artificial aroma sensing. * Subjectivity may appear when untrained laic human panels and animal preference tests are used where individual deviations impair consistent profiling.

This rapid technique has become necessary because of the cost and laborious work associated with conventional laboratory methods. At the initial stages of sensory science, the evaluation of sensory properties of food was done mainly by the use of the nine-point hedonic scale developed by Peryam and Girardot (1952), which involved the use of a team of trained human sensory evaluators. This form of sensory analysis can be challenged with subjectivity on the side of less trained or unprofessional panelists, the sensitivities of smell receptors (Bliss et al. 1996) and taste buds (Tuorila and Monteleone 2009), since the sense of smell and taste varies with age, and in some cases, sex (Peelle 2019; Bliss et al. 1996) and lifestyle activities such as smoking (Da Ré et al. 2018). These and other challenges, such as the intensive work required in training professional evaluators, developing questionnaires and evaluating the report from the panelist, and the difficulty on the side of evaluators to determine the least smell or aroma of products, led to the development of a rapid model aroma sensing machine known as the e-nose, which was used to discriminate aroma of different foods, and became famous in the 1980s after a publication by Persaud and Dodd (1982).

Figure 1 demonstrates the analogies and differences of the biological and artificial aroma sensing and evaluation, highlighting the benefits of the quality assessment based on the e-nose technology contrary to human sensory tests. Despite the challenges associated with sensory panel tests, such tests cannot be entirely neglected, because the determination of product preferences can be well explained using the outcomes of sensory tests (Hower 2015), and when done by professional human sensory panelists or in carefully designed animal preference trials, those can be highly accurate and accepted as reference methods. Therefore, the e-nose at times serves as the best complementary technique to validate conventional methods and substitute those when technical conditions require (e.g. continuous or rapid feedback needed; poisonous environment or sample exclude biological sensory test).

The e-nose consists of three major parts which are the sample delivery system, the detection system, and the computing system (Pearce et al. 2006). The detection systems are mostly made of metal oxide semiconductor sensors (MOS), metal-oxide-semiconductor field-effect transistor (MOSFET), conducting polymers composites and intrinsically conducting polymers (Scott, James, and Ali 2006; Gardener and Bartlett 1999; Loutfi et al. 2015). Gas detection has also been achieved by the use of optical sensors (Li, Askim, and Suslick 2019), surface acoustic wave sensors (Shiokawa and Kondoh 2004), and quartz microbalance (QMB) (Buttry and Ward 1992). Micro-electro-mechanical systems (MEMS) coupled with nanotechnologies are the recent most promising emerging technologies in the multiarray system (Kang et al. 2020; Loutfi et al. 2015; Scott, James, and Ali 2006). Recent studies (Gao et al. 2019, 2018) developed and investigated dual transduction sensor, that is two transduction mechanisms in one sensor gas (a film bulk acoustic wave resonator (FBAR) together with interdigitated electrodes) which has showed very promising results of simultaneous detection of gas-induced variations in the sensing material. The technology has also been used to characterize systems where ultra-fast gas chromatography (GC) or mass spectrometry (MS) was employed in the detection processes (Loutfi et al. 2015) giving the chance not only to record a smell fingerprint but to identify the volatile compounds of an odorant mixture.

Figure 2. Advantages and disadvantages of electronic nose technologies.

The working principle of the sensor-based e-noses relies on the changes of the sensor(s), caused by volatile compounds (VCs). In most applications, sealed vials are used to contain defined amount of a liquid or solid substance, and the headspace generated with or without incubation is tested. The VCs of the headspace interfere with a sensor or an array of sensors, and change their physico-chemical properties, e.g. electric resistance or optical properties, that are translated to electric signals. These signals per sensor form the multidimensional data representing the odor pattern of the certain sample tested.

In recent advancement, the e-nose device has been designed to include sensors that measure the ambient gas atmosphere or environment based on the general principle that changes in the gaseous atmosphere influence the sensory properties in a characteristic way (Loutfi et al. 2015).

Although sensory panel tests are the most used tools to evaluate the organoleptic properties of food, some sensory parameters may also be analyzed by analytical chemical approaches. Most of these methods are highly precise however they focus on single ingredients influencing a fraction of the complex sensory appearance of a sample. Furthermore, the conventional analytical methods require *a priori* knowledge of what to be analyzed, which in many applications checking the flavor variations of foods is not obvious. Aouadi et al. (2020) compared the major conventional analytical methods with e-nose along with various criteria. The major advantages and disadvantages of the e-nose technology are summarized in Figure 2, while Figure 3 shows the main parameters that characterize the quality of the applied sensors (Llobet 2020). Partial selectivity or sensitivity and cross selectivity or sensitivity of the various sensors arranged into an array are also important characteristics influencing the multivariate data recorded with the certain sensor array (Pearce et al. 2006).

The main advantage of the e-nose technology is that once calibrated, it can continuously assess the aroma pattern of foods throughout the whole production process at minimal costs (Kauer and White 2009), whiles the major constraints are that most e-nose does not provide information on the nature of constituting compounds, except in the case of GC and MS-based e-noses, but only give a digital fingerprint of the food that can be investigated employing multivariate statistical analysis. Again, the high initial cost of purchase and the skilled labor required for its operation can be challenging (Aouadi et al. 2020; Ampuero and Bosset 2003).

Despite its enormous contribution to sensory science, technology has faced some constructive criticisms from authors like Boeker (2014). On the nomenclature of the technology (electronic nose), Boeker argued that the devices named "electronic nose" as suggested by many, are not exactly feasible tools to measure odor qualities, and,

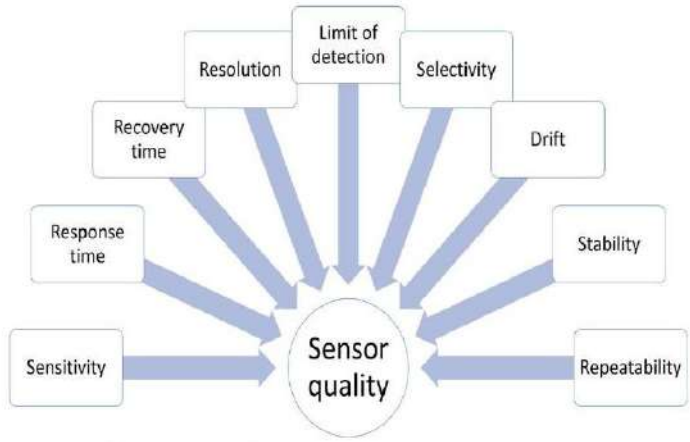


Figure 3. The main parameters characterizing the sensors applied in electronic noses.

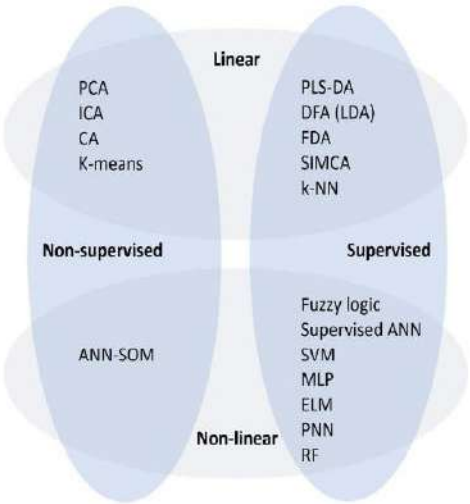


Figure 4. The overview of multivariate data analysis tools used in the qualitative evaluation of electronic nose results. PCA: principal component analysis (Bedoui et al. 2013); ICA: independent component analysis (Yong, Wenkai, and Huichun 2014; Martinelli et al. 2002); CA: cluster analysis (Scott, James, and Ali 2006); K-means (Scott, James, and Ali 2006); PLS-DA: partial least square discriminate analysis (Gromski et al. 2014); DFA: discriminant function analysis including LDA: linear discriminant analysis (Gromski et al. 2014); FDA: Fisher discriminant analysis (Zhang et al. 2007); SIMCA: soft independent modeling by class analogy (Tominaga 1999); k-NN: k-nearest neighbor (Tominaga 1999); ANN-SOM: artificial neural network self-organizing map (Scott, James, and Ali 2006); Fuzzy logic (Llobet et al. 1999); Supervised ANN (Khoshgoftaar, Van Hulse, and Napolitano 2010); SVM: support vector machine (Gromski et al. 2014); MLP: multilayer perceptron (Zhang and Tian 2014); ELM: extreme learning machine (Chen et al. 2017); PNN: probabilistic neural network (Nishanth and Ravi 2016); RF: random forest (Gromski et al. 2014)

therefore, the apparent similarity of the biological functional property of the sense of smell and the design of e-noses falls short. Notwithstanding, he believes that e-noses are chemical measurement systems, and therefore, measure chemical properties of sample gases, not odor properties. Based on this, Boeker (2014) suggested a new terminology for the "electronic nose" to be "chemical correlate" systems. Boeker's opinion maybe legitimate, however, by convention, everyone uses the term "e-nose," because the primary goal of the device is to mimic human olfaction.

In the current and most widely accepted terminology, data collected from e-noses are mostly pretreated or preprocessed using various mathematical algorithms, such as baseline correction or subtraction, relative scaling, standard normal variate transformation, range scaling, multiplicative signal correction (Banerjee et al. 2016), to analyze and interpret the e-nose data meaningfully. The multivariate data recorded with e-nose devices are mostly evaluated with multivariate data analytical tools, i.e. the application of mathematical and statistical techniques to extract valuable information from the complex, collinear and bulky multidimensional data (Qu 2007).

In combination with e-nose, mostly qualitative data analyses are performed (Figure 4) where the objective is to find the differences or similarities among certain groups of samples based on the multivariate data describing their odor patterns. However, there is also a possibility to run quantitative calibrations, like principal component regression (PCR) or partial least squares regression (PLSR) using the sensor signals and reference variables such as compositional data or human sensory parameters (Hines et al. 2006). In the most applied linear qualitative and quantitative multivariate data analyses approaches the original variables, mostly being the signals of multiple sensors describing the odor patterns of samples, are mathematically transformed to latent variables. During this process, the dimensions of the dataset are reduced, while the few generated latent variables describe large amount of the variance of the original variables, thus, hold most of the useful information on the odor patterns. The latent variables are then used in regression or classification models, where the relationship with continuous variables or class variables are revealed, and the models are optimized with successive approximations of various validation approaches (Naes et al. 2002). Chemometric approaches are further translating the statistical information to chemical information (Lavine and Workman 2008) as the

contributions of the latent variables and weight vectors of the multivariate data analyses are evaluated based on the *a priori* chemical knowledge. The nonlinear multivariate modeling approaches generate functions which are nonlinear combinations of the original multidimensional data describing odor and depend on the qualitative or quantitative reference data. The models are generally fitted by iterative trainings and validations.

The most used multivariate methods are the principal component analysis (PCA), independent component analysis (ICA), linear discriminate analysis (LDA), partial least squares discriminant analysis (PLS-DA), cluster analysis (CA), fuzzy logic or artificial neural network (ANN), such as a probabilistic neural network (PNN). Among these techniques, PCA, ICA, LDA, PLS-DA, and CA are based on a linear approach while fuzzy logic, ANN and PNN are regarded as nonlinear methods (Hines et al. 2006). Another difference among the techniques is the application of background knowledge during the analysis of the multivariate data (Figure 4). Non-supervised modellings describe the odor patterns with reduced and uncorrelated latent variables which hold most of the variance of the original variables. Supervised modellings use reference knowledge, i.e. class identifications of the samples, and discriminate the predefined groups based on the most useful variance of the original variables describing the odor. Multivariate models predicting quantitative or qualitative properties based on the sensor signals may be tested with cross-validation or independent validation (Naes et al. 2002).

There is a clear and sometimes offending difference between the approaches of classical analytical chemistry and correlative multivariate technologies like e-nose. Classical analytical chemistry is "knowledge-driven," focuses very much on prior assumed knowledge. The substance to be measured is well defined, the method is selective and sensitive to the targeted substance, and the number of measurements is restricted. On the other hand, correlative multivariate modeling is "data-driven," where lots of empirical measurements are performed, and background knowledge is only used for the design of experiments to obtain the empirical data, or in the data evaluation process as well as in the graphical interpretation of the results. The knowledge about, for example, pure constituents is not used as an integral part of the mathematical modeling of the mixture data obtained, still, for effective multivariate calibration modeling, it is important to combine prior knowledge and empirical data in a balanced way (Martens and Stark 1991).

During the learning process, the background knowledge of group identities or chemical composition is correlated with empirical measurements of odor profiles, and the complex relationship revealed is converted into models that can predict the quality of future products based on their odor profiles. This data-driven modeling based on data of various technologies may allow reliable correlative analysis in systems where classical analysis has failed (Martens 2015), however, the results of an e-nose data evaluation do not always provide the reason of differences among groups, only the indication that difference does exist and can be proven. Consequently, for users familiar with analytical chemistry only, e-nose results often seem offendedly speculative, because the answers on "why?" are mostly missing, at the same time, argumentations include more "mathematical massaging" instead of evidential statements which makes the technology even more mysterious for those being not familiar with it.

Still, the application of e-nose technology is fast gaining popularity in the field of dairy science, because of its advantages mentioned in the previous paragraphs. In dairy production and processing, the results obtained from e-nose analysis can quickly inform the producer or the management about the aroma pattern of their products, which helps to properly supply consumers with products of acceptable and standard aroma and quality. The aroma pattern or profile may also help to develop new products with the desired aroma, to evaluate the possible shelf life or storage time of the products in a rapid and precise manner (Labreche et al. 2005).

This review aims to critically evaluate the advances made in the application of the e-nose or artificial sensory system in the dairy industry, focusing on the evaluation of milk, yoghurt and cheese properties earlier mentioned, which are the main quality properties of these products, and the trends and future development of the technology.

The application of e-nose in dairy production

The ever-changing conditions of the market and the need for standardized food products in the global market have forced stakeholders of the dairy industry to apply the most recent and advanced technologies in the evaluation of the flavor of their products. This has inspired significant progress in the dairy industry when it comes to the application of e-nose in testing aroma profiles, shelf life or quality parameters. The next section of this review will focus on the advancements of the technology related to the quality control of milk, yoghurt and cheese production.

Milk production

The perpetual interaction of key aroma compounds in milk is an important determinant of milk quality (Tian, Xu, et al. 2020). Accordingly, the most relevant studies considering the application of e-nose to evaluate milk are reviewed in the following section.

Milk produced for human consumption is generally the product of dairy ruminants. Otherwise mentioned, the milk reviewed under this section describes that of the dairy cow. The e-nose technique has fast been accepted in the aroma pattern, shelf life, and rancid flavor evaluation of milk, because of its rapid and precise information delivery (Magan, Pavlou, and Chrysanthakis 2001). The e-nose technology has been used to ensure high-quality standards and integrity of dairy products, such as milk (Korel and Balaban 2002).

Eriksson et al. (2005) investigated how the MOSFET and CO_2 based e-nose could be used to differentiate between

milk produced by healthy and mastitic cows. The study used milk from dairy cows with acute clinical mastitis, and milk from healthy cows as a reference sample, which were analyzed using a gas-sensor array system under different incubating temperature of 60 °C and 40 °C, respectively. A discriminant analysis based on partial least-squares regression (PLS-DA) revealed 100% classification of the mastitic milk samples and the milk from healthy cows, under 60 °C treatment, compared with the 40 °C, which did not show any distinct classification. The volatile substances in mastitic milk as identified by GC-MS were mainly sulfides, ketones, amines and acids, while milk from healthy cows was characterized with products of lipids. The results showed that mastitic milk from cows suffering from acute clinical mastitis could be differentiated from healthy milk using MOSFET sensor array technology in combination with a CO_2 sensor.

Ali, O'Hare, and Theaker (2003) examined bacterially contaminated milk with a QMB-based e-nose. In the experiment, the sensors were exposed to the headspace of uncontaminated milk and samples contaminated with *Pseudomonas fragi*or *Escherichia coli*. PCA analysis was used to analyze the sensor array fingerprints. In the PCA scores, there was no separation between uncontaminated milk samples and those contaminated with *P. fragi*. This was explained by authors to mean *P. fragi* was perhaps a poor fermenter of milk. However, the opposite was found for the differentiation between the control milk samples and those contaminated with *E. coli*, as these groups were separated along PC1 describing the largest portion of the variance of the odor pattern. This indicates that *E. coli* is a better fermenter of milk compared with *P. fragi*.

Korel and Balaban (2002) also evaluated the microbial and sensory properties of milk using a conductive polymer-based e-nose and human sensory test. The study was done on milk inoculated with odor-producing bacteria, *Pseudomonas fluorescens* or *Bacillus coagulans*, under different storage temperatures, and the odors produced were correlated with microbial loads and sensory scores. The results of the human sensory test showed that the sensory scores for control (UHT treated milk) were significantly different ($p < 0.01$) from milk samples treated with *Pseudomonas fluorescens* or *Bacillus coagulans*. A DFA analysis of the e-nose data also discriminated the odors produced by the microbial inoculants with accuracies of 100%. The study concluded that thee-nose has the potential to differentiate the odor change of milk samples inoculated with *Pseudomonas fluorescens* or *Bacillus coagulans*, by using DFA as the pattern recognition technique, and results can be obtained similar to those from sensory panels (Korel and Balaban 2002).

Magan, Pavlou, and Chrysanthakis (2001) employed a portable conductive polymer-based e-nose (BH-114) to recognize spoilage bacteria and yeasts in milk. The study used e-nose to detect the volatile profiles produced by uninoculated skimmed milk media, or that inoculated with bacteria (*Pseudomonas aureofaciens* and *Bacillus cereus*) or yeasts (*Candida pseudotropicali* sand *Kluyveromyces lactis*). The results showed that the sensor array employed in the study was able to differentiate the microbial volatile profiles produced by the different strains. A quantitative differentiation between three different concentrations of *P. aureofaciens* (1×10^6 cells ml^{-1}: low), (3.5×10^8 cells ml^{-1}: medium) and (8×10^8 cells ml^{-1}: high) was also studied and showed that the system could effectively discriminate between the inoculants. Using an initial inoculum of about 10^3-10^4 cells ml^{-1} it was possible to differentiate between unspoiled milk, yeasts and bacterial species. The results of a PCA plot revealed that e-nose data explained the differences of skim milk treated with the bacterial and yeast species, showing two separate clusters for the yeasts (*Candida pseudotropicalis* and *Kluyveromyces lactis*) and the bacteria (*Pseudomonas spp.* and *Bacillus cereus*), while with DFA, a clear classification of the unspoiled milk, yeasts, and bacteria was achieved based on the volatile profile data. The study confirmed the ability to differentiate volatile profiles of raw milk from milk treated with yeast and bacteria. Microbial milk spoilage can severely influence the industrial process, quality and commercial value of dairy products due to off-odors or flavors, physical defects and secondary metabolite toxicity from the microbes, i.e. mycotoxins (Sørhaug and Stepaniak 1997), and the e-nose technology can evaluate the above-mentioned milk spoilage features (Korel and Balaban 2002).

Kang et al. (2014) examined volatile flavor compounds in milk using an e-nose system equipped with GC-MS. The results showed that thirty (30) volatile compounds (VCs) were recorded from off-flavor milk. Some of the VCs detected include aldehyde, 2, 2, 3-trimethyl-3-oxetanol, 1-pentanol, 1-hexanol, 3-methyl-1-butanol, 1-octanol, 4-methyl-1-penten-3, butyric acid, benzoic acid, and heptanoic acid. The rancidity potential of the milk was also analyzed, through the identification of five VCs which have the potential to cause rancidity in milk (acetaldehyde, 1-hexanol, 1-pentanol, butyric and heptatonic acids). A PCA plot of off-flavor milk samples showed 72% variance in the volatile profile scores along PC1, and 15% along PC2. PC1 showed the variation between the aldehyde group (acetaldehyde) and the alcohols (1-hexanol, and 1-pentanol), while PC2 showed the variation between the carboxylic acids (butyric and heptatonic acids), the aldehyde, and alcohols.

Wang, Xu, and Sun (2010) also studied the application of the e-nose in differentiating milk flavorings. The milk samples were five (5) commercial milk flavorings (3 natural milk flavorings and 2 synthetic flavorings) and one self-made enzyme induced milk flavoring prepared by lipolyzed milk fat. The results showed that the e-nose can clearly and rapidly show a separation between the synthetic milk flavorings, the natural milk flavorings and the enzyme induced milk flavoring. Through PCA score plot evaluation, PC1 explained 94.16% of the variance of odor describing e-nose signals and showed the difference between the commercial milk flavorings and the enzyme induced flavorings, whiles PC2 covering 5.55% variance was responsible for the difference between the natural commercial flavorings and the synthetic flavorings. The results indicate that the e-nose sensors can clearly and rapidly distinguish the difference amongst

synthetic milk flavorings, natural milk flavorings and enzyme induced milk flavoring.

The application of the e-nose in the detection of milk adulteration was also studied by Yu, Wang, and Xu (2007). The study was to evaluate the adulteration of milk with water or reconstituted milk powder using MOS-based e-nose. The samples studied were whole fluid milk, reconstituted milk powder and whole fluid milk adulterated with different proportions of water. Samples were measured during 7 days of storage, and data were evaluated with LDA. The study concludes that MOS based e-nose can discriminate the originality (whole or reconstituted) and purity of milk (whole milk versus adulterated) at different days in storage, and also the amount of added water can be determined.

Milk sensory properties are influenced by feeding (Hadjipanayiotou 1995), though may not be adversely influenced in situations where some feed supplements are rumen-protected (Tóth et al. 2019).In an experiment to determine the effect of feed supplementation based on extruded linseed meal and fish oil on the composition and sensory properties of raw milk and ultra-high temperature (UHT) treated milk, Tóth et al. (2019) employed the e-nose to discriminate experimental UHT milk with 2.8% fat (E-UHT) and commercial UHT (C-UHT) milks with different fat contents (2.8% and 1.5%, w/w) based on the volatile profiles obtained from e-nose measurement. A DFA of e-nose data explained the difference in fat content (separation of E-UHT 2.8% and C-UHT 2.8% from C-UHT 1.5% along DF1), and the separation between the E-UHT 2.8% and C-UHT 2.8% milk samples, along DF2. Overall, E-UHT samples formed a distinguishable group separated from commercial milks. In a cross-validation test, the multivariate discrimination model based on odor profiles classified 89% of the samples correctly, indicating that there is a connection between the volatile profile of milk samples and the grouping variables (i.e., experimental versus commercial products) (Tóth et al. 2019).

Falchero et al. (2009) analyzed milk produced by cows pasture-fed on two different Alpine vegetation types (*Trifolium alpinum* and *Festuca nigrescens*) with the e-nose. The results showed a very good classification of the milks, according to the two vegetation types. A PCA performed on the complete dataset showed 97.76% of the cumulated variance between the milks from different feeding regime, and LDA made using scores of 4 identified PCs correctly classified 95.7% of the e-nose data into respective feeding regime.

In a study to differentiate raw milk from three different farm sources (farms: 1, 2 and 3) and milks stored at four different storage periods (days: 1, 2, 3 and 4), Amari, Bari, and Bouchikhi (2009) employed a portable e-nose made of MOS and taguchi gas sensors (TGS) to measure the volatile profiles of milks. A three-dimensional PCA score plot of the measured volatile profiles showed three distinct clusters according to the farm sources, and the level of odor differences was indicated by the extent of the explained variance along the respective separations. PC1 explained the 96.61% of the e-nose data and was responsible for the separation of farm 3 milk from that of farm 1, while PC2 (3.21% explained variance) and PC3 (0.13% explained variance) were responsible for the separation of farm 3 milk from farm 2, and farm 2 from farm 1, respectively. A PCA score plot concerning the storage days showed that days 1 and 2 were not so distinctly different, since they combined in one group. However, days 3 and 4 were separated to form different groups respectively. PC1 explained 93.03% variance of the overall e-nose data, along which days 1 and 2 samples separated from that of day 3. The separation of day 4 samples from the rest occurred along PC2 with 4.90% explained variance. Amari, Bari, and Bouchikhi (2009) concluded that portable MOS and TGS-based e-nose could differentiate between different sources of milk and at different storage times.

From the above-reviewed section on the application of the e-nose in analyzing the sensory properties of milk, it is evident that the electronic nose technology has been widely applied or used to evaluate microbial contamination and shelf life of milk. It is also possible to use the technology for classification or discrimination of milk-based on the feeding regimes, and the heat treatment applied to the raw milk. E-nose also proved to be applicable to discriminate original milk from those adulterated with foreign materials. In the data processing and evaluation, PCA and LDA were mostly used.

Yoghurt production

Apart from milk aroma evaluation, yoghurt's sensory properties have been studied using the e-nose devices and has so far enabled the rapid determination of yoghurt quality (Mortazavian, Rezaei, and Sohrabvandi 2009). Volatile compounds such as lactic acid, acetaldehyde, diacetyl, acetoin, acetone, and 2-butanone contribute most to the typical aroma and flavor of yoghurt (Cheng 2010). It is important to work toward obtaining a good yoghurt flavor during production because this will likely improve the consumer acceptability levels (high or moderate acceptance) of the product (Mortazavian, Rezaei, and Sohrabvandi 2009). Most of the flavor compounds in yoghurt are generated from lipolysis of milk fat and microbiological conversion of lactose and citrate (Cheng 2010).

In a study by Tian, Liu, et al. (2020) a combined application of e-nose analysis and back-propagation neural network (BPNN) with random forest models (RF) (Liu et al. 2013) were used to assess satisfactory and unsatisfactory yoghurt flavors among consumers. The preference (satisfactory versus unsatisfactory) of yoghurt samples were first evaluated by human sensory panelists. The e-nose was then employed to see how the technology confirms the validity or otherwise of the different flavors evaluated by the panelists. The results indicated that a PCA could not distinguish the satisfactory or unsatisfactory yoghurt flavors. However, BPNN and RF models clearly discriminated between the two categories of flavors, with accuracy values close to 100%. The RF model showed better discrimination than the BPNN model, with an accuracy of 93.75% for the samples with unsatisfactory

flavor. Tian, Liu, et al. (2020) concluded that the combination of an electronic nose and a nonlinear chemometric model, such as BPNN and RF, is an effective system for the evaluation of yoghurt flavor acceptability amongst consumers.

A recent study by Demarigny et al. (2021) investigated the possible utilization of a portable e-nose, known as NeOse Pro, to examine the microbial fermentation of yoghurt. The study employed the NeOse Pro to discriminate between yoghurt before fermentation and after fermentation using two mixes of lactic acid bacteria containing strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*. A PCA score plot obtained on milk before and after fermentation using *Lactobacillus delbrueckii subsp bulgaricus* only, revealed a clear separation, with described total cumulative variance of 96.41%. A PCA score plot of the milk fermented with *Lactobacillus delbrueckii subsp bulgaricus* or *Streptococcus thermophilus* also showed a clear separation according to the two bacteria used. A GC/MS analysis performed on the produced yoghurt detected acetaldehyde as the abundant volatile compound. Authors concluded that NeOse Pro is a precise tool to study the fermentation of yoghurt during production.

Tian et al. (2017) also studied the effects of four probiotic strains (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosus*, and *Lactobacillus casei*) in coculture with traditional starters on the flavor profile of yoghurt using a GC-MS equipped with MOS based e-nose. A total of 45 VCs were identified by headspace solid-phase microextraction followed by GC-MS. Among these compounds, ketones and aldehydes were the most abundant. *L. rhamnosus* or *L. plantarum* did not significantly affect the major VCs identified, however, *L. casei* and *L. acidophilus* were found in the formation of minor VCs. A radar fingerprint of the e-nose data exhibited good differentiation of samples that contained different probiotics.

Gutiérrez-Méndez et al. (2008) applied an electronic sensory device in the assessment of aroma generation of *Lactococcus lactis*, a bacterium used in the industrial fermentation of milk in yoghurt production. In the study, twenty-three (23) strains of *Lactococcus lactis* isolated from different dairy sources were used. The aroma produced by each strain was evaluated by human sensory panelists and an e-nose. In the analysis of e-nose data, the first 3 principal components that accounted for most of the variation (92.53%) were plotted for each of the samples to determine whether certain samples form groups or whether they were independent. Thus, the evaluation of milk inoculated with different *Lactococci* strains using the e-nose, using PCA, allowed the identification of 4 different groups within the *Lactococci* strains (Gutiérrez-Méndez et al. 2008). The results showed that the *Lactococcus lactis* strains exhibited clear phenotypic differences related to their isolation source and that there was not a clear relationship between the source of isolation and the ability of *Lactococci* strains to produce aroma. To understand the aroma nature of samples in these 4 different groups generated by PCA, using the e-nose data, sensory data were analyzed by plotting odor intensity scores. One of the identified groups was characterized by the low intensity of both odor descriptors; strains isolated from commercial dairy starter cultures dominated this group. Two groups within the 4 identified strains showed a more intense yoghurt-like odor than the rest. In those two groups, strains isolated from nondairy sources and commercial dairy starter cultures were present. The last group showed the most dispersed data, where yoghurt-like odor and Fresco cheese-like odor were the most intense descriptors (Gutiérrez-Méndez et al. 2008).

Marilley et al. (2004) also studied aroma-producing lactic acid bacteria in fermented milk with an e-nose. In the study, thirty-four (34) reference strains and sixty-two (62) *Lactobacillus casei* strains from 5 dairy products were isolated for incubation. The isolated strains were classified into 7 different genotypes by repetitive extragenic palindromic polymerase chain reaction (REP-PCR). The strains were incubated in UHT milk supplemented with casamino acids. The VCs of incubated milk were analyzed after 10 days of incubation with the e-nose. The volatile profiles of the strains (reference versus isolated) were analyzed by a PCA plot. The plot showed that the VCs produced by the reference strains (*Lactobacillus helveticus, Streptococcus thermophiles* and strains of *Propionibacterium freudenreichii*) were separated from the other strains which grouped, with PC1 describing 56.3% variance responsible for the differences between the VCs produced by the reference strains and 25.9% variance, along PC 2, explaining the difference in VCs within the reference strains.

Navrátil, Cimander, and Mandenius (2004) studied on-line multisensor monitoring of yoghurt and Filmjö lk (Swedish yoghurt-like sour milk) fermentations on the production scale. The e-nose signals were selected by evaluation of PCA loading vectors and further analyzed by studying the variability of the selected PCs. The loading vectors for yoghurt and Filmjölk indicate the importance of the signals, in all, 16 out of 19 most contributing sensor response signals were selected from the evaluation of PCA. The first PC of the e-nose signals was used for on-line generation of a process trajectory plot visualizing the actual state of fermentation. The authors concluded that e-nose signals have a potential for rapid on-line monitoring and assessment of the yoghurt fermentation process.

Kovacs et al. (2020a) applied an e-nose based on ultrafast GC technology to differentiate milks fermented with probiotic, moderately probiotic, and non-probiotic *Lactobacillus* strains. The GC chromatograms were transformed into signals of a sensor array by selecting the peaks and handling the retention time of a peak as a sensor, and the area under the peak as the intensity value. Using the recorded odor patterns, classification models were developed for the different bacteria groups and fermentation stages, and the formation of the aromatic compounds during the fermentation process was monitored. Based on a public database of retention indices of volatiles, the authors attempted to identify some specific aroma compounds formed during the fermentation, which include 2-methyl-1-propanol, acetic acid, and heptan-2-ol. Results showed that

aroma monitoring of the fermentation process with the e-nose was a promising and reliable analytical method for rapid classification of bacteria strains according to their probiotic activity and for the monitoring of aroma changes during the fermentation process of milk.

The applied method of data evaluation of the GC-based e-nose could be the harmonization of data originating from different measurements, as the chromatogram peaks may be different, resulting in a different sensor array – as peaks are referred to as sensors. Accordingly, every sample having a different odor profile is described with a different sensor array, which makes the comparison of odor profiles difficult.

This section of the review focused on the analysis of yoghurt sensory properties with the e-nose. The most analyzed yoghurt property was flavor, produced either from the different strains of probiotics or bacteria used during the fermentation and end-stage of yoghurt production and from commercial and synthetic flavorings. The literature sources indicated that the e-nose technique may also be incorporated into the quality control process of yoghurt production. This section of the review also captured an on-line method of multisensory monitoring of different yoghurt fermentation processes. The PCA and back-propagation neural network (BPNN) with random forest models (RF) as machine learning techniques were incorporated into the studies describing the successful evaluation of the yoghurt flavors.

Cheese production

In a recent study, Štefániková et al. (2019) investigated the applicability of e-nose with ultra-fast GC for the characterization of steamed cheese and for the evaluation of steamed cheese quality during 14-day storage. As part of the study, samples of smoked and unsmoked steamed cheese varieties from five Slovak enterprises were used. The PCA of the acquired e-nose data revealed a separation of the stored smoked steamed cheese samples from the stored unsmoked samples. Based on the GC chromatograms and database of retention indices volatile compounds (acetaldehyde, 1-propanal, propanoic acid, ethyl hexanoate, furfural, butan-2-one, isovaleric acid, 1-hexanol or α-pinene) causing the group separations were identified.

Sberveglieri et al. (2016) used a portable novel MOS nanowire gas sensor device (S3) and GC-MS-based approach for the characterization of grated Parmigiano Reggiano cheeses. The volatile profiles of the cheeses at different ripening times (12, 13, 16, 17, 18 and 36 months) were measured. Samples were also evaluated by trained sensory panelists based on the organoleptic qualities (undegraded and degraded). PCA of the S3 sensor data showed a clear separation of the degraded and undegraded cheese samples. The result was also in positive correlation with that of the triangular test done by the trained panelists. In a separate PC plot based on ripening time, 12 and 13 months of ripening were grouped together, 14, 17, and 18 months of ripening also formed a cluster, and the samples with 36 months of ripening were separated from all the others. The reason for the observed separation, according to Sberveglieri et al. (2016), was due to the difference in the aromatic profile during the aging process, as a result of biochemical changes reflected in the cheese aroma and organoleptic properties.

Haddi et al. (2010) used a portable e-nose made up of 6 MOS sensors, jointly with pattern recognition methods, to discriminate cheeses made from goat's, cow's milk and their mixtures. DFA was used to discriminate the different categories of aroma profiles and multivariate analysis of variance (MANOVA) was performed to test the significance of the differences between fingerprints of cheeses (100% goat's cheese, 10% cow's + 90% goat's cheese, 25% cow's + 75% goat's cheese, 50% cow's + 50% goat's cheese and 100% cow's cheese). The DFA approach achieved a success rate of 91.66%, which shows that the samples were correctly classified in their original groups, with only two misclassified samples amongst 24. MANOVA, conducted on fingerprints from individual cheeses, confirmed that the five groups, already discriminated by DFA, were significantly different (Wilks_Lambda $F = 21.34$, $P < 0.05$), indicating the presence of distinct odor profiles of the five cheese types.

Benedetti et al. (2005) studied the shelf life of Crescenza cheese as measured by e-nose. In the study, 14 packets of Crescenza cheese at the beginning of their commercial life were used for the e-nose tests after 0, 6, 13, and 20 days storage at 8 and 15 °C. The results of the study showed that the first 2 PCs explained 98% of the total variance, and their score plot indicated a separation of the samples according to the storage conditions (age and storage temperature). Samples were distributed along PC1 and PC2 according to the storage time and storage temperature, respectively. A DFA done on the sensor responses gave 100% recognition for all Crescenza cheese. Clear discrimination between "fresh (0 days)," "aged (6 and 13 days)," and "very aged (20 days)" samples were reported by the authors.

Trihaas, Vognsen, and Nielsen (2005) studied the application of a GC-MS based e-nose to model the ripening of Danish blue cheese. The study used 96 Danish blue cheeses produced in weeks 35, 37, 39 and 43, and stored at 10 °C for 4 weeks then at 4 °C for 8 weeks. A chemical analysis of volatile compounds was also done by employing the GC-MS. A PCA plot of the e-nose data showed that the first PC (93%) explained the changes in cheese odors as it occurs with storage time (4 and 8 weeks), while PC2 accounted for 6% of the total occurred variance indicating variations among the different batches (production weeks). Cheeses produced in weeks 35 and 43, respectively, were considerably different from each other. The authors concluded that the e-nose could be an efficient tool to determine the variation between cheeses of different ripening period.

Drake et al. (2003) studied e-nose correlation with descriptive sensory analysis of aged Cheddar cheese. In the study, sensory panelists assessed 17 flavor terms for 11 aged Cheddar cheese (≥6 months). The aroma patterns of the cheeses were then analyzed with the e-nose. The sensory and e-nose data were analyzed by PCA. The PCA revealed three components that described 79% of the variance among the cheeses. PC1 explained 39% of the variance and it was responsible for the aged flavors (sulfur, catty). PC2 which

Table 1. The list of electronic nose devices in the reviewed studies and their application modes in the odor analysis of dairy products.

E-nose model	Manufacturer	Type	Sensing system	Number of sensors	Application	Data analysis and presentation	Authors
BH-114	Bloodhound Sensors Ltd., Leeds, UK	Portable	Conductive polymer	14	Milk, classifiaction of milk spoilage bateria and yeast	PCA, DFA	Magan, Pavlou, and Chrysanthakis (2001)
e-NOSE 4000	EEV Inc., Amsford, U.S.A		Conductive polymer	12	Milk, classification microbial and sensory properties	DFA	Korel and Balaban (2002)
QCM based E-nose	SES Piezo Ltd., Portsmouth, England		QCM	6	Milk, evaluation of bacteria-contaminated milk	PCA	Ali, O'Hare, and Theaker (2003)
3320 Applied Sensor Lab Emission Analyzer	Applied Sensor Co. Ltd, Linkoping, Sweden	Portable	MOSFET sensor array, MOS sensor array, CO2 sensor	12,10 and 1	Milk, differetiation of mastitic from healthy milk	PCA, PLS-DA	Eriksson et al. (2005)
FOX 4000	Alpha MOS, Toulouse, France	Benchtop	MOS sensor array	18	Milk, shelf-life evaluation	Vector norm function analysis	Labreche et al. (2005)
e-nose PEN 2	WMA Airsense Analysentechnik GmbH, Schwerin, Germany	Portable	MOS sensor array	10	Milk, identification of adulterated milk	LDA	Yu, Wang, and Xu (2007)
e-nose PEN 2	WMA Airsense Analysetechnik GmbH, Schwerin, Germany	Portable	MOS sensor array	10	Milk, analysis of milk from cows fed on 2 different pasture	PCA, LDA	Falchero et al. (2009)
TGS 8XX fabricated e-nose		Prototype	MOS sensor array	8	Milk, idenfication of source and evalaution of storage time	PCA	Amari, Bari, and Bouchikhi (2009)
FOX 4000	Alpha MOS, Toulouse, France	Benchtop	MOS sensor array	18	Milk, identification of different flavorings	PCA	Wang, Xu, and Sun (2010)
GC-MS equipped with polymer based sensors		Prototype	Conductive polymer	16	Milk, classification of volatile flavor compounds	PCA	Kang et al. (2014)
FOX 4000	Alpha MOS, Toulouse, France	Benchtop	MOS sensor array	18	Milk, discrimination of commercial milk from n-3 enriched milk	DFA	Tóth et al. (2019)
NeOse Pro	Aryballe, Grenoble, France	Portable	Peptides arrayed on gold layer	64	Milk, fermentation	PCA	Demarigny et al. (2021)
On-line multisensor			MOSFET sensor array, MOS sensor array, SnO_2 sensor	10, 19 and 1	Yoghurt, monitoring of yoghurt fermentation	PCA, sensor vector analysis	Navrátil, Cimander, and Mandenius (2004)
GC-MS equipped with polymer based sensors		Prototype	Conductive polymer	12	Yoghurt, screening of aroma-producing lactic acid bacteria	PCA	Marilley et al. (2004)
FOX 3000	Alpha MOS, Nantes, France	Benchtop	MOS sensor array	12	Yoghurt, evaluation of aroma generation of *Lactococcus lactis*	ANOVA, PCA	Gutiérrez-Méndez et al. (2008)
FOX 4000	Alpha MOS, Toulouse, France	Benchtop	MOS sensor array	12	Yoghurt, flavor profile of yoghurt	PCA	Tian et al. (2017)
FOX 4000	Alpha MOS, Toulouse, France	Benchtop	MOS sensor array	18	Yoghurt, evaluation of flavors	PCA, BPNN, RF	Tian, Liu, et al. (2020)
Heracles NEO	Alpha MOS, Toulouse, France	Benchtop	ultra-fast GC, double column, 2 FID	2 flame ionization detectors	Yoghurt, monitoring of fermentation	LDA	Kovacs, et al. (2020a)
eNose 5000	Marconi Applied Technologies; JVA Analytical Ltd., Dublin, Ireland	Benchtop	MOS sensor array	8	Cheese, characterization of commercial Cheddar cheese	PCA, ANOVA	O'Riordan and Delahunty (2003)

3320 Applied Sensor Lab Emission Analyzer	Applied Sensor Co. Ltd, Linkoping, Sweden	Portable	MOSFET sensor array, MOS sensor array	10 and 22	Cheese, shelf-life evaluation	PCA, LDA	Benedetti et al. (2005)
FOX-3000	Alpha MOS, Nantes, France	Benchtop	MOS sensor array	12	Cheese evaluating ripening	PCA	Trihaas, Vognsen, and Nielsen (2005)
E-nose Figaro TGS 8XX		Prototype	MOS sensor array	6	Cheese, discriminate and identify cheeses	DFA, MANOVA	Haddi et al. (2010)
Small Sensor System (S3) equiped with SPME-GC-MS	developed at SENSOR Laboratory, Brescia, Italy	Portable	MOS, ZnO, SnO_2 sensors	6	Cheese, characterization of grated parmigiano cheese	PCA	Sberveglieri et al. (2016)
Heracles II	Alpha MOS, Toulouse, France	Benchtop	ultra-fast GC, double column	2 flame ionization detectors	Cheese, characterization of steamed smoked and steamed unsmoked cheese	PCA	Štefániková et al. (2019)

MOS: metal oxide semiconductor; MOSFET: metal-oxide-semiconductor field-effect transistor; GC-MS: gas chromatograph-mass spectrometer; ZnO: Zinc oxide; SnO2: Tin (IV) oxide; QCM: conducting polymers composites and quartz microbalance; PCA: principal component analysis; LDA: linear discriminant analysis; DFA: discriminant factor analysis; RF: random forest; BPNN: backpropagation neural network; PLS-DA: partial least squares discriminant analysis; ANOVA: analysis of variance; MANOVA: multivariate analysis of variance.

Table 2. Summary of some key properties and identified volatile compounds of different dairy products for evaluation of their quality.

Product	Property studied	Volatile compound / properties	References
Milk	Mastitic milk	sulfides, ketones, amines and acids	
Milk	Healty	lipids	Eriksson et al. (2005)
Milk	Rancidity	acetaldehyde, 1-hexanol, 1-pentanol, butyric and heptatonic acids	Kang et al. (2014)
Yoghurt	Fermentation, lactic acid bacteria	acetaldehyde	Demarigny et al. (2021)
Yoghurt	Fermentation, lactic acid bacteria	2-methyl-1-propanol, acetic acid, heptan-2-ol, decane,2,4 heptadienal 2-octanol	Kovacs et al. (2020a)
Cheese	Smoked and unsmoked steamed	acetaldehyde, 1-propanal, propanoic acid, ethyl hexanoate, furfural, butan-2-one, isovaleric acid, 1-hexanol or α-pinene	Štefániková et al. (2019)
Cheese	Flavors	sulfur, catty, fruity, umami, and brothy odors	Drake et al. (2003)

explained 26% of the variance was associated with fruity, match-like flavor, sweetness and umami, whereas PC3 (14% of the variance) was positively associated with cooked, whey, diacetyl, and brothy flavors and salty and sour tastes.

From the above-reviewed section on e-nose application in cheese production, it was observed that the authentication of cheese from different milk sources, processing effect (steamed smoked versus steamed unsmoked), shelf life or storage time and ripening time can be analyzed based on the volatile profile scores measured by the e-nose. Data analysis techniques such as PCA and LDA or DFA were mostly used in the evaluation of the e-nose data.

Trends and future development of e-nose technology

Table 1 shows a summary of the most prominent publications mentioned in the previous sections concerning the odor analysis of milk, yoghurt and cheese, with the indication of the applied hardware and data analytical protocols.

There has been significant improvement in the e-nose technology since it was first described by Persaud and Dodd, in 1982, as an odor discriminant tool. A historical overview of the evolution of e-nose technology with some of the main milestones was presented by Aouadi et al. (2020). The technology has witnessed the development of sensors from a wide range of materials, which are capable of detecting odors or volatile profiles under various conditions, amongst them are temperature and humidity. New sensors have been developed from semiconducting oxides, conducting polymers, polymer or dielectric coatings, biological films, colorimetric and fluorometric sensors. New transducers such as piezoelectric and catalytic gate metal-oxide-semiconductor field-effect transistor devices have also emerged. Hybrid noses with mixed sensors have been developed to improve the ability of e-nose discrimination.

The technology has also seen the possibility of predicting odors from sensors' signals or data, due to the advanced knowledge people now have in multivariate data analysis and chemometrics. With the help of other analytical methods or tools, such as the electronic tongue, near-infrared spectroscopy, gas chromatography, mass spectrometry and nuclear magnetic resonance, the precision and accuracy of the e-nose technology in the evaluation of dairy foods is increasingly being observed. The estimation time or time of analysis has also improved over the period. The current evolution of portable and micro e-nose devices in the market seems very promising for the olfaction industry since it is now possible to measure substances out of the laboratory. These smaller devices also have low electricity demand, small space demand and low cost compared with the bench-top devices.

The technological future of olfaction depends largely on improving the current devices which cannot detect the chemical composition of odors, reduction or elimination sensors drift, or redundancy, and signal to noise ratio. Among these, drift corrections are highly necessary as these may influence the long-term usability of e-nose based evaluation models. Besides the development of improved hardware providing less drift, advanced mathematical methods and measurement practices can be developed to minimize these negative effects as shown in other fields using taste sensors (Kovacs et al. 2020b).These drawbacks of the technology need to be tackled to increase precision and accuracy.

The appearance of ultra-fast GC-based e-noses provides the possibility of identification of certain volatiles (Table 2), and the miniaturization of the GC technology with gas cartridges allows the development of portable aroma sensing and identification applications (Contreras et al. 2008; Leary et al. 2019). Advanced data evaluation techniques, such as spectrum-like evaluations, are needed for analyzing the chromatograms as sensors, making the generated sensor array signals comparable even in the case of different odor profiles. Further decreasing the influence of some environmental factors, such as humidity and temperature, on the sensor responses will also improve the efficiency of the technology in the rapid quality evaluation of the dairy product.

Conclusions

The electronic nose technology has gained popularity in sensory science, notwithstanding the controversies surrounding the nomenclature of the technology. The application of the technology to evaluate the aroma pattern, shelf life, microbial composition, and the authentication, classification or discrimination of dairy products based on the composition of the volatile compounds (VCs) have been extensively reviewed in this paper. This review revealed that the sensors mostly employed include metal oxide semiconductor sensors (MOS), metal-oxide-semiconductor field-effect transistor (MOSFET), conducting polymers composites and

intrinsically conducting polymers surface acoustic wave sensors, and quartz microbalance (QMB). The mostly applied multivariate classification methods were principal component analysis (PCA), linear discriminate analysis (LDA), and partial least squares discriminant analysis (PLS-DA). The e-nose proved to be an efficient tool in the rapid determination of milk, yoghurt and cheese quality, based on the aroma pattern. In recent advances, portable and prototype e-noses have been used to investigate the quality of dairy products. The limitation of the technology, however, is the inability to provide information on the nature of constituting compounds, except in gas chromatography (GC) or GC and mass spectrometer (GC-MS) based electronic noses.

Acknowledgements

Authors acknowledge the support of the Ministry of Innovation and Technology and the Ministry of Human Capacities, Hungary.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the Ministry of Innovation and Technology [GINOP-2.3.2-15-2016-00046], and the Ministry of Human Capacities [EFOP-3.6.3-VEKOP-16-2017-00005]

ORCID

Haruna Gado Yakubu http://orcid.org/0000-0002-9606-7140
Zoltan Kovacs http://orcid.org/0000-0003-0641-8830
Tamas Toth http://orcid.org/0000-0003-4457-1473
George Bazar http://orcid.org/0000-0001-9829-4366

References

Ali, Z., W. T. O'Hare, and B. J. Theaker. 2003. Detection of bacterial contaminated milk by means of a quartz crystal microbalance based electronic nose. *Journal of Thermal Analysis and Calorimetry* 71 (1): 155–61. https://doi.org/10.1023/A. :1022274419166. doi: 10.1023/A:1022274419166.

Amari, A., N. E. Bari, and B. Bouchikhi. 2009. Conception and development of a portable electronic nose system for classification of raw milk using principal component analysis approach. *Sensors & Transducers* 102 (3):33–44. http://www.sensorsportal.com.

Ampuero, S., and J. O. Bosset. 2003. The electronic nose applied to dairy products: A review. *Sensors and Actuators B: Chemical* 94 (1): 1–12. https://doi.org/10.1016/S0925-4005. (03)00321-6. doi: 10.1016/S0925-4005(03)00321-6.

Aouadi, B., J. L. Zinia Zaukuu, F. Vitális, Z. Bodor, O. Fehér, Z. Gillay, G. Bazar, and Z. Kovacs. 2020. Historical evolution and food control achievements of near infrared spectroscopy, electronic nose, and electronic tongue—Critical overview. *Sensors* 20 (19):5479–42. doi: 10.3390/s20195479.

Banerjee, M. B., T. N. Chatterjee, R. B. Roy, B. Tudu, R. Bandyopadhyay, and N. Bhattacharyya. 2016. Multivariate preprocessing techniques towards optimising response of fused sensor from electronic nose and electronic tongue. International Conference on Computing, Communication and Automation (ICCCA), Noida, 949–54. https://doi.org/doi: doi: 10.1109/CCAA.2016.7813875.

Bedoui, S., R. Faleh, H. Samet, and A. Kachouri. 2013. Electronic nose system and principal component analysis technique for gases identification. 2013 10th International Multi-Conference on Systems, Signals and Devices, SSD 2013, 1–6. Tunisia: IEEE. doi: 10.1109/SSD.2013.6564152.

Benedetti, S., N. Sinelli, S. Buratti, and M. Riva. 2005. Shelf life of crescenza cheese as measured by electronic nose. *Journal of Dairy Science* 88 (9):3044–51. https://doi.org/10.3168/jds.S0022-0302. (05)72985-4. doi: 10.3168/jds.S0022-0302(05)72985-4.

Biolatto, A., G. Grigioni, M. Irurueta, A. M. Sancho, M. Taverna, and N. Pensel. 2007. Seasonal variation in the odour characteristics of whole milk powder. *Food Chemistry* 103 (3):960–7. doi: 10.1016/j.foodchem.2006.09.050.

Bliss, P. J., T. J. Schulz, T. Senger, and R. B. Kaye. 1996. Odour measurement — Factors affecting olfactometry panel performance. *Water Science and Technology* 34 (3-4):549–56. doi: 10.1016/0273-1223(96)00595-1.

Boeker, P. 2014. On 'electronic nose' methodology." *Sensors and Actuators B: Chemical* 204:2–17. doi: 10.1016/j.snb.2014.07.087.

Buttry, D. A., and M. D. Ward. 1992. Measurement of interfaclal processes at electrode surfaces with the electrochemical quartz crystal microbalance. *Chemical Reviews* 92 (6):1355–79. doi: 10.1021/cr00014a006.

Chen, C., K. Li, M. Duan, and K. Li. 2017. *Extreme learning machine and its applications in big data processing. Big data analytics for sensor-network collected intelligence*. Amsterdam, Netherlands: Elsevier Inc. doi: 10.1016/B978-0-12-809393-1.00006-4.

Cheng, H. 2010. Volatile flavor compounds in yogurt: A review. *Critical Reviews in Food Science and Nutrition* 50 (10):938–50. doi: 10.1080/10408390903044081.

Contreras, J., J. Murray, S. Tolley, J. Oliphant, H. Tolley, S. Lammert, E. Lee, D. Later, and M. Lee. 2008. Hand-portable gas chromatograph-toroidal ion trap mass spectrometer (GC-TMS) for detection of hazardous compounds. *Journal of the American Society for Mass Spectrometry* 19 (10):1425–34. doi: 10.1016/j.jasms.2008.06.022.

Da Ré, A. F., L. G. Gurgel, G. Buffon, W. E. R. Moura, D. C. G. M. Vidor, and M. A. P. Maahs. 2018. Tobacco influence on taste and smell: Systematic review of the literature. *International Archives of Otorhinolaryngology* 22 (1):81–7. https://doi.org/DOI. doi: 10.1055/s-0036-1597921.

Demarigny, Y., E. Legrand, J. Sanchez, A. Hallier, N. Laurent, S. Slimani, T. Livache, and D. Picque. 2021. Utilisation of a portable electronic nose, NeOse Pro, to follow the microbial fermentation of a yoghurt. *Food and Nutrition Sciences* 12 (01):90–105. doi: 10.4236/fns.2021.121008.

Drake, M. A., P. D. Gerard, J. P. Kleinhenz, and W. J. Harper. 2003. Application of an electronic nose to correlate with descriptive sensory analysis of aged cheddar cheese. *LWT - Food Science and Technology* 36 (1):13–20. https://doi.org/10.1016/S0023-6438. (02)00216-5. doi: 10.1016/S0023-6438(02)00216-5.

Eriksson, Ä., K. Persson Waller, K. Svennersten-Sjaunja, J. E. Haugen, F. Lundby, and O. Lind. 2005. Detection of mastitic milk using a gas-sensor array system (electronic nose). *International Dairy Journal* 15 (12):1193–201. doi: 10.1016/j.idairyj.2004.12.012.

Falchero, L., G. Sala, A. Gorlier, G. Lombardi, M. Lonati, and G. Masoero. 2009. Electronic nose analysis of milk from cows grazing on two different alpine vegetation types. *The Journal of Dairy Research* 76 (3):365–71. doi: 10.1017/S0022029909004105.

Gao, F., F. Boussaid, W. Xuan, C. Y. Tsui, and A. Bermak. 2018. Dual transduction surface acoustic wave gas sensor for VOC discrimination. *IEEE Electron Device Letters* 39 (12):1920–3. doi: 10.1109/LED.2018.2874821.

Gao, F., W. Xuan, A. Bermak, F. Boussaid, C. Y. Tsui, and J. Luo. 2019. Dual transduction on a single sensor for gas identification. *Sensors and Actuators B: Chemical* 278:21–7. doi: 10.1016/j.snb.2018.09.029.

Gardener, J., and P. Bartlett. 1999. *Electronic noses, principles and application*. New York, NY: Oxford University Press.

Gardner, J. W., and P. N. Bartlett. 1994. A brief history of of electronic noses. *Sensors and Actuators, B: Chemical* 18 (1-3):210–1. doi: 10.1016/0925-4005(94)87085-3.

Gómez, A. H., J. Wang, G. Hu, and A. Garcia Pereira. 2006. Electronic nose technique potential monitoring mandarin maturity. *Sensors and Actuators B: Chemical* 113 (1):347–53. doi: 10.1016/j.snb.2005.03.090.

Gromski, P. S., E. Correa, A. A. Vaughan, D. C. Wedge, M. L. Turner, and R. Goodacre. 2014. A comparison of different chemometrics approaches for the robust classification of electronic nose data. *Analytical and Bioanalytical Chemistry* 406 (29):7581–90. doi: 10.1007/s00216-014-8216-7.

Gutiérrez-Méndez, N., B. Vallejo-Cordoba, A. F. González-Córdova, G. V. Nevárez-Moorillón, and B. Rivera-Chavira. 2008. Evaluation of aroma generation of Lactococcus lactis with an electronic nose and sensory analysis. *Journal of Dairy Science* 91 (1):49–57. doi: 10.3168/jds.2007-0193.

Haddi, Z., F. Annanouch, A. Amari, A. Hadoune, B. Bouchikhi, and N. E. Bari. 2010. Application of a portable electronic nose device to discriminate and identify cheeses with known percentages of cow's and goat's milk. Proceedings of IEEE Sensors, 771–74. doi: 10.1109/ICSENS.2010.5690762.

Hadjipanayiotou, M. 1995. Composition of ewe, goat and cow milk and of colostrum of ewes and goats. *Small Ruminant Research* 18 (3):255–62. https://doi.org/10.1016/0921-4488. (95)00697-3. doi: 10.1016/0921-4488(95)00697-3.

Hines, E. L., P. Boilot, J. W. Gardner, and M. A. Gongora. 2006. Pattern analysis for electronic noses. In *Handbook of machine olfaction*, ed. T. C. Pearce, S. S. Schiffman, H. T. Nagle, and J. W. Gardner, 133–160. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Hower, D. 2015. The science of sensory evaluation: An ethnographic critique. In The Social Life of Materials, ed. A. Drazer and S. Küchler, 81–97. London: Bloomsbury.

Kang, H., S. Y. Cho, J. Ryu, J. Choi, H. Ahn, H. Joo, and H. T. Jung. 2020. Multiarray nanopattern electronic nose (e-nose) by high-resolution top-down nanolithography. *Advanced Functional Materials* 30 (27):2002486. doi: 10.1002/adfm.202002486.

Kang, N. K., T. Sun Jun, Y. S. Yang, and Y. S. Kim. 2014. Analysis of volatile flavor compounds in milk using electronic nose system. *Journal of Sensor Science and Technology* 23 (5):320–5. doi: 10.5369/JSST.2014.23.5.320.

Kauer, J. S., and J. White. 2009. *Electronic nose. Encyclopedia of neuroscience*, 871–877. doi: 10.1016/B978-008045046-9.01694-6..

Khoshgoftaar, T. M., J. Van Hulse, and A. Napolitano. 2010. Supervised neural network modeling: An empirical investigation into learning from imbalanced data with labeling errors. *IEEE Transactions on Neural Networks* 21 (5):813–30. doi: 10.1109/TNN.2010.2042730.

Korel, F., and M. Ö. Balaban. 2002. Microbial and sensory assessment of milk with an electronic nose. *Journal of Food Science* 67 (2):758–64. doi: 10.1111/j.1365-2621.2002.tb10672.x.

Kovacs, Z., Z. Bodor, J.-L. Zinia Zaukuu, T. Kaszab, G. Bazar, T. Tóth, and C. Mohácsi-Farkas. 2020a. Electronic nose for monitoring odor changes of Lactobacillus species during milk fermentation and rapid selection of probiotic candidates. *Foods* 9 (11):1539–16. doi: 10.3390/foods9111539.

Kovacs, Z., D. Szöllősi, J.-L Z. Zaukuu, Z. Bodor, F. Vitális, B. Aouadi, V. Zsom-Muha, and Z. Gillay. 2020b. Factors influencing the long-term stability of electronic tongue and application of improved drift correction methods. *Biosensors* 10 (7):74. doi: 10.3390/bios10070074.

Labreche, S., S. Bazzo, S. Cade, and E. Chanie. 2005. Shelf life determination by electronic nose: Application to milk. *Sensors and Actuators B: Chemical* 106 (1):199–206. doi: 10.1016/j.snb.2004.06.027.

Larmond, E. 1997. *Laboratory methods for sensory evaluation of foods*, 1–1637. Ottawa, Canada: Department of Agriculture Publication.

Lavine, B. K., and J. Workman. 2008. Chemometrics. *Analytical Chemistry* 80 (12):4519–31. doi: 10.1021/ac800728t.

Leary, P. E., B. W. Kammrath, K. J. Lattman, and G. L. Beals. 2019. Deploying portable gas chromatography-mass spectrometry (GC-MS) to military users for the identification of toxic chemical agents in theater. *Applied Spectroscopy* 73 (8):841–58. doi: 10.1177/0003702819849499.

Li, Z., J. R. Askim, and K. S. Suslick. 2019. The optoelectronic nose: Colorimetric and fluorometric sensor arrays. *Chemical Reviews* 119 (1):231–92. doi: 10.1021/acs.chemrev.8b00226.

Liu, M., M. Wang, J. Wang, and D. Li. 2013. Comparison of random forest, support vector machine and back propagation neural network for electronic tongue data classification: Application to the recognition of orange beverage and Chinese vinegar. *Sensors and Actuators B: Chemical* 177:970–80. doi: 10.1016/j.snb.2012.11.071.

Llobet, E. 2020. *Advanced nanomaterials for inexpensive gas microsensors*. Amsterdem: Elsevier Inc.

Llobet, E., E. L. Hines, J. W. Gardner, P. N. Bartlett, and T. T. Mottram. 1999. Fuzzy ARTMAP based electronic nose data analysis. *Sensors and Actuators B: Chemical* 61 (1-3):183–9. https://doi.org/10.1016/S0925-4005. (99)00288-9. doi: 10.1016/S0925-4005(99)00288-9.

Loutfi, A., S. Coradeschi, G. Kumar Mani, P. Shankar, and J. Bosco Balaguru Rayappan. 2015. Electronic noses for food quality: A review. *Journal of Food Engineering* 144:103–11. doi: 10.1016/j.jfoodeng.2014.07.019.

Magan, N., A. Pavlou, and I. Chrysanthakis. 2001. Milk-sense: A volatile sensing system recognizes spoilage bacteria and yeasts in milk. *Sensors and Actuators B: Chemical* 72 (1):28–34. https://doi.org/10.1016/S0925-4005. (00)00621-3. doi: 10.1016/S0925-4005(00)00621-3.

Marilley, L., S. Ampuero, T. Zesiger, and M. G. Casey. 2004. Screening of aroma-producing lactic acid bacteria with an electronic nose. *International Dairy Journal* 14 (10):849–56. doi: 10.1016/j.idairyj.2004.02.013.

Martens, H. 2015. Quantitative big data: Where chemometrics can contribute. *Journal of Chemometrics* 29 (11):563–81. doi: 10.1002/cem.2740.

Martens, H., and E. Stark. 1991. Extended multiplicative signal correction and spectral interference subtraction: New preprocessing methods for near infrared spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* 9 (8):625–35. doi: 10.1016/0731-7085(91)80188-F.

Martinelli, E., C. Falconi, A. D'Amico, and C. Di Natale. 2002. Preprocessing of electronic nose data by independent component analysis. *Proceedings of IEEE Sensors* 1 (2):1339–42. doi: 10.1109/icsens.2002.1037313..

Mortazavian, A. M., K. Rezaei, and S. Sohrabvandi. 2009. Application of advanced instrumental methods for yogurt analysis. *Critical Reviews in Food Science and Nutrition* 49 (2):153–63. doi: 10.1080/10408390701764807.

Mottram, D. S., and J. S. Elmore. 2003. Sensory evaluation | Aroma. *Encyclopedia of Food Sciences and Nutrition* :5174–80. doi: 10.1016/b0-12-227055-x/01068-3.

Naes, T., T. Isaksson, T. Fearn, and T. Davies. 2002. *Multivariate calibration and classification*. Chichester, UK: NIR Publictions.

Navrátil, M., C. Cimander, and C. F. Mandenius. 2004. On-line multisensor monitoring of yogurt and filmjölk fermentations on production scale. *Journal of Agricultural and Food Chemistry* 52 (3):415–20. doi: 10.1021/jf0304876.

Nishanth, K. J., and V. Ravi. 2016. Probabilistic neural network based categorical data imputation. *Neurocomputing* 218:17–25. doi: 10.1016/j.neucom.2016.08.044.

Pearce, T. C, S. S Schiffman, H. T Nagle, and J. W Gardener, eds. 2006. *Handbook of machine olfaction: Electronic nose technology*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Peelle, J. E. 2019. Age-related sensory deficits and their consequences. In *The handbook of cognitive aging: A life course perspective*, ed. A. K. Thomas and A. Gutchess, 2–19. Cambridge, UK: Cambridge University Press. doi: 10.31234/OSF.IO/3CPTQ.

Persaud, K., and G. Dodd. 1982. Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. *Nature* 299 (5881):352–5. doi: 10.1038/299352a0.

Peryam, D. R., and N. F. Girardot. 1952. Advanced taste test method. *Food Engineering* 24 (194):58–61.

Pu, D., Y. Zhang, H. Zhang, B. Sun, F. Ren, H. Chen, and Y. Tang. 2020. Characterization of the key aroma compounds in traditional

hunan smoke-cured pork leg (Larou, THSL) by aroma extract dilution analysis (AEDA), odor activity value (OAV), and sensory evaluation experiments. *Foods* 9 (4):413–6. doi: 10.3390/foods9040413.

Qu, X. 2007. Multivariate data analysis. *Technometrics* 49 (1):103–4. doi: 10.1198/tech.2007.s455.

Santonico, M., P. Pittia, G. Pennazza, E. Martinelli, M. Bernabei, R. Paolesse, A. D'Amico, D. Compagnone, and C. D. Natale. 2008. Study of the aroma of artificially flavoured custards by chemical sensor array fingerprinting. *Sensors and Actuators B: Chemical* 133 (1): 345–51. doi: 10.1016/j.snb.2008.02.053.

Sberveglieri, V., M. Bhandari, E. Núñez Carmona, G. Betto, and G. Sberveglieri. 2016. A novel MOS nanowire gas sensor device (S3) and GC-MS-based approach for the characterization of grated Parmigiano Reggiano cheese. *Biosensors* 6 (4):60. doi: 10.3390/bios6040060.

Scott, S. M., D. James, and Z. Ali. 2006. Data analysis for electronic nose systems. *Microchimica Acta* 156 (3-4):183–207. doi: 10.1007/s00604-006-0623-9.

Shiokawa, S., and J. Kondoh. 2004. Surface acoustic wave sensors. *Japanese Journal of Applied Physics* 43 (5B):2799–802. doi: 10.1143/JJAP.43.2799.

Sørhaug, T., and L. Stepaniak. 1997. Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends in Food Science & Technology* 8 (2):35–41. https://doi.org/10.1016/S0924-2244.(97)01006-6. doi: 10.1016/S0924-2244(97)01006-6.

Štefániková, J., V. Nagyová, M. Hynšt, V. Victoris, P. Martišová, and L. Nagyová. 2019. Application of electronic nose for determination of Slovak cheese authentication based on aroma profile. *Potravinarstvo Slovak Journal of Food Sciences* 13 (1):262–7. doi: 10.5219/1076.

Tian, H., H. Liu, Y. He, B. Chen, L. Xiao, Y. Fei, G. Wang, H. Yu, and C. Chen. 2020. Combined application of electronic nose analysis and back-propagation neural network and random forest models for assessing yogurt flavor acceptability. *Journal of Food Measurement and Characterization* 14 (1):573–83. doi: 10.1007/s11694-019-00335-w.

Tian, H., Y. Shen, H. Yu, Y. He, and C. Chen. 2017. Effects of 4 probiotic strains in coculture with traditional starters on the flavor profile of yogurt. *Journal of Food Science* 82 (7):1693–701. doi: 10.1111/1750-3841.13779.

Tian, H., X. Xu, X. Sun, C. Chen, and H. Yu. 2020. Evaluation of the perceptual interaction among key aroma compounds in milk fan by gas chromatography–olfactometry, odor threshold, and sensory analyses. *Journal of Dairy Science* 103 (7):5863–73. doi: 10.3168/jds.2019-17880.

Tominaga, Y. 1999. Comparative study of class data analysis with PCA-LDA, SIMCA, PLS, ANNs, and k-NN. *Chemometrics and Intelligent Laboratory Systems* 49 (1):105–15. https://doi.org/10.1016/S0169-7439. (99)00034-9. doi: 10.1016/S0169-7439(99)00034-9.

Tóth, T., P. J. Mwau, G. Bázár, G. Andrássy-Baka, H. Hingyi, É. Csavajda, and L. Varga. 2019. Effect of feed supplementation based on extruded linseed meal and fish oil on composition and sensory properties of raw milk and ultra-high temperature treated milk. *International Dairy Journal* 99:104552. doi: 10.1016/j.idairyj.2019.104552.

Trihaas, J., L. Vognsen, and P. V. Nielsen. 2005. Electronic nose: New tool in modelling the ripening of Danish Blue cheese. *International Dairy Journal* 15 (6-9):679–91. doi: 10.1016/j.idairyj.2004.07.023.

Tuorila, H., and E. Monteleone. 2009. Sensory food science in the changing society: Opportunities, needs, and challenges. *Trends in Food Science & Technology* 20 (2):54–62. doi: 10.1016/j.tifs.2008.10.007.

Wang, B., S. Xu, and D. W. Sun. 2010. Application of the electronic nose to the identification of different milk flavorings. *Food Research International* 43 (1):255–62. doi: 10.1016/j.foodres.2009.09.018.

Yong, Y., W. Wenkai, and Y. Huichun. 2014. Drift elimination method of electronic nose signals based on independent component analysis coupled with wavelet energy threshold value. *Transactions of the Chinese Society of Agricultural Engineering* 30 (24):325–30.

Yu, H., J. Wang, and Y. Xu. 2007. Identification of adulterated milk using electronic nose. *Sensors and Materials* 19 (5):275–85.

Zhang, L., and F. Tian. 2014. Performance study of multilayer perceptrons in a low-cost electronic nose. *IEEE Transactions on Instrumentation and Measurement* 63 (7):1670–9. doi: 10.1109/TIM.2014.2298691.

Zhang, S., C. Xie, C. Fan, Q. Zhang, and Q. Zhan. 2007. An alternate method of hierarchical classification for e-nose: Combined fisher discriminant analysis and modified Sammon mapping. *Sensors and Actuators B: Chemical* 127 (2):399–405. doi: 10.1016/j.snb.2007.04.045.

2. CONCLUSIONS DRAWN FROM THE REVIEWED LITERATURE

The application of NIR spectroscopy in the dairy industry has evolved over the years. Its usefulness in the evaluation of animal feed compared with wet chemistry methods has proven to be fast and gaining more accuracy in practical application. The rapid determination of moisture, ash, EE, CP, NDF, ADF and ADL of forages or TMR, using NIR spectroscopy could give a fair understanding of the nutritional composition of the feed and could support feeding specialist aiming precision feeding, especially, in highly sensitive periods of cows when the metabolic balance is compromised. The review has also shown the possibility of NIRS as a rapid method to evaluate milk quantitative and qualitative qualities. On the other hand, regarding the application of e-nose in dairy production, the application of the technology to evaluate the odor pattern, shelf life, microbial composition, and the authentication, classification or discrimination of milk based on the composition of the volatile compounds (VCs) have been extensively studied.

Though literature on the application of NIR spectroscopy to evaluate the quality of forages exist, its application to evaluate forage mixtures (winter cereals and Italian ryegrasses) harvested at various phases, and the influence of ensiled periods on the forage mixture quality remain limited. Similarly, although the e-nose proved to be an efficient tool in the rapid evaluation of milk quality based on the odor fingerprint, its application to profile silages is virtually not available or very limited, and, therefore, will be a novelty. In addition to this, the application of the technology to profile milk odor from different feeding regimes or diets, and its application to test the odor profile of fortified functional dairy products such as DHA enriched milkshakes, remain limited, hence making this current study very relevant.

3. OBJECTIVES OF THE THESIS

From the background, the following specific objectives which formed the main chapters of this thesis were considered:

1. To demonstrate the applicability of near-infrared spectroscopy in the evaluation of the qualitative and quantitative characteristics of newly introduced forage mixtures in dairy cattle farming (Study 1: As a rapid monitoring tool, can NIR spectroscopy be used for checking differences of mixture forages during harvest? Can NIR spectroscopy be used to identify differences and/or similarities among different mixture silages? Can NIR spectroscopy detect the chemical composition of mixture forages and/or silages?).
2. To check the feasibility of electronic nose technologies to evaluate the quality of some feeds and dairy products, i.e. describing the odor profile of:

a. alfalfa and rye silages used in dairy feeding (Study 2: Can the odor profile of silages be related to the harvesting phase and/or ensiling methodology? Can the odor profile recorded with a gas-sensors-based electronic nose be used to assess the quality of the silages?),
b. raw bovine milk as affected by feeding (Study 3: Can milks produced by cows fed with different diets be differentiated based on the aroma patterns recorded with a gas-chromatography-based electronic nose? Can evidence on the different aroma modifying effects of the winter cereals and Italian ryegrass mixture silage supplementations be found? Can the supplementations with the less effect on the aroma profile of the produced milk be detected?)
c. value-added functional food fortified with health promoting additives (Study 4: Can the off-odor caused by micro-encapsulated microalgae oil supplementation in newly developed milkshake be detected using a gas-chromatography-based electronic nose?).

4. METHODOLOGICAL SUMMARY OF THE THESIS

The methodological summary will focus on the **four (4) experiments** studied under the overall PhD theme.

4.1. Study 1

4.1.1. Samples (forage mixtures)

The compositions of the four forage mixtures used in the study were (commercial products, producer: Agroteam S.p.a., Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy):

Mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat.

Mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat.

Mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat.

Mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

The percentages of the forage mixtures composition reflect the seed weight ratio of the various winter cereals (WC) and Italian ryegrasses (IRG) used in the study. The forages were cultivated on an experimental farm (Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Kaposvár, Hungary.

4.1.2. Harvesting of the forages

The four forage mixtures (Mixture A, B, C and D) were harvested on five (5) phases, with one week interval between each harvest (Cut 1, 2, 3, 4 and 5), at each harvest, 5 samples of each of the 4 mixture (510 g) were taking on the

field, totaling 20 samples per each cut. The total number of samples at the end of the harvest period was 100 (20 by 5).

4.1.3. Forage preservation, storage and preparation

One part of the forage samples (fresh) were stored frozen at -20 °C in sealed polyethylene bags (ca. 1000 g), and the other part was dried at 60°C until mass constancy (ca. 600 g) using a Memmert UFE400 oven (Memmert GmbH, Buechenbach, Germany). The dried samples were ground with a Retsch Rotor Beater Mill SR 200 with a bottom sieve of 1 mm aperture size (Retsch GmbH, Haan, Germany).

4.1.4. Ensiling of forages

Wilted and chopped materials of 510 g were packed into laboratory silos using a mechanical hand packer into anaerobic glass jars capacity of 0.72 litre [729 kg/m3]. After ensiling 0, 7, 14 and 90 days, five laboratory silos per mixtures (A, B, C and D) were opened and samples of silages were stored frozen at –20 °C in sealed polyethylene bags. Samples were thawed at 4 °C for 24 hours until being measured with NIR spectroscopy in fresh or moist form, without grinding the chopped samples. Then the silages were dried and ground analogously to the forages, and NIR spectra of the dried silages were also recorded.

4.1.5. Chemical analyses of forages

Chemical analyses were performed in the analytical laboratory of Kaposvár Campus. The CP, EE, crude ash, CF and total sugar were determined according to AOAC (2006). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to the procedure of Van Soest et al. (1991). The statistical evaluation of the chemical data was performed with one-way analysis of

variance (ANOVA) including Tukey's post-hoc test to check the group differences, using the SPSS v26 (IBM Corp., Armonk, USA).

4.1.6. Near-infrared spectroscopic measurement of forages and silages

NIR spectroscopy measurements were performed in the NIRS laboratory of Kaposvár Campus. In all cases, samples were at room temperature (25 °C) during the NIRS measurements. The reflectance NIR spectra (log R^{-1}) of the moist silages were recorded with a FOSS DS2500 spectrometer (FOSS Analytical A/S, Hillerod, Denmark) (Photo 1a) in the range of 400-2500 nm, at 2 nm spectral steps, with 8 nm nominal bandpass, as the average of 32 successive scans. To avoid spectral variation caused by heterogeneity of the silages, the large cup (FOSS 60056582) was rotated eight times during the successive scans, and the acquired spectrum of an individual sample was the average of spectra of two refilled subsamples. FOSS Mosaic Solo v.8.0.4.10 (FOSS Analytical A/S, Hillerod, Denmark) and FOSS ISIScan Nova (FOSS Analytical A/S, Hillerod, Denmark) software packages were used for the operation of the spectrometer and data acquisition.

The NIR spectra of the dried and ground forages (n = 100) and ensiled forages (n = 80) were collected in reflectance mode using a NIRSystems 6500 spectrometer (FOSS NIRSystems, Laurel, MD, USA) (Photo 1b) equipped with a sample transport module and small ring cup (FOSS IH-0307). Log R^{-1} spectra were recorded in the 400-2500 nm range with 2 nm spectral steps. The WinISI II version 1.5 spectral analytical software (InfraSoft International LLC, State College, PA, USA), was utilized for the operation of the spectrometer and acquiring data.

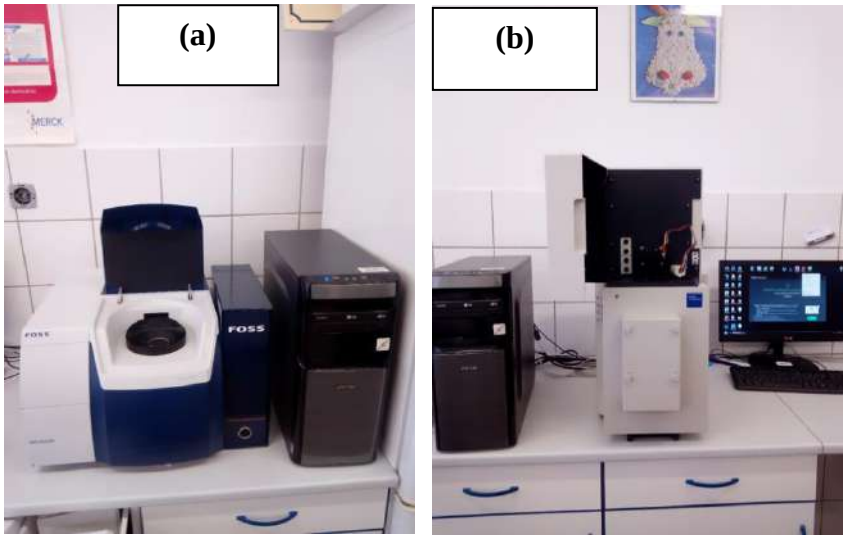


Photo 1: FOSS DS2500 spectrometer **(a)** (FOSS Analytical A/S, Hillerod, Denmark) and NIRSystems 6500 spectrometer **(b)** (FOSS NIRSystems, Laurel, MD, USA)

4.2. Study 2

4.2.1. Samples (alfalfa and rye silages)

The study used alfalfa and rye silage (n = 22 and 38, respectively) derived from large scale commercial dairy farms in Hungary. Alfalfa forages were prepared for ensiling with and without wilting (n = 10 and 12, respectively), while rye silages were harvested before heading and in heading (n = 17 and 21, respectively). The fresh samples were dried at 70 °C for 8 hours, then homogenized with a laboratory mill (Peppink Mills, Netherlands) and analyzed for DM, CP, EE, CF, crude ash, pH, acetic acid and lactic acid concentrations by means of NIR spectroscopy using a Quant FT-NIR spectrometer (Q-Interline, Denmark) and an internationally recognized calibration database (Samplinq®, Eurofins Agro Inc., Wageningen, Netherlands) at Livestock Performance Testing Ltd, Gödöllő, Hungary. The quality of samples was evaluated based on the pH and the lactic acid/acetic

acid ratio. Each freshly collected sample was equally distributed into six plastic bags for the odor measurement. The prepared sub-samples (n = 360) were stored at –20 °C in sealed bags under vacuum, until the odor measurements. Frozen storage of any sample did not last longer than 30 days.

4.2.2. Odor measurement of alfalfa and rye silages

The odor measurement was performed at Kaposvár University (now Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Kaposvár, Hungary) for six days where the six sets of sub-samples were analyzed daily (n = 60 per day). The odor of each sample was examined with an Alpha MOS FOX4000 electronic nose based on the metal oxide semiconductor (MOS) sensor array technology (Alpha M.O.S., Toulouse, France) within 30 days after sampling. 2 g of subsamples (n=360) were filled into 20 mL glass vials then sealed with polytetrafluoroethylene septa. The headspace samples containing the volatiles of the silages were generated above the solid sample during three-minute incubation at 40 °C. 5 mL of the headspace was injected into the continuous flow of synthetic air. The relative resistance changes (ΔR/R0) of 18 MOS sensors caused by the injected volatiles were measured after each injection and saved as sensitivity values. One measurement cycle took 20 minutes, including two minutes data acquisition and 18 minutes cleaning phase when pure synthetic air was used to rinse the sensors and the syringe. The maximal sensitivity experienced on each sensor was saved in each measurement cycle, thus, all sub-samples were described with 18 variables.

4.3. Study 3

4.3.1. Design and sample collection (milk)

The experimental total mixed ratios (TMRs) used in the feed trail were formulated using the forage mixtures in study 1. The study was carried out at the dairy farm (Fészerlak) of the Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár Campus. The experimental design used was a single-blinded randomized efficacy study divided into 5 periods (Figure 4.3.1-1.), conducted between August 2019 and March 2020.

The dairy cows used for the study were 32 multiparous Holstein-Friesian cows (>150 days in milking, average milk production: <25 kg/day), fed with 4 experimental diets (EXP-1, 2, 3, 4) and a control (CTR) as shown in Table 1. Mix A, B, C, D within the experimental diets as mentioned below are identical with those described in section 4.1.1.

Figure 4.3.1-1. A single-blinded randomized efficacy study divided into 5 feeding periods.

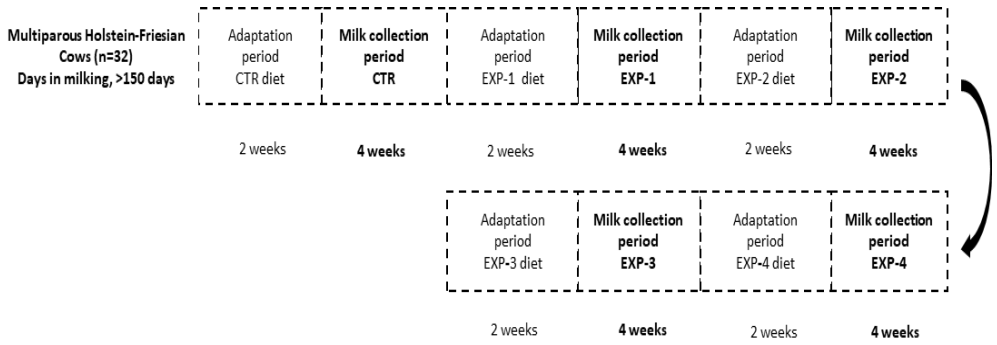


Figure 4.3.1-1. A single-blinded randomized efficacy study divided into 5 feeding periods.

Table 1. The control (CTR) and experimental diets (EXP-1, 2, 3 and 4)

Parameter	CTR	EXP-1	EXP-2	EXP-3	EXP-4
Ingredient, kg/ cow/ day					
Corn silage	11	11	11	11	11
Alfalfa haylage	7	7	7	7	7
Vetch-triticale haylage	7	-	-	-	-
Mix A[1]	-	-	-	-	7
Mix B[2]	-	-	-	7	-
Mix C[3]	-	7	-	-	-
Mix D[4]	-	-	7	-	-
Concentrate[5]	6	6	6	6	6
Grass hay	2	2	2	2	2
Molasses (liquid)	1.2	1.2	1.2	1.2	1.2

[1]Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat

[2]Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat

[3]Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat)

[4]Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

[5]Vitafort Co., Dabas, Hungary ("533-614", dry matter: 88.00%, crude protein: 16.00%, NE_l MJ kg^{-1}: 6.74, crude fiber: 5.00%, ether extract: 2.90%, ash: 8.30%, starch: 42.71%, sugar: 2.34%, calcium: 1.71%, phosphorus: 0.57%, sodium: 0.66%, magnesium: 0.37%, vitamin A: 22,800 IU kg^{-1}, vitamin D: 4,500 NE kg^{-1}, vitamin E: 128 mg kg^{-1}

Milk collection started after 2 weeks of dairy cow adaptation to each feeding trial (Figure 5). During this adaptation period, cows were fed their assigned diet and then studied for two weeks in order to facilitate the cows' physiological adjustment to the feed, as well as to avoid the influence of a preceding trial on a succeeding one. The cows' udder health was continually monitored with Mastatest (Mastaplex Ltd., Dunedin, New Zealand) in order to rule out mastitis. The milk of unhealthy cows was not included in the test sample. For each trial or period (5 trials in total), 8 separate collections of homogenized milk from each group were sampled into 0.5 L bottles (n = 40), which were then stored frozen (-20 °C) for subsequent e-nose measurement.

4.3.2. Odor measurement of milks

Heracles Neo (Alpha MOS, Toulouse, France) (Photo 2) flash gas chromatograph with two columns (MXT-5 and MXT-1701, Restek, USA) equipped with a HS 100 (PAL Systems, Switzerland) auto-sampler was used for the measurement in the Correltech® Laboratory of ADEXGO Kft., Herceghalom, Hungary. The hydrogen used as carrier gas during the measurement was in FID Grade.

Photo 2: Heracles Neo Electronic Nose (Alpha MOS, Toulouse, France)

After 5-minute incubation (50 °C) of 20 mL sealed vials with UltraCleanTM polytetrafluoroethylene/silicone septum (Supelco, Inc., Merck KGaA, Darmstadt, Germany) containing 1 mL of individual milk samples (5 measurement per sample (n=25)). A 5 mL headspace injection was done, and acquisition time per sample was 110 s, with analysis period per sample being 9 minutes, respectively.

4.3.3. Analysis of milk fatty acids

Milk (n=40) was homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in a 20-fold volume of chloroform:methanol (2:1 v:v). Furthermore, the total lipid content was extracted (Folch et al., 1957). The solvents were ultrapure grade (Sigma-Aldrich, St. Louis, MO, USA); moreover, 0.01% w/v butylated hydroxytoluene was added in order to prevent FA oxidation. The samples were then evaporated to dryness under a nitrogen stream, and were transmethylated through a base-catalyzed $NaOCH_3$ method (Christie, 1982).

After cooling, the total lipid was extracted with chloroform and then trans-methylated with an acid-catalyzed method (Christie, 2003), while using H_2SO_4 (1 v/v%) in methanol as a methyl donor, and toluene as a solvent at 50 ^oC overnight. FA methyl-esters were extracted into 300 μL ultrapure n-hexane for the purposes of gas chromatography (AOC 20i automatic injector; Shimadzu 2030, Kyoto, Japan); then equipped with a Phenomenex Zebron ZB-WAXplus capillary GC column (30 m × 0.25 mm ID, 0.25 μm film, Phenomenex Inc., Torrance, CA, USA); and also subjected to a flame ionization detector (FID). The characteristic operating conditions were as follows: injector temperature: 220 °C; detector temperature: 250 °C; and helium flow: 28 cm/s. The oven temperature was then graded from 60 (2 min holding) to 150 °C, from 150 to 180 °C; 2 °C/min and 10 min at 180 °C, from 180 to 220 °C; and then 2 °C/min and 16 min at 220 °C. In addition, the makeup gas used was nitrogen. Calculation was then performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration. FA results were expressed as the weight % of the total FA-methyl esters.

4.4. Study 4

4.4.1. Samples (fortified milkshake)

Milk-based vanilla shake powder was fortified with micro-encapsulated microalgae oil (brand: S17-P100, life's DHA, DSM Nutritional Products Inc., Heerlen, Netherlands) to increase the omega-3 (n3) fatty acid content. A 10-step oil-enrichment protocol was performed from 0.2 up to 2 w/w% inclusion rate (Table 2), to increase the DHA in the shake product, and based on this a flash-GC-based e-nose was used to describe the product's odor.

Table 2. The graded food additive enrichment protocol (product composition)

	shake powder	S17-P100	total mass			calc. EPA+DHA
No.				calc. DHA	calc. EPA	
				mg in 10 g		
1	9980	20	10000	4.9	1.5	6.4
2	9960	40	10000	9.8	3.0	12.8
3	9940	60	10000	14.7	4.4	19.1
4	9920	80	10000	19.6	5.9	25.5
5	9900	100	10000	24.5	7.4	31.9
6	9880	120	10000	29.4	8.9	38.3
7	9860	140	10000	34.3	10.4	44.7
8	9840	160	10000	39.2	11.8	51.0
9	9820	180	10000	44.1	13.3	57.4
10	9800	200	10000	49.0	14.8	63.8

S17-P100, brand name of the primarily docosahexaenoic acid enrichment used; EPA, eicosapentaenoic acid (C20:5 n3); DHA, docosahexaenoic acid (C22:6 n3)

4.4.2. Odor measurement of milkshakes

The same electronic nose was used as described in 4.3.2. Three-times 1 g aliquots of each sample were placed into three 20-mL headspace vials (n=30), sealed with a magnetic cap and an UltraCleanTM

polytetrafluoroethylene/silicone septum (Supelco, Inc., Merck KGaA, Darmstadt, Germany), with 5 mL headspace injection. Incubation: 80°C for 10 min.

4.4.3. Analysis of fatty acids

Samples (shake powder, FA additive, complemented shake powder) were homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in a 20-fold volume of chloroform:methanol (2:1 vol:vol) and total lipid content was extracted according to (Folch et al. 1957). Solvents were ultrapure-grade (Carl Roth, Karlsruhe, Germany) and 0.01 % w:v butylated hydroxytoluene was added to prevent FA oxidation. Directly to the raw, dry sample, C19:0 internal standard was added (Merck cat. No.: 72332). The internal standard used was a solution of 1 mg/ml in chloroform:methanol (2:1 vol:vol). The total amount added was ca. 1/20 mass of the extracted fat, i.e. to 1 g raw sample (ca. 100 mg crude extract) 5 mg C19:0 was added.

Total lipid extract (including the internal standard as well) was dried fully on a rotary evaporator, under a nitrogen stream and was trans-methylated with the acid-catalyzed method (Christie, 2003), using H2SO4 (1 vol/vol%) in methanol as a methyl donor, and toluene was used as a solvent. For the quantitative analysis, C19:0 methyl ester standard calibration was used at 6 points (Merck cat. No.: 74208) to assess detector response, and the concentration of analyte in the calibration was between 5 and 500 microg/ml. The correlation coefficient was not less than 0.999, proving the linearity of analysis. Fatty acid methyl-esters were extracted into ultrapure n-hexane for gas chromatography. This was performed on a Shimadzu GCMS-QP2010 apparatus (AOC 20i automatic injector), equipped with a Phenomenex Zebron ZB-WAX Capillary GC column (30 m x 0.25 mm ID, 0.25 micrometer film, Phenomenex Inc., Torrance, CA, USA). Characteristic operating conditions were: injector temperature: 270 °C, detector

temperature: 300 °C, helium flow: 28 cm/sec. The oven temperature was graded: from 80 to 205 °C: 2.5 °C/min, 5 min at 205 °C, from 205 to 250 °C 10 °C/min and 5 min at 210 °C. FA results were expressed as mg/g of raw sample mass and as well as weight% of the total FAs. All samples were analyzed in duplicate, and results are means of 2 analyses. The Limit of Detection was determined as three times the signal-to-noise ratio (3S/N), while the Limit of Quantification 10S/N. The range of LOD was between 0.1– 0.5 μg/ml for the FAs; C4:0 and C24:0).

4.5. Statistical Analysis

4.5.1. Multivariate analysis of NIR data (study 1)

The multivariate data of the NIR spectroscopic measurements were exported from WinISI and Mosaic software packages in text file format and The Unscrambler 11.0 (CAMO Analytics AS, Oslo, Norway) was applied for data processing and analysis. Moving average smoothing was applied with 5 spectral points before further data pre-treatments. SNV transformation (Barnes et al., 1989) and 2^{nd} order Norris derivatives with 5-point gap (Hopkins, 2001; Norris, 2001) were calculated to decrease additive and multiplicative effects of light scatter. PCA (Cowe and McNicol, 1985) was used to describe the basic multidimensional characteristics of the NIR data matrix and to visualize the differences of the sample groups. Calibrations for the chemical constituents were conducted with the forage NIR data and chemical (reference) PLS regression, and models were tested with full cross-validation (Næs et al., 2002). The precision and accuracy of the chemometric models were evaluated by the determination coefficient (R^2, R^2_{CV}) and the root mean square error (RMSEC, RMSECV) of calibration and cross-validation, respectively (Næs et al., 2002).

4.5.2. Multivariate analysis of e-nose data (studies 2, 3, and 4)

In study 2, the recorded data of the electronic nose were analyzed with multivariate classification methods using AlphaSoft v12 (Alpha M.O.S., Toulouse, France). The DFA was applied to test the possibility of group identification based on the odor properties (Naes et al., 2002). Cross-validation was used to test the supervised classification models when sub-samples of a single sample were left out of the modelling iteratively and were used for testing the classification capability of the model. The classification models were evaluated based on the confusion matrices where hit rates and misclassifications were indicated.

In studies 3 and 4, the AlphaSoft v12 (Alpha M.O.S., Toulouse, France) was again used for multivariate data analysis approaches. The chromatogram peaks were used as sensor data representing the smell fingerprints of the milk samples. Data were analyzed with multivariate data classification methods: PCA and DFA, as unsupervised and supervised classification methods, respectively. The PCA was used to describe the general multidimensional patterns of e-nose data, and the DFA to establish the possibility of group identification based on odor properties of the milk and shake samples (Næs et al., 2002). The PLSR (Næs et al., 2002) was used to fit calibration models describing the relation of the odor signals and the concentration of S17-P100 food additive.

The accuracy of the DFA and PLSR models was tested with leave-one-out cross-validation, when a single record was left out of the modeling process and was used for testing by predicting its qualitative or quantitative properties; this process was repeated iteratively until all samples had been used for validation once (Næs et al., 2002). The sensor selection function of AlphaSoft was used to identify the most distinctive variables during the qualitative and quantitative approaches. In addition, DFA and PLSR

calculations based on the selected sensors were performed. The volatile compounds described by the selected sensors were identified using the AroChemBase database. The retention time, defined as the amount of time a compound spends in the column after it has been injected (Burian et al., 2010) was converted to Kováts retention index (RI) (Guardino et al., 1976). The most abundant compounds were also identified based on the retention indices using the AroChemBase (Alpha MOS, Toulouse, France) database.

5. PUBLICATIONS INCLUDED IN THE THESIS

5.1. Near-infrared spectroscopy for rapid evaluation of winter cereals and Italian ryegrass forage mixtures

Title	Near-infrared spectroscopy for rapid evaluation of winter cereals and Italian ryegrass forage mixtures
Authors	Haruna Gado Yakubu, Alemayehu Worku, Róbert Tóthi, Tamás Tóth , Szilvia Orosz, Hedvig Fébel, László Kacsala, Balázs Húth, Richárd Hoffmann, George Bazar
Journal	Animal Science Journal
Year Volume Issue	2023 94 1
Pages Quartile IF	1-13 Q2 2.000
Doi	10.1111/asj.13823

Received: 24 March 2022 | Revised: 15 December 2022 | Accepted: 17 February 2023

DOI: 10.1111/asj.13823

RESEARCH ARTICLE

Animal Science Journal WILEY

Near-infrared spectroscopy for rapid evaluation of winter cereals and Italian ryegrass forage mixtures

Haruna Gado Yakubu[1] | Alemayehu Worku[2] | Róbert Tóthi[1] | Tamás Tóth[3,4] | Szilvia Orosz[5] | Hedvig Fébel[6] | László Kacsala[1] | Balázs Húth[3] | Richárd Hoffmann[7] | George Bazar[4]

[1]Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvár, Hungary

[2]Department of Animal and Range Science, College of Agricultural Sciences, Arba Minch University, Arba Minch, Ethiopia

[3]Agricultural and Food Research Centre, Széchenyi István University, Győr, Hungary

[4]ADEXGO Kft., Balatonfüred, Hungary

[5]Livestock Performance Testing Ltd., Gödöllő, Hungary

[6]Nutrition Physiology Research Group, Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Herceghalom, Hungary

[7]Institute of Plant Production Sciences, Hungarian University of Agriculture and Life Sciences, Kaposvár, Hungary

Correspondence
George Bazar, ADEXGO Kft., 13 Lapostelki str., 8230 Balatonfüred, Hungary.
Email: bazar@agrilab.hu

Haruna Gado Yakubu, Hungarian University of Agriculture and Life Sciences, 40 Guba Sándor str., 7400 Kaposvár, Hungary.
Email: harunagado12@gmail.com

Funding information
Hungarian government and EU, Grant/Award Number: GINOP-2.3.4.-15-2016-00005

Abstract

Near-infrared (NIR) spectroscopy was employed to determine the differences between forage mixtures of winter cereals and Italian ryegrass and to evaluate fermentation characteristics of mixed silages. Forages were harvested on five phases (Cuts 1–5), with 1 week interval ($n = 100$). The yield of the last harvest (Cut 5) was ensiled and analyzed on four different days (D0, D7, D14, and D90) ($n = 80$). Principal component analysis based on the NIR data revealed differences according to the days of harvest, differences between winter cereals and Italian ryegrass forages, and differences in the fermentation stages of silages. The partial least square regression models for crude protein (CP), crude fiber (CF), and ash gave excellent determination coefficient in cross-validation ($R^2_{CV} > 0.9$), while models for ether extract (EE) and total sugar content were weaker ($R^2_{CV} = 0.87$ and 0.74, respectively). The values of root mean square error of cross-validation were 0.59, 0.76, 0.22, 0.31, and 2.36 % DM, for CP, CF, EE, ash, and total sugar, respectively. NIR proved to be an efficient tool in evaluating type and growth differences of the winter cereals and Italian ryegrass forage mixtures and the quality changes that occur during ensiling.

KEYWORDS
chemical composition, chemometrics, forage evaluation, NIR technology, quality assessment

1 | INTRODUCTION

Forages are the most important sources of dietary fiber for dairy cows (Wilkinson & Rinne, 2018). Forages that are harvested and fed to livestock over a period of time include hay and silage. Corn silage has become the major forage component in the ration of dairy cows over the last few decades especially in areas or climates where corn is well established or adapted (Allen et al., 2003). Corn is a suitable silage material because of the easiness to ensile and its high energy content (Allen et al., 2003). Crops such as winter cereals (Geren, 2014; Leão et al., 2017; Siefers et al., 1996), grasses (Amaral et al., 2020; Harrison et al., 1994; Rinne et al., 2020), and, especially,

ryegrasses (Hoffman et al., 1998; Soundharrajan et al., 2019; Yan et al., 2019) have also been extensively studied and proved to be good forage materials.

In the past years, forage mixtures have been found to provide significant nutritive value compared with whole-crop silages when used in animal feed formulation (Emile et al., 2005). Again, the poor establishment of corn in areas where climate change have impacted hugely has made it imperative to explore more about the potential of forage mixtures in those areas, in order to advance dairy nutrition (Worku, Tóth, et al., 2021; Worku, Tóthi, Orosz, Fébel, Kacsala, Húth, et al., 2021; Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, & Tóth, 2021). Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, and Tóth (2021) established that both Italian ryegrass and winter cereal mixtures were well fermented without the use of additives and therefore contributed to eliminating the cost of additives for dairy farmers. Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, and Tóth (2021) also established that the ensiled mixtures have high potentially degradable neutral detergent fiber (NDF) and effective protein degradability, which improved dry matter intake and milk production, which could increase the net returns of dairy farms.

Over these years, the nutritive qualities of forages have been analyzed by conventional methods, mainly, the proximate or Weende system of analysis. The system consists of the analytical determinations of water (moisture), crude ash, crude fat (ether extract [EE]), crude protein (CP), and crude fiber (CF). Nitrogen-free extract (NFE), more or less representing sugars and starches, is calculated by difference rather than measured by analysis. A newer method for evaluating the fiber fraction of feeds was developed in the 1960s by P.J. Van Soest. This system was developed because it was determined that CF did not accurately estimate the energy content of forages for ruminants. This method consists of measuring NDF and acid detergent fiber (ADF) fractions in forages.

The challenges associated with such conventional methods of forage evaluation include the need for reagents, long time for analysis, high cost, skilled labor needed to operate laboratory protocols, and potential differences or variation in results when standards are not followed. These challenges led to the development and application of a more rapid and cost efficient method of analysis such as the near-infrared (NIR) spectroscopy (Marten et al., 1984; Norris, 1985) to complement the existing conventional laboratory methods.

In the application of NIR to evaluate forage or silage qualities, the NIR region (800–2500 nm wavelength interval or 12,500–4000 cm^{-1} wavenumber interval) of the electromagnetic spectrum is usually achieved by shining NIR light of known intensity on the prepared forage or silage sample and measuring the intensity of the reflected or transmitted light, which gives data on the main chemical bonds (C–H, N–H, O–H, and C–O) in the analyzed samples (Marten et al., 1984; Norris, 1985; Yakubu et al., 2022), and by means of multivariate data analysis and chemometrics (Fearn, 2005; Martens, 2015), the data are evaluated qualitatively or quantitatively.

In its application, NIR has also been reported to have enabled the identification of faulty wet laboratory techniques used in silage or forage analysis (Shenk & Westerhaus, 1994). NIR has been employed to study the various nutritive composition of forages, characterization of the various forages used in forage mixtures, and silages (García & Cozzolino, 2006; Parrini et al., 2018; Vranić et al., 2020; Yakubu et al., 2022).

For the evaluation of the nutritive value of clover and oats mixed forages, Alomar et al. (2009) and Rushing et al. (2016) reported high determination coefficients of cross-validation models and low standard errors for CP, NDF, and ADF. Rukundo et al. (2020) also reported robust prediction equation for CP in composite animal feeds, while García and Cozzolino (2006) recorded high coefficient of determination and low standard error for CF (%) in broad based forage mixtures. In a similar analysis with herbage mixture, Parrini et al. (2018) reported good coefficients of determination with low error for acid detergent lignin (ADL). Like the above-mentioned studies, the development of NIR calibration or prediction equations to evaluate new forage will go a long way to improve quality evaluation of such mixtures. Although there is vast information about the applicability of NIR spectroscopy in feed analysis, it is still important to discover what information NIR may provide in the analysis of novel ensiled forage mixtures, especially the nutritive prediction, characterization of the main forage types included in mixtures, and the ensiling characteristics of these forages.

The aim of this research was to characterize forage mixtures (with or without Italian ryegrass) harvested at different growth stages, develop starter calibration models for predicting some essential nutritive values of the forage mixtures, and finally characterize the fermentation stages of the ensiled forage mixtures, by the means of NIR spectroscopy.

2 | MATERIALS AND METHODS

2.1 | Samples

The forages were cultivated on an experimental farm (Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Kaposvár, Hungary−46.3666687 N, 17.7999992 E, at an altitude of 153 m above sea level). The compositions of the four cultivated forage mixtures used in the study are commercial products that are used nowadays for cattle feeding in maize-based regions especially in the summer feeding period.

Mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat. Mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat. Mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat. Mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

The percentages of the forage mixtures' compositions reflect the seed weight ratio of the various winter cereals and Italian ryegrasses used in the study. The producer of the mixtures was Agroteam S.p.a., Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy.

The experimental field allotted 3 ha to each mixture. Before sowing on sandy soil, 351 kg/ha of artificial fertilizer (NPK: 16:16:16) was applied to improve the fertility of the soil. The seedbed was prepared with a cultivator. The four different forage mixtures were sown on September 29, 2018 (sowing rate of 75 kg seed/ha for each mixture) at a depth of 3 cm by a seed drill. Plant protection treatment was not applied during the growing season. The precipitation was 288 mm from September 2018 to April 2019. Cutting at a 10 cm stubble height was carried out on different stages based on the existing extended BBCH scale which is a standard code system used to describe the phenological growth stages of plants (Meier et al., 2009).

The four forage mixtures (Mixtures A–D) were harvested on five phases, with 1 week interval between each harvest (Cuts 1–5). Harvesting dates and phenological stage were Cut 1, 2019.04.03. (BBCH: 32–33); Cut 2, 2019.04.10. (BBCH: 33–34); Cut 3, 2019.04.17. (BBCH: 35–38); Cut 4, 2019.04.24. (BBCH: 39–45); Cut 5, 2019.04.30. (BBCH: 49–58).

At each harvest, five samples (average weight of samples: 510 g) of each of the four mixtures were collected on the field, totaling 20 samples per each cut. The total number of samples at the end of the harvest period was 100 (20 by 5).

Forage samples were stored frozen at −20°C in sealed polyethylene bags until being processed for NIR measurements. Samples were dried at 60°C until mass constancy using a Memmert UFE400 oven (Memmert GmbH, Buechenbach, Germany), and the dried samples were ground with a Retsch Rotor Beater Mill SR 200 with a bottom sieve of 1 mm aperture size (Retsch GmbH, Haan, Germany).

Wilted and chopped materials of 510 g were packed into laboratory silos using a mechanical hand packer into anaerobic glass jars capacity of 0.72 L (729 kg/m^3). After ensiling 0, 7, 14, and 90 days, five laboratory silos per mixtures (A–D) were opened, and samples of silages were stored frozen at −20°C in sealed polyethylene bags. Samples were thawed and stored at 4°C for 24 h until being measured with NIR spectroscopy in fresh or moist form, without grinding the chopped samples. Then, the silages were dried and ground analogously to the forages, and NIR spectra of the dried silages were also recorded.

The samples used in this study have also been applied in different studies (Worku, Tóth, et al., 2021; Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, & Tóth, 2021) which evaluated the nutritive value of the silage mixtures and aroma profiling using electronic nose.

2.2 | Chemical analyses

The CP, EE, crude ash, CF, and total sugar were determined according to Association of Official Analytical Chemist (2006). The statistical evaluation of the chemical data was performed with one-way analysis of variance (ANOVA) including Tukey's post hoc test to check the group differences, using the SPSS v26 (IBM Corp., Armonk, USA).

2.3 | Measurement with NIR spectroscopy

In all cases, samples were at room temperature (25°C) during the NIRS measurements. The frozen silages ($n = 80$) were thawed at constant ambient room temperature (25°C), and no additional chopping was performed before scanning. The reflectance NIR spectra ($\log R^{-1}$) of the moist silages were recorded with a FOSS DS2500 spectrometer (FOSS Analytical A/S, Hillerod, Denmark) in the range of 400–2500 nm, at 2 nm spectral steps, with 8 nm nominal bandpass, as the average of 32 successive scans. To avoid spectral variation caused by heterogeneity of the silages, the large cup (FOSS 60056582) was rotated eight times during the successive scans, and the acquired spectrum of an individual sample was the average of spectra of two refilled subsamples. FOSS Mosaic Solo v.8.0.4.10 (FOSS Analytical A/S, Hillerod, Denmark) and FOSS ISIScan Nova (FOSS Analytical A/S, Hillerod, Denmark) software packages were used for the operation of the spectrometer and data acquisition.

The NIR spectra of the dried and ground forages ($n = 100$) and ensiled forages ($n = 80$) were collected in reflectance mode using a NIRSystems 6500 spectrometer (FOSS NIRSystems, Laurel, MD, USA) equipped with a sample transport module and small ring cup (FOSS IH-0307). $\log R^{-1}$ spectra were recorded in the 400–2500 nm range with 2 nm spectral step. The WinISI II version 1.5 spectral analytical software (InfraSoft International LLC, State College, PA, USA) was utilized for the operation of the spectrometer and acquiring data. Each sample was scanned twice applying one refilling, and the average spectrum of the two scans was saved for further data evaluation.

2.4 | Multivariate data analysis

The multivariate data of the NIR spectroscopic measurements were exported from WinISI and Mosaic software packages in text file format and The Unscrambler 11.0 (CAMO Analytics AS, Oslo, Norway) was applied for data processing and analysis. Moving average smoothing was applied with 5 spectral points before further data pre-treatments. In general, spectra treatments are done to overcome problems associated with light scattering by samples which were measured by reflectance and other spectrum base-line-affecting phenomena and therefore does not affect the chemical information carried by the spectra (Fearn, 2009). Standard normal variate (SNV) transformation (Barnes et al., 1989) and second-order Norris derivatives with 5-point gap (Hopkins, 2001; Norris, 2001) were calculated to decrease additive and multiplicative effects of light scatter. Principal component analysis (PCA) (Cowe & McNicol, 1985) was used to describe the basic multidimensional characteristics of the NIR data matrix and to visualize the differences of the sample groups. Calibrations for the chemical constituents were conducted using partial least squares (PLS) regression, and models were tested with full cross-validation, also known as leave-one-out cross-validation, where in an iterative process the spectrum of each sample was omitted from the calibration set once, and the model was tested on the samples left out (Næs et al., 2017). The precision and accuracy of the chemometric models were evaluated by

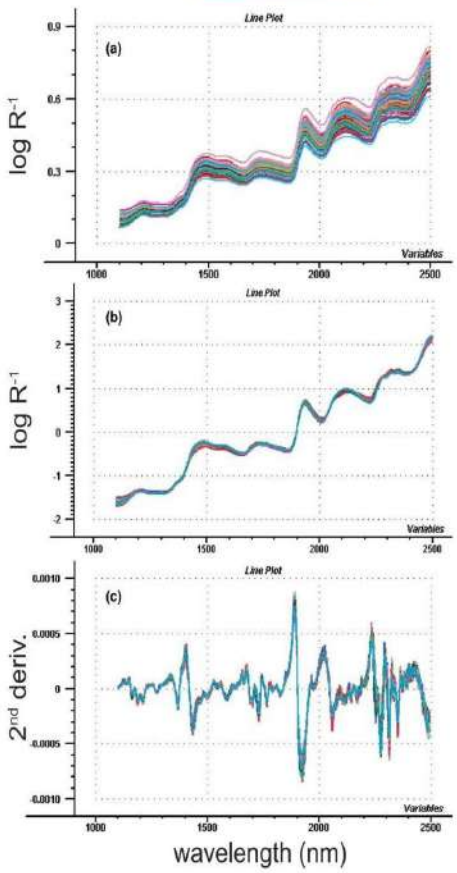


FIGURE 1 (a) Raw, (b) SNV-corrected, and (c) second derivative near-infrared reflectance spectra of the dried forages (Mixtures A–D) harvested on five different dates (Cuts 1–5) ($n = 100$).

the determination coefficient (R^2, R^2_{CV}) and the root mean square error (RMSEC, RMSECV) of calibration and cross-validation, respectively (Næs et al., 2017). The number of latent variables (LVs) (Schneeweiss, 1991) within the calibration models was increased until reaching the minimal RMSECV; at the same time, it was maximized at 10 to avoid overfitting.

3 | RESULTS AND DISCUSSION

3.1 | Harvesting study

The raw spectra obtained from the measurement of the dried and ground forages ($n = 100$) within 1100–2500 nm NIR region are shown in Figure 1a, while the result of the application of spectral pre-treatments, standard normal variant (SNV), and second derivative are represented in Figure 1b,c, respectively. The application of the spectral pre-treatments resulted in the reduction of the variation in the y axis of the raw spectra. Figure 1c revealed absorption bands in the NIR region of the spectrum at 1450 and 1930 nm related to the O—H (moisture) stretch first overtone and combination bands, respectively (Yang et al., 2017), at 1725 nm related to the C—H (fiber fraction) stretch first overtone (Yang et al., 2017), and at 2300 nm related to C—H combination band (Workman & Weyer, 2012). The N—H (protein) absorption occurs at 1590 and 2100 nm (Cozzolino et al., 2001; Murray, 1986; Yang et al., 2017).

The PCA score plot of the dried forages shows group dependent variations. In Figure 2a, where the harvesting groups are indicated, Cuts 1 and 2 clustered in the negative region of PC2, whereas Cuts 4 and 5 clustered within the positive region of PC2. Scores of Cut 3 samples dispersed within the above-mentioned two clusters. This can be related to the stage of maturity and chemical composition of the plants at this phase of growth. The third cut in Mixtures A and B was closer to a less mature stage, while in Mixtures C and D, the third cut was closer to a more mature stage as observed by Worku, Tóth, et al. (2021) in an earlier study involving the nutritional composition of these forage mixtures at different phenological phases (Table 1). Cut 3 represented the stage where the samples containing Italian ryegrass separated the most from those containing winter cereals only as also demonstrated by Figure 2b showing the same PCA scores colored by the four different mixtures. Separation of the groups is along PC1, as Mixtures A and B are clustered in the negative region, while forage mixtures containing Italian ryegrass (Mixtures C and D) are in the positive region of PC1. Figure 2c shows the loadings of PC1 and PC2, indicating that protein-related N—H absorptions (1450–1650 and 1900–2200 nm) are dominating PC1, while spectral regions representing fiber (C—H) are dominant in PC2 (1600–1800 and 2200–2400 nm), as described by Yang et al. (2017), Workman and Weyer (2012), and Murray (1986). These observations can be related to the changes of chemical composition during that phase of harvest or cut. Chemical composition of the plant is different in the different stages of plant growth. In the boot stage, energy percentage and CP are highest but dry matter yield is low, and as forage approaches to mid to early bloom, the chemical composition differs significantly from the early growth stage, whereas fiber fraction (NDF, ADF, and ADL) may increase in the late maturity or final phase of growth (Johnston et al., 1998). Maturity level affects the chemical composition of forages more than other factors (Johnston et al., 1998).

Figure 2a,b highlights that the biggest difference between mixtures containing Italian ryegrass (Mixtures C and D) and not containing it (Mixtures A and B) appeared in the time of the third cut.

A PCA was performed with the NIR spectroscopic data of the four mixtures at the fifth harvesting day (Figure 3a) to compare the forages at the stage of harvesting for ensiling. The mixtures containing Italian ryegrass (Mixtures C and D) again clustered separately from those containing winter cereals only (Mixtures A and B), similarly as observed in the previous PCA (Figure 2b). Figure 3c shows the scores of the different harvesting days (Cuts 1–5) of Mixture D, the five different harvesting days separated. Although all cuts are different, Cuts 1 and 2 differ from Cuts 4 and 5 along PC1, while Cut 3 is different

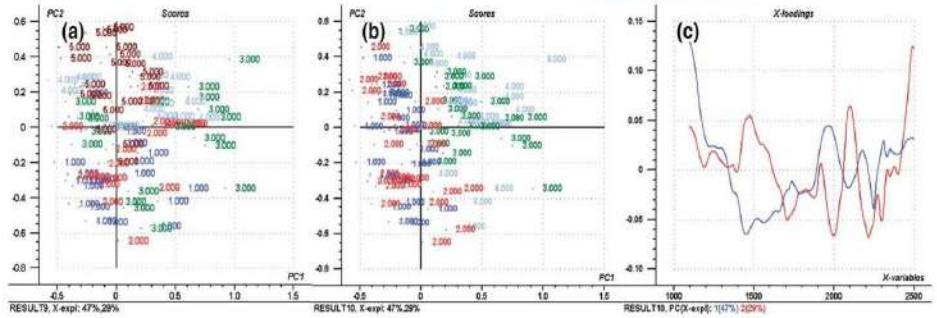


FIGURE 2 PCA score plots of the dried forages calculated from the SNV-corrected NIR spectra (1100–2500 nm) colored (a) by the five harvesting days (1: Cut 1, 2: Cut 2, 3: Cut 3, 4: Cut 4, 5: Cut 5) or (b) by the four mixtures (1: Mixture A, 2: Mixture B, 3: Mixture C, 4: Mixture D) and (c) the loading vectors of the first two principal components (PC1 and PC2) highlighting the dominant spectral regions responsible for the distribution of samples along PC1 and PC2 in the score plots.

TABLE 1 Crude fiber (%DM) composition of forage mixtures at five (5) cutting phases ($n = 100$) adapted and extended from Worku, Tóth, et al. (2021).

	Mixture A	Mixture B	Mixture C	Mixture D
Cut 1	15.89 ± 0.379^{a}	17.33 ± 0.342^{a}	17.15 ± 0.682^{a}	17.31 ± 1.289^{a}
Cut 2	20.97 ± 0.892^{b}	22.07 ± 0.560^{b}	22.16 ± 1.769^{b}	21.29 ± 1.033^{b}
Cut 3	21.80 ± 1.344^{b}	22.93 ± 0.427^{b}	$23.06 \pm 0.341b^{c}$	23.33 ± 1.365^{c}
Cut 4	24.79 ± 0.203^{c}	26.00 ± 0.943^{c}	25.78 ± 1.358^{c}	24.24 ± 0.265^{c}
Cut 5	29.84 ± 0.207^{d}	31.38 ± 0.465^{d}	29.20 ± 2.473^{d}	27.90 ± 0.485^{d}

Note: Mean values of crude fiber ± standard deviation with different superscripts within columns show differences within mixtures on cutting days ($P < 0.05$).

from the rest along PC2. Figure 3d shows that the difference described by PC1 is influenced by water (O—H, 1450 and 1950 nm) and fiber (C—H, 1730 and 2300 nm) absorptions, while PC2 is dominated by fiber (C—H, 1730 and 2300 nm) absorptions. Mixture D had different CF content at Cut 3 compared with Cut 1, 2, and 5; however, there was no significant difference ($P > 0.05$) between Cuts 3 and 4 (Worku, Tóth, et al., 2021) (Table 1).

Table 2 represents the descriptive statistics of the forage chemical data used as reference data in the calibration and cross-validation process. The RMSEC and the RMSECV values of Table 2 were compared with the SD of Table 2 and together with the coefficient of determination (R^2), decision on best models was taken.

To establish a relationship between the NIR data and the chemical laboratory data (reference data) of the forage mixtures, PLS regression models were built using the 1100–2500 nm regions of the measured spectra. The optimal calibration equation between reference data and NIR data was chosen based on the highest R^2_{CV}, smallest RMSECV.

The results of the best PLS models showed in Table 3 were built by applying a second derivative math treatment to the NIR data. The least precise model was built for the total sugar content, even though that was the constituent with the largest range. The R^2 and R^2_{CV} for the other constituents were greater than 0.9, except the R^2_{CV} for EE (0.87). The RMSECV ranged between 0.2 and 2.4, lower than the standard deviations (SDs) of the reference chemical data and with LV values (3–7) lower than 1/10th of the total sample number used in the study, representing robust models. These results were similar to what Acosta et al. (2020) and Alomar et al. (2009) reported for CP, García and Cozzolino (2006) for CF, and Cozzolino et al. (2001) for ash in NIR silage quality prediction studies.

The calibration and cross-validation regression lines (Y-fit) of CP and CF models are shown in Figure 4. The black diagonal line shows the optimal Y-fit, while blue and red lines show the calibration and cross-validation Y-fits, respectively. The blue dots show the NIR predicted composition values of samples during calibration in the function of the laboratory reference values, and red dots show the NIR predicted values at cross-validation testing, again, in the function of the reference values measured in the chemical lab. The closer the dots are to the regression line and the less the regression line deviates from the optimal Y-fit, the better the calibration model.

3.2 | Fermentation study

Figure 5 shows various spectral pre-processing or math treatments applied to the spectra data ($N = 80$) obtained from the silage mixtures

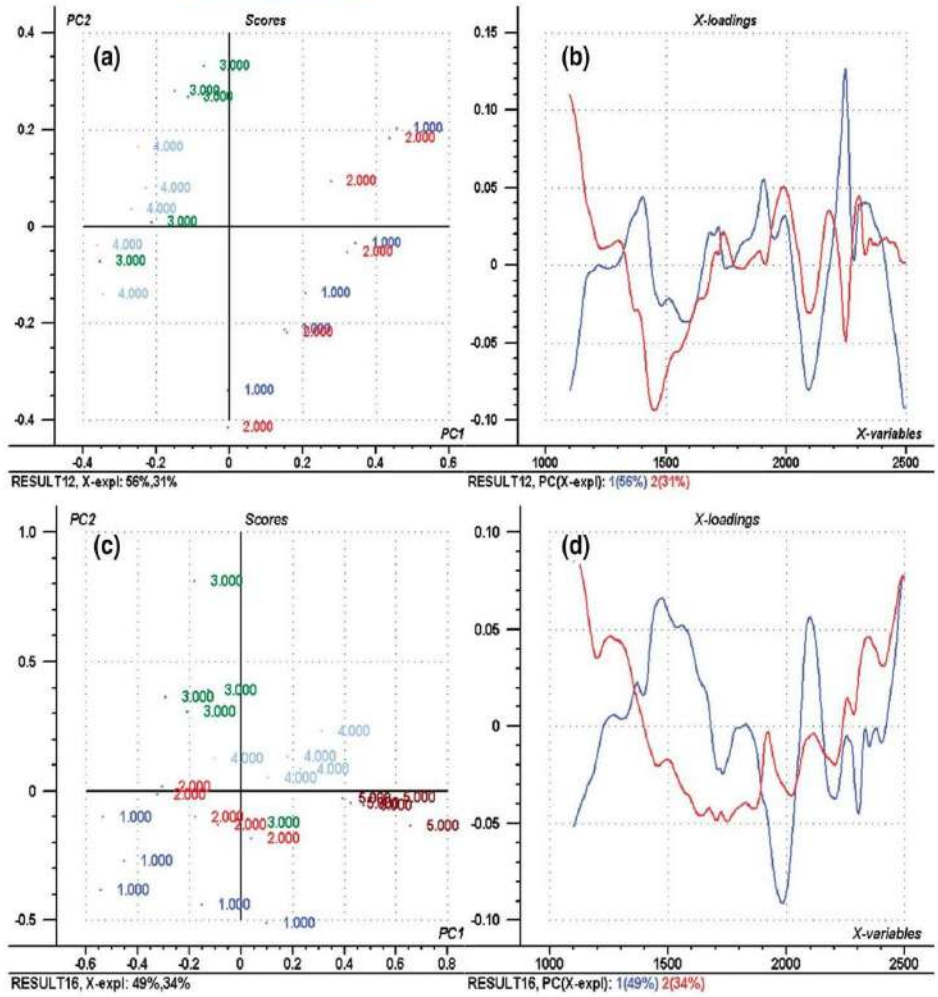


FIGURE 3 (a,c) PCA score plots calculated from the SNV-corrected NIR spectra (1100–2500 nm) showing (a) the clustering of samples of the four mixtures (1: Mixture A, 2: Mixture B, 3: Mixture C, 4: Mixture D) on the fifth harvesting day ($n = 20$) and (c) the clustering of Mixture D samples according to the five harvesting days ($n = 25$) and (b,d) the loading vectors of the first two principal components defining the planes of the score plots.

TABLE 2 Parameters of the forage chemical data used in calibration and cross-validation.

Constituent (%DM)	Min value	Max value	Mean	SD
CP	10.3	23.1	15.2	2.71
CF	15.5	27.5	21.6	3.18
EE	2.00	4.72	3.39	0.60
Ash	7.03	11.5	9.00	1.18
Total sugar	8.12	30.5	19.2	4.45

Abbreviations: CF, crude fiber; CP, crude protein; EE, ether extract; Max value, maximum value of reference dataset; Min value, minimum value of reference dataset; SD, standard deviation of reference dataset.

in the moist (Figure 5a,c,e) and dried (Figure 5b,d,f) states, respectively. The spectra obtained from the moist silages revealed sharp water absorption bands (O–H) or peaks at 1450 and 1950 nm at the first overtone and combination band, respectively (Murray, 1986; Workman & Weyer, 2012; Yang et al., 2017). The different math treatments also reduced the influence of spectroscopic noise on the NIR spectral data obtained (Fearn, 2009).

From Figure 6, in wet samples, only the D0 is different, the others (D7, D14, and D90) did not separate within the plane described by the first two principal components (PCs) that described 96% of the total variance of the NIR spectral data. The absorption regions of water are dominant in the loadings responsible for the two PCs. This

TABLE 3 The PLSR calibration and cross-validation results for the chemical constituents of the dried forages (n = 100) on dry weight basis (%) with second derivative (smoothing 5, gap 5) pre-treatment.

Constituent (%DM)	LV	R^2	RMSEC (%)	R^2_{CV}	RMSECV (%)
CP	3	0.96	0.57	0.95	0.59
CF	3	0.95	0.70	0.95	0.76
EE	7	0.93	0.16	0.87	0.22
Ash	6	0.96	0.25	0.93	0.31
Total sugar	3	0.77	2.19	0.74	2.36

Abbreviations: CF, crude fiber; CP, crude protein; EE, ether extract; LV, number of latent variables in the PLSR calibration model; R^2, determination coefficient of calibration; R^2_{CV}, determination coefficient of cross-validation; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross-validation.

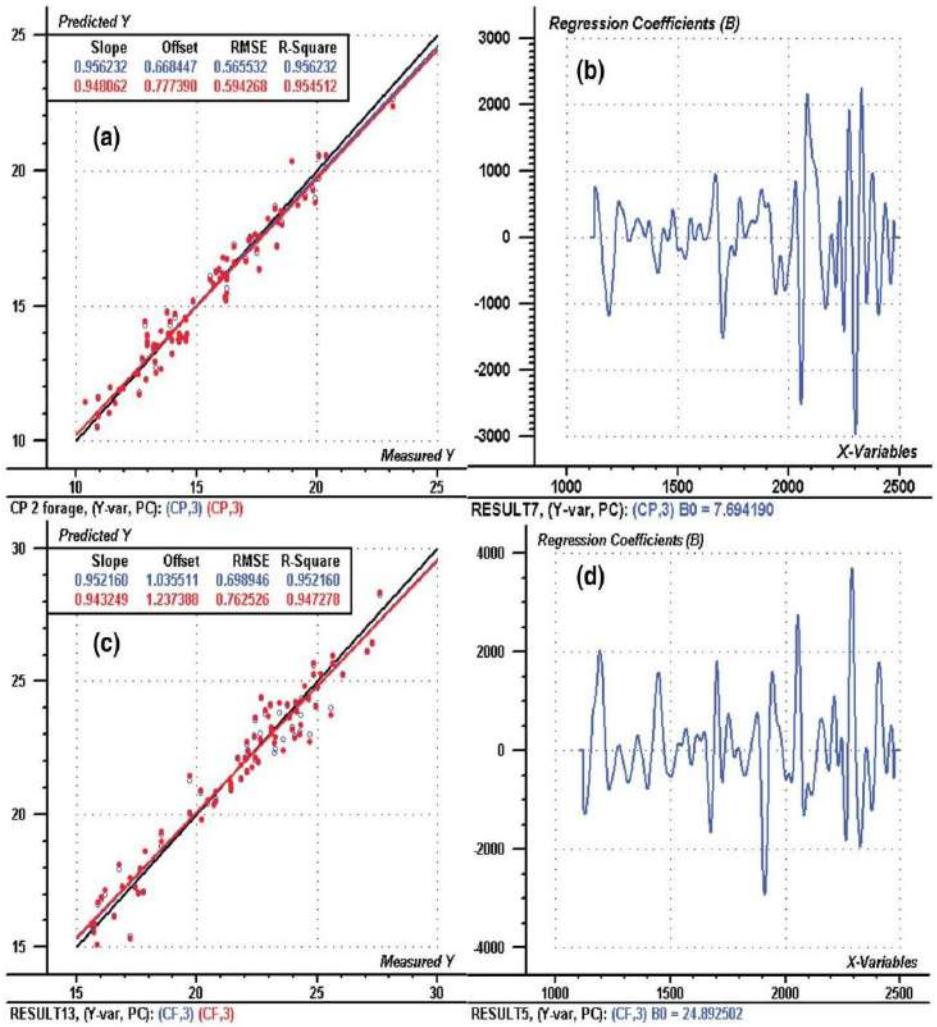


FIGURE 4 The Y-fit graphs of the PLSR models calibrated on the (a) crude protein and (c) crude fiber content of the dried forages with the regression coefficient vectors (b and d, respectively) highlighting the spectral regions exploited in the relevant calibrations.

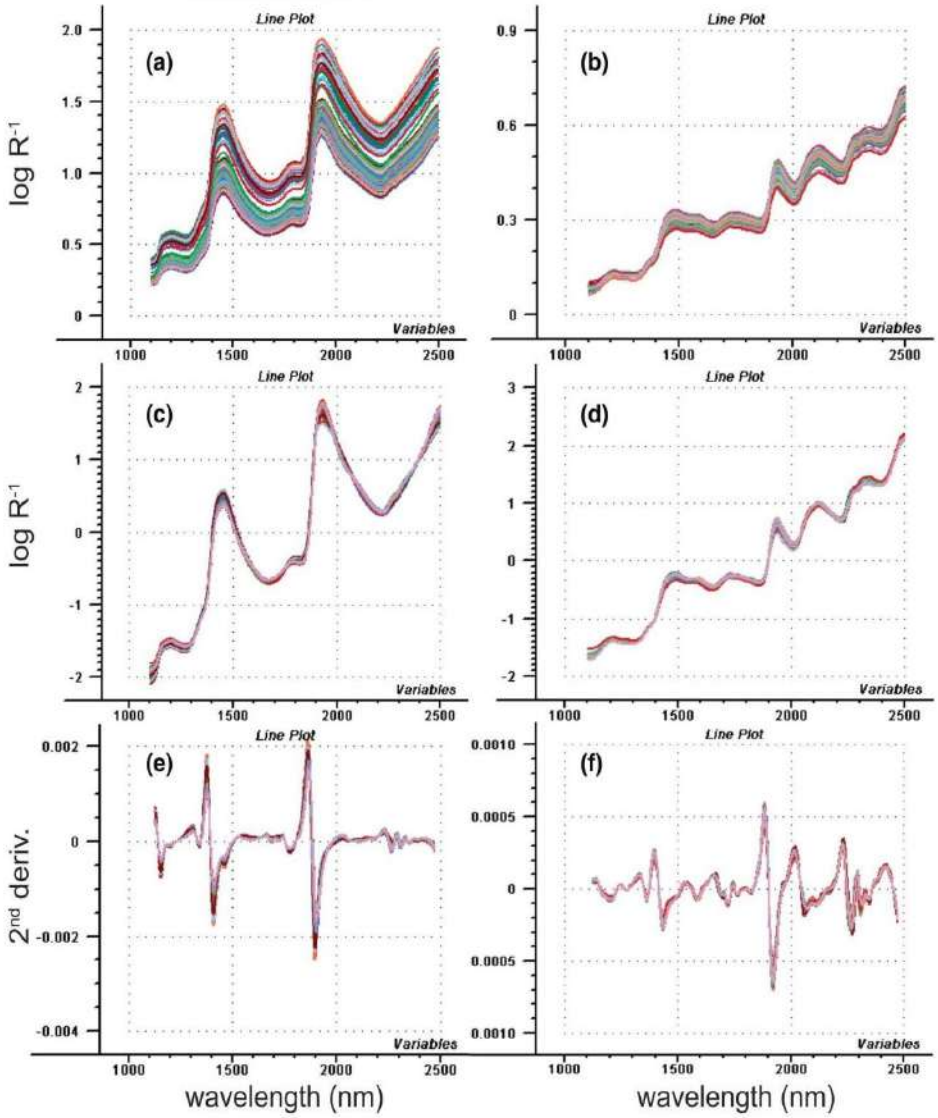


FIGURE 5 (a,b) Raw, (c,d) SNV-corrected, and (e,f) second derivative spectra of the (a,c,e) wet and (b,d,f) dried silages, containing data of the four mixtures (A–D) and 4 days of fermentation (D0, D7, D14, and D90).

reflects that the biggest difference seen in the wet samples was related to the change of water content or the quality of water (i.e., changes in water soluble content) between the initial stage and the sampling days during the fermentation. The characteristics of water and its spectral pattern did not change significantly during the fermentation (D7, D14, and D90); therefore, the NIR spectra of silages dominated by water absorption did not show age-dependent pattern. In the dry samples, however, the different fermentations days separated within the PCA. The separation of D0 occurred in both moist and dried silage samples.

To observe the characteristics of the silages from early phase of fermentation to the last phase, Days 0 and 90 were compared. From Figure 7a, it could be observed that all silage mixtures were similar on Day 0 of the fermentation. At Day 90 (Figure 7c), Mixtures A and C

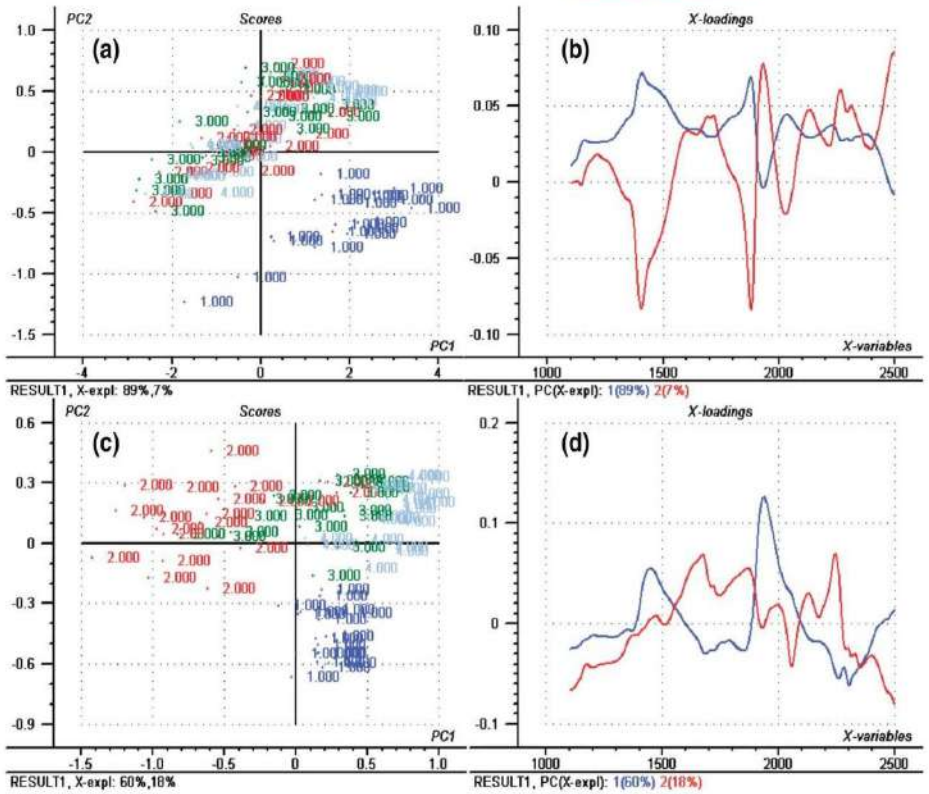


FIGURE 6 PCA score plots calculated from the SNV-corrected NIR spectra (1100–2500 nm) of the (a) wet and (c) dried silages indicating the fermentation days (1: Day 0; 2: Day 7; 3: Day 14; 4: Day 90) and (b,d) the loading vectors of the first two principal components defining the planes of the score plots.

clustered together, while Mixtures B and D were different. For most winter cereals and ryegrasses, complete fermentation could be achieved on Day 60, from first fermentation (Mohd-Setapar et al., 2012), this perhaps explains why complete separation of the silage mixtures were observed on Day 90 of the fermentation. The water (O—H) absorbance or peaks at 1450 and 1950 nm (Murray, 1986) were pronounced in both Days 0 and 90, explaining a possible stable moisture levels of the silages from the initial phase to final phase of fermentation. In Figure 7c, Mixture D shows difference from the rest of the samples along PC2; this is dominated by fiber absorptions (Figure 7d, 1700 and 2300 nm regions). As already reported by Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, and Tóth (2021), at Day 0, the CF content of Mixture D was significantly different from other opening days, with the rest being similar in CF content. Again, at Day 90, a high CF content that was associated with high pH and low lactic acid concentration 4.08 (%DM) was recorded (Worku, Tóthi, Orosz, Fébel, Kacsala, Vermeire, & Tóth, 2021), probably caused by restricted fermentation which mostly result in low acidification (Kung, 2018). In a related study by Worku, Tóth, et al. (2021) that evaluated the aroma profile of the same ensiled forages by means of electronic nose, Mixture D was similarly found to have different aroma profile when compared with the other mixtures at the end of the 90-day fermentation. The winter cereal mixture silages (Mixtures A and B) had similar aroma profile, as Mixture C was closer to that of Mixtures A and B, based on the PCA score plot and linear discriminant analysis. Overall, the electronic nose results showed that the mixture of Italian ryegrass and winter cereals (Mixture D) was significantly different from the mixture of Italian ryegrass and oats (Mixture C) and the mixtures without Italian ryegrass (Mixtures A and B). The characteristic difference of Mixture D could also be observed in this current study employing NIR spectroscopy.

The PCA of the dried silage samples (Figure 8) showed clear separation of Mixtures A and B from Mixtures C and D, both at the beginning (Day 0) and the end (Day 90), along PC1 in both plots. From the loading plots (b,d), it could be observed that sugar content (C—H bonds, at 2350 nm) (Workman & Weyer, 2012) was the most prominent component causing the difference between the two clusters, not including and including Italian ryegrass (Mixtures A and B and Mixtures C and D, respectively). As confirmed by the chemical analysis, Mixture B had significantly lower CF and NDF content on Day 90, compared with Mixtures A, C, and D (Worku, Tóth, et al., 2021).

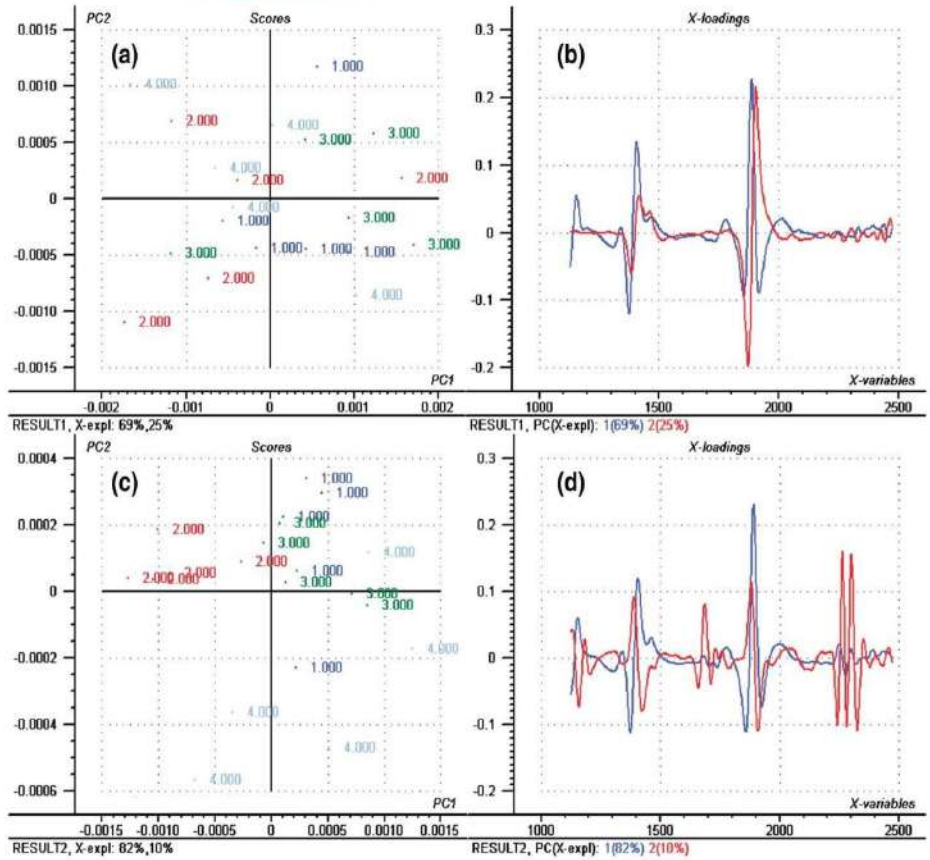


FIGURE 7 (a,c) PCA score plots and (b,d) corresponding loading plots calculated from the second derivative NIR spectra (1100–2500 nm) of the moist silages (1: Mixture A; 2: Mixture B; 3: Mixture C; 4: Mixture D) at (a,b) the beginning (Day 0) and (c,d) the end (Day 90) of the fermentation trial.

In this study, NIR coupled with chemometrics have proven to be efficient in the analysis of qualitative and quantitative qualities of forage mixtures and qualitative evaluation of ensiled forage mixtures. In the qualitative analysis, the application of chemometric tools such as PCA to forage NIR data revealed the differences between forages of different composition, harvested at different phases. Loadings of the PCAs revealed associated absorption bands responsible for the differences in the fresh and ensiled forage mixtures, mostly O—H, N—H, and C—H, representing moisture, protein, and fiber or sugar fractions, respectively. NIR spectroscopy also revealed differences of forages mixtures at the transitional maturity phase, as assessed based on the phase of harvest.

The application of PLS modeling between NIR data and chemical data of forages mixtures resulted in models for predicting CP, CF, EE, ash, and total sugar in dried forage mixtures. NIR range of 1100–2500 nm treated with second derivative (smoothing 5, gap 5) gave robust models in predicting nutritive composition of the forage mixtures.

Compared with earlier studies mentioned in Section 1, which predicted the nutritive value of forage mixtures, this current research analyzed the quality changes in growth (harvested or cut stages) of the forage mixtures and the quality changes during different fermentation phases of the forage mixtures. The findings of the NIRS analysis agree with those of the previous electronic nose measurements (Worku, Tóth, et al., 2021), that is, the mixture silage of winter cereals and Italian ryegrass (Mixture D) differed both from the mixture of oats and Italian ryegrass (Mixture C) and from the mixtures of winter cereals without Italian ryegrass (Mixtures A and B). The findings of the NIRS and electronic nose results could not be clearly confirmed with the chemical data (no significant differences in major chemical components were found), but the fact of similarities of the results of these independent analyses is reassuring. The benefit of these nontargeted rapid analytical approaches lay especially in the ability to identify complex patterns causing differences that might be difficult or impossible to find with targeted analyses.

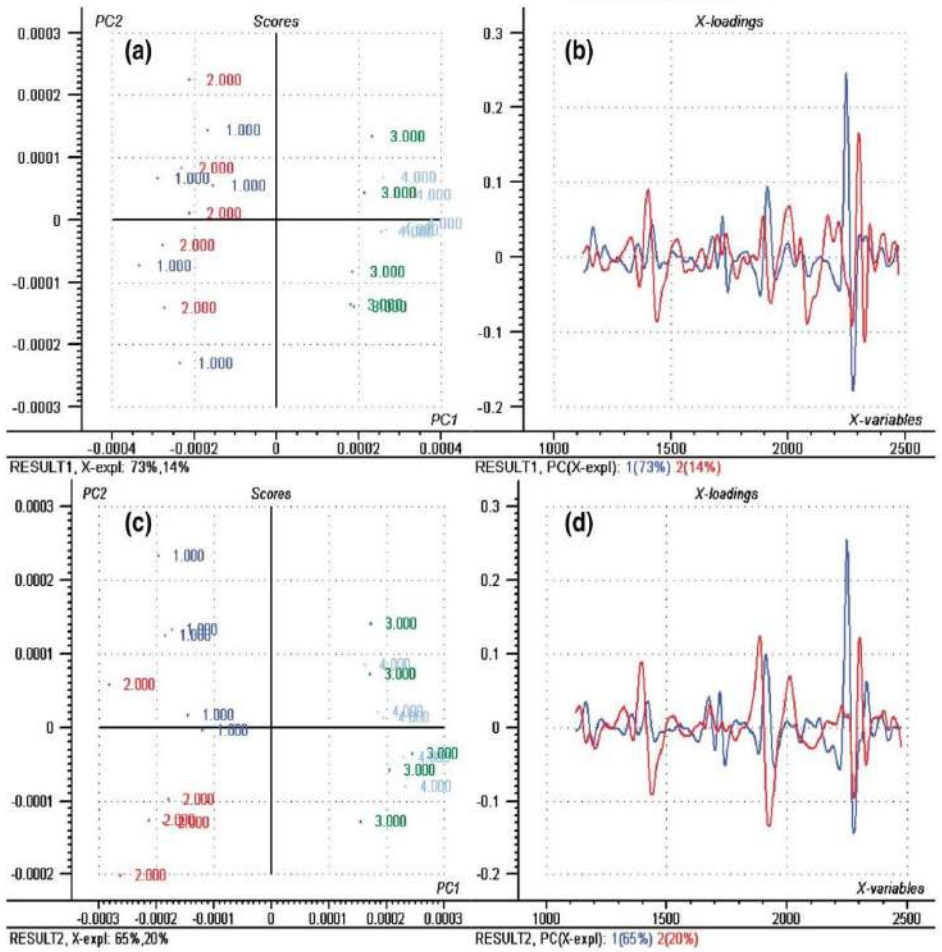


FIGURE 8 (a,c) PCA score plots and (b,d) corresponding loading plots calculated from the second derivative NIR spectra (1100–2500 nm) of the dried silages (1: Mixture A; 2: Mixture B; 3: Mixture C; 4: Mixture D) at (a,b) the beginning (Day 0) and (c,d) the end (Day 90) of the fermentation trials.

ACKNOWLEDGMENTS

The authors gratefully acknowledge research funding support from the Hungarian government and EU (Project No. GINOP-2.3.4.-15-2016-00005).

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

ORCID

Haruna Gado Yakubu https://orcid.org/0000-0002-9606-7140
Tamás Tóth https://orcid.org/0000-0003-4457-1473
Hedvig Fébel https://orcid.org/0000-0002-9867-0664
George Bazar https://orcid.org/0000-0001-9829-4366

REFERENCES

Acosta, J. J., Castillo, M. S., Hodge, G. R., & Resources, E. (2020). Comparison of benchtop and handheld near infrared spectroscopy devices to determine forage nutritive value. *Crop Science*, *60*, 3410–3422. https://doi.org/10.1002/csc2.20264

Allen, M. S., Coors, J. G., & Roth, G. W. (2003). Corn silage. In D. R. Buxton, R. E. Muck, & J. H. Harrison (Eds.), *Silage science and technology* (42nd ed.). Agronomy Monograph. (pp. 547–608). American Society of Agronomy. https://doi.org/10.2134/agronmonogr42.c12

Alomar, D., Fuchslocher, R., Cuevas, J., Mardones, R., & Cuevas, E. (2009). Determination of quality parameter of pasture by NIR reflentance spectroscopy. *Chilean Journal of Agricultural Research*, *69*(2), 198–206. https://doi.org/10.4067/S0718-58392009000200009

Amaral, R. C., Carvalho, B. F., Costa, D. M., Morenz, M. J. F., Schwan, R. F., & Ávila, C. L. D. S. (2020). Novel lactic acid bacteria strains enhance the conservation of elephant grass silage cv. BRS

Capiaçu. *Animal Feed Science and Technology*, *264*(July 2019), 114472. https://doi.org/10.1016/j.anifeedsci.2020.114472

Association of Official Analytical Chemist. (2006). *Official methods of analysis* (18th ed.). AOAC International.

Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy*, *43*(5), 772–777. https://doi.org/10.1366/0003702894202201

Cowe, I. A., & McNicol, J. W. (1985). The use of principal components in the analysis of near-infrared spectra. *Applied Spectroscopy*, *39*(2), 257–266. https://doi.org/10.1366/0003702854248944

Cozzolino, D., Fassio, A., & Gimenez, A. (2001). The use of near-infrared reflectance spectroscopy (NIRS) to predict the composition of whole maize plants. *Journal of the Science of Food and Agriculture*, *81*(1), 142–146. https://doi.org/10.1002/1097-0010(20010101)81:1<142::AID-JSFA790>3.0.CO;2-I

Emile, J., Jacobs, F., Al Rifaï, M., Le Roy, P., & Faverdin, P. (2005). Triticale and mixtures silages for feeding dairy cows. *Grassland Science in Europe*, *13*, 804–806.

Fearn, T. (2005). Chemometrics: An enabling tool for NIR. *NIR News*, *16*(7), 17–19. https://doi.org/10.1255/nirn.856

Fearn, T. (2009). The effect of spectral pre-treatments on interpretation. *NIR News*, *20*(6), 15–16. https://doi.org/10.1255/nirn.1146

García, J., & Cozzolino, D. (2006). Use of near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration models Uso de la espectroscopía de reflectancia en el infrarrojo cercano (NIR) para predecir la composición química de forrajes e. *Agricultura Tecnic (Chile)*, *66*(1), 41–47.

Geren, H. (2014). Dry matter yield and silage quality of some winter cereals harvested at different stages under Mediterranean climate conditions. *Turkish Journal of Field Crops*, *19*(2), 197–202. https://doi.org/10.17557/tjfc.55884

Harrison, J. H., Blauwiekel, R., & Stokes, M. R. (1994). Fermentation and utilization of grass silage. *Journal of Dairy Science*, *77*(10), 3209–3235. https://doi.org/10.3168/jds.S0022-0302(94)77264-7

Hoffman, P. C., Combs, D. K., & Casler, M. D. (1998). Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. *Journal of Dairy Science*, *81*(1), 162–168. https://doi.org/10.3168/jds.S0022-0302(98)75563-8

Hopkins, D. W. (2001). What is a Norris derivative? *NIR News*, *12*(3), 3–5. https://doi.org/10.1255/nirn.611

Johnston, J., McKinlay, J., & Wheeler, B. (1998). *Forage production from spring cereals and cereal-pea mixtures*. OMAF Publication.

Kung, L. (2018). Silage fermentation and additives. *Latin American Archives of Animal Production*, *26*(3), 61–66.

Leão, G. F. M., Jobim, C. C., Neumann, M., Horst, E. H., Dos Santos, S. K., Venancio, B. J., & Santos, L. C. (2017). Nutritional composition and aerobic stability of winter cereal silage at different storage times. *Acta Scientiarum - Animal Sciences*, *39*(2), 131–136. https://doi.org/10.4025/actascianimsci.v39i2.34270

Marten, G. C., Brink, G. E., Buxon, D. R., Halgerson, J. L., & Hornstein, J. S. (1984). Near infrared reflectance spectroscopy analysis of forage quality in four legume species. *Crop Science*, *24*(6), 1179–1182. https://doi.org/10.2135/cropsci1984.0011183X002400060040x

Martens, H. (2015). Quantitative Big Data: Where chemometrics can contribute. *Journal of Chemometrics*, *29*(11), 563–581. https://doi.org/10.1002/cem.2740

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Heß, M., Lancashire, D. P., Schnock, U., Reinhold, S., van den Boom, T., Weber, E., & Zwerger, P. (2009). The BBCH system to coding the phenological growth stages of plants—History and publications. *Journal für Kulturpflanzen*, *61*(2), 41–52.

Mohd-Setapar, S. H., Abd-Talib, N., & Aziz, R. (2012). Review on crucial parameters of silage quality. *APCBEE Procedia*, *3*(May 2012), 99–103. https://doi.org/10.1016/j.apcbee.2012.06.053

Murray, I. (1986). The NIR spectra of homologous series of organic compounds. In *Proceedings of the International NIR/NIT Conference* (pp. 13–28). Akadémiai Kiadó.

Næs, T., Isaksson, T., Fearn, T., & Davies, T. (2017). *A user-friendly guide to Multivariate Calibration and Classification* (2nd ed.). IM Publications LLP. https://doi.org/10.1255/978-1-906715-25-0

Norris, K. H. (1985). Definition of NIRS Analysis, Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. In G. C. Marten, J. S. Shenk, & F. E. Barton, II (Eds.), *USDA Agriculture Handbook No. 634* (1st ed.) (p. 6). United Stated Department of Agriculture.

Norris, K. H. (2001). Applying Norris derivatives. Understanding and correcting the factors which affect diffuse transmittance spectra. *NIR News*, *3*(12), 6–9.

Parrini, S., Acciaioli, A., Crovetti, A., & Bozzi, R. (2018). Use of FT-NIRS for determination of chemical components and nutritional value of natural pasture. *Italian Journal of Animal Science*, *17*(1), 87–91. https://doi.org/10.1080/1828051X.2017.1345659

Rinne, M., Winquist, E., Pihlajaniemi, V., Niemi, P., Seppälä, A., & Siika-aho, M. (2020). Fibrolytic enzyme treatment prior to ensiling increased press-juice and crude protein yield from grass silage. *Bioresource Technology*, *299*(December 2019), 122572. https://doi.org/10.1016/j.biortech.2019.122572

Rukundo, I. R., Danao, M.-G. C., MacDonald, J. C., Wehling, R. L., & Weller, C. L. (2020). Performance of two handheld NIR spectrometers to quantify crude protein of composite animal forage and feedstuff. *AIMS Agriculture and Food*, *6*(2), 463–478. https://doi.org/10.3934/agrfood.2021027

Rushing, J. B., Saha, U. K., Lemus, R., Sonon, L., & Baldwin, B. S. (2016). Analysis of some important forage quality attributes of Southeastern Wildrye (*Elymus glabriflorus*) using near-infrared reflectance spectroscopy. *American Journal of Analytical Chemistry*, *7*(9), 642–662. https://doi.org/10.4236/ajac.2016.79060

Schneeweiss, H. (1991). Models with latent variables: LISREL versus PLS. *Statistica Neerlandica*, *45*(2), 145–157. https://doi.org/10.1111/j.1467-9574.1991.tb01300.x

Shenk, J. S., & Westerhaus, M. O. (1994). The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In G. C. Fahey, Jr. (Ed.), *Forage quality, evaluation and utilization*. ASA, CSSA, and SSSA Books. (pp. 406–449). John Wiley & Sons, Inc.. https://doi.org/10.2134/1994.foragequality.c10

Siefers, M. K., Huck, G. L., Young, M. K., Turner, J. E., Pendergraft, J. S., & Bolsen, K. K. (1996). Agronomic and silage quality traits of winter cereals. In *Cattlemen's Day* (pp. 68–71). Kansas State University.

Soundharrajan, I., Kuppusamy, P., Park, H. S., Kim, J. H., Kim, W. H., Jung, J. S., & Choi, K. C. (2019). Lactic acid bacteria mixture as inoculants on low moisture Italian ryegrass silage fermentation. *Journal of the Korean Society of Grassland and Forage Science*, *39*(3), 127–131. https://doi.org/10.5333/kgfs.2019.39.3.127

Vranić, M., Bošnjak, K., Rukavina, I., Glavanović, S., Pintić Pukec, N., Babić, A., & Vranić, I. (2020). Prediction of forage chemical composition by NIR spectroscopy. *Journal of Central European Agriculture*, *21*(3), 554–568. https://doi.org/10.5513/JCEA01/21.3.2839

Wilkinson, J. M., & Rinne, M. (2018). Highlights of progress in silage conservation and future perspectives. *Grass and Forage Science*, *73*(1), 40–52. https://doi.org/10.1111/gfs.12327

Workman, J. Jr., & Weyer, L. (2012). *Practical guide and spectral atlas for interpretive near-infrared spectroscopy*. CRC Press. https://doi.org/10.1201/b11894

Worku, A., Tóth, T., Orosz, S., Fébel, H., Kacsala, L., Húth, B., Hoffmann, R., Yakubu, H. G., Bazar, G., & Tóthi, R. (2021). Aroma profile, microbial and chemical quality of ensiled green forages mixtures of winter cereals and Italian ryegrass. *Agriculture (Switzerland)*, *11*(6), 512. https://doi.org/10.3390/agriculture11060512

Worku, A., Tóthi, R., Orosz, S., Fébel, H., Kacsala, L., Húth, B., Hoffmann, R., & Tóth, T. (2021). In situ ruminal degradability and

fermentation characteristics of novel mixtures of winter cereal and Italian ryegrass plus winter cereal silages. *Czech Journal of Animal Science*, *66*(8), 302–314. https://doi.org/10.17221/12/2021-CJAS

Worku, A., Tóthi, R., Orosz, S., Fébel, H., Kacsala, L., Vermeire, D., & Tóth, T. (2021). Novel mixtures of Italian ryegrass and winter cereals: Influence of ensiling on nutritional composition, fermentation characteristics, microbial counts and ruminal degradability. *Italian Journal of Animal Science*, *20*(1), 749–761. https://doi.org/10.1080/1828051X.2021.1924883

Yakubu, H. G., Kovacs, Z., Toth, T., & Bazar, G. (2022). The recent advances of near-infrared spectroscopy in dairy production–A review. *Critical Reviews in Food Science and Nutrition*, *62*(3), 810–831. https://doi.org/10.1080/10408398.2020.1829540

Yan, Y., Li, X., Guan, H., Huang, L., Ma, X., Peng, Y., Li, Z., Nie, G., Zhou, J., Yang, W., Cai, Y., & Zhang, X. (2019). Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. *Bioresource Technology*, *279*, 166–173. https://doi.org/10.1016/j.biortech.2019.01.107

Yang, Z., Nie, G., Pan, L., Zhang, Y., Huang, L., Ma, X., & Zhang, X. (2017). Development and validation of near-infrared spectroscopy for the prediction of forage quality parameters in Lolium multiflorum. *PeerJ*, *5*, e3867. https://doi.org/10.7717/peerj.3867

How to cite this article: Yakubu, H. G., Worku, A., Tóthi, R., Tóth, T., Orosz, S., Fébel, H., Kacsala, L., Húth, B., Hoffmann, R., & Bazar, G. (2023). Near-infrared spectroscopy for rapid evaluation of winter cereals and Italian ryegrass forage mixtures. *Animal Science Journal*, *94*(1), e13823. https://doi.org/10.1111/asj.13823

5.2. Description of the odor profile of alfalfa and rye silages using an electronic nose

Title	Description of the odor profile of fermented alfalfa and ryegrass silages using an electronic nose
Authors	Yakubu Haruna Gado, Bázár György, Radó-Nyiczky Éva, Orosz Szilvia, Tóth Tamás
Journal	Hungarian Journal of Animal Production
Year Volume Issue	2022 71 1
Pages Quartile IF	1-10 - -
Doi	-

DESCRIPTION OF THE ODOR PROFILE OF FERMENTED ALFALFA AND RYE SILAGES USING AN ELECTRONIC NOSE

YAKUBU HARUNA GADO BÁZÁR GYÖRGY RADÓ-NYICZKY ÉVA
OROSZ SZILVIA TÓTH TAMÁS

ABSTRACT

Silages, as conserved forages, form a basic component of ruminant diets. The odor of silage is highly informative and helps in determining the quality. Electronic nose (e-nose) technology provides rapid and objective measurement of odors and may be applicable for quick screening of various silages. Aroma profile analysis of alfalfa and rye silages (n = 22 and 38, respectively) collected from Hungarian dairy farms, produced with different harvest technologies, and covering a wide range of quality, was performed with an e-nose utilizing the metal oxide semiconductor sensor array technology. The odor patterns of the quality categories based on the pH and the lactic acid / acetic acid ratio were compared. The applied e-nose was not suitable for distinguishing between samples made from different plant materials with average compositional parameters, because the effect of species on the odor was less characteristic than that of the diverse processing conditions. However, on the basis of the aroma profiles described by the measured sensor signals, alfalfa-based forages prepared with different processing (direct-cut *vs.* wilted) could be accurately identified, and rye samples were identifiable according to the different phenological phases at harvest (before heading *vs.* heading). In the supervised classification (discriminant analysis) of groups of samples based on pH and lactic acid / acetic acid ratio, 65-75% of the samples selected for validation were correctly identified, which indicates reliability of the method. Based on the results, the presented instrumental aroma testing methodology proved to be promising in the field of quality testing of fermented forages.

ÖSSZEFOGLALÁS

Yakubu, H. G. – Bázár, Gy. – Radó-Nyiczky, É. – Orosz, Sz. – Tóth, T.: LUCERNA ÉS ROZS ALAPÚ ERJESZTETT TÖMEGTAKARMÁNYOK ILLATPROFILJÁNAK LEÍRÁSA ELEKTRONIKUS ORRAL

Minden változás, ami az erjesztett tömegtakarmányok készítése és kitárolása során történik, hatással van a takarmány illóanyag tartalmára, beleértve a tejsavat, ecetsavat és vajsavat, amelyek erősen befolyásolják a szilázs minőségét. Ezek a komponensek erősen kihatnak a szilázs beltartalmi értékeire, szennyezettségére és érzékszervi (organoleptikus) karakterére. Így a tömegtakarmányok erjedés-dinamikája főleg a tejsav, a rövid szénláncú illózsírsavak (ecetsav, propionsav, vajsav) és a romlást jelző fehérjék, aminosavak és ammónia jelenléte alapján értékelhető. Ismert, hogy a tejelő tehenek szárazanyag-felvétele korlátozott, így a gyengébb táplálóanyag-tartalmú takarmány nem ellensúlyozható a mennyiséggel, míg a felvehető rossz minőségű takarmánnyal nem vihető be a szükséges mennyiségű táplálóanyag- és energiatartalom, a nem megfelelő minőségű takarmány pedig akár teljes takarmány-visszautasítást is eredményezhet. Éppen ezért a minőség megítélésében nem csak a beltartalmi paraméterek folyamatos nyomonkövetése szükséges, hanem a romlásból származó, nem kívánatos aroma anyagoké is. Mindezek alapján a tömegtakarmányok rutinszerű, gyakori érzékszervi vizsgálata fontos, de összetett feladat. Az erjesztett tömegtakarmányok minőségének megítélése során még napjainkban is a humán érzékszervi bírálat a legáltalánosabb módszer, amely azonban számos egyedi faktor által torzított, szubjektív, nem automatizálható döntéshozatalt tesz lehetővé. Az elektronikus orr (e-orr) technológiákat egyre gyakrabban használják az iparban a körülményes és sokszor szubjektív eredményeket adó, folyamatirányításra kevéssé alkalmas humán érzékszervi bírálatok kiváltására. Az e-orr technológia gyors és objektív módszer lehet a minőség

ellenőrzésére mind a silózás, mind a depózás, mind a kitárolás során. A minták nem igényelnek különösebb előkészítést, emellett az e-orr gyorsasága, alacsony működtetési költségei lényeges előnyt jelenthetnek, ugyanakkor a sokféle növényi alappal, eltérő technológiával készült tömegtakarmányok vizsgálatához specifikus analitikai protokollok felállítása szükséges. Szerzők magyarországi szarvasmarha-telepekről származó, eltérő technológiákkal előállott, széles minőségi skálán mozgó lucerna- (n = 22) és rozsszilázsok, valamint -szenázsok (n = 38) aromavizsgálatát végezték el e-orr berendezéssel, majd az illatmintázatokat összevetették a pH-ra, valamint tejsav/evetsav arányra alapozott minőségi besorolásokkal. Eredményeik alapján az alkalmazott fémoxid félvezető (metal oxide semiconductor, MOS) gázérzékelősoros technológián alapuló e-orr nem alkalmas az eltérő növényi alapanyagból készült, átlagos beltartalmi paraméterekkel rendelkező minták megkülönböztetésére, mivel az egyéb kezeléshatások a fajtahatásnál jelentősebb illatváltozásokat eredményeznek. A mért szenzorjelekkel leírt aromaprofilok alapján ugyanakkor megbízhatóan azonosíthatóak voltak az eltérő agrotechnikával előkészített (fonnyasztott *vs.* fonnyasztás nélküli) lucerna alapú tartósított tömegtakarmányok, és ugyanez elmondható a rozsmintákról, amelyek a betakarításkori fenológiai fázis szerint (kalászhányás előtti, illetve utáni betakarítás) mutattak eltérő illatprofilt. A minták pH és tejsav/ecetsav arány alapján kialakított csoportjainak irányított osztályozása (diszkriminancia analízise) során a tesztelésre kiválasztott minták 65-75%-át sikerült helyesen rangsorolni. A bemutatott műszeres aromavizsgálati módszertan az eredmények alapján ígéretesnek bizonyult az erjesztett tömegtakarmány minőségvizsgálata terén.

INTRODUCTION

Worldwide, hay, grasses, whole-crop silage (e.g. corn, alfalfa and rye silages), fermented mixed-crop forages, cereals and legumes are the major crops used as fresh or conserved forage for the nutrition of ruminants (*Keady et al.,* 2012). Silage making is one of the most important sources of conserved forages. In particular, silage forms a basic component of ruminant diets (*Keady et al.,* 2012). This approach is widely used for conserving forage for feeding milk- and meat-producing ruminants (*Cheli et al.,* 2013). Silage making has increased considerably and has become economically important for many farms, especially in temperate areas of the world (*Reynolds and Frame*, 2006).

Due to the climatic conditions of Hungary, the annual amount of feed required for livestock must be produced in the period from April to November (*Schmidt*, 2001). Thus, the preservation of different forage (e.g. alfalfa, corn, grass, winter cereals, Italian ryegrass) is an intensively researched area in Hungary (*Baintner and B. Kissné*, 1989; *Schmidt et al.*, 2001; *B. Kissné and Bana*, 2002; *Szűcsné et al.*, 2005; *Avasi et al.*, 2008; *Orosz*, 2009; *Lehel et al.*, 2011; *Orosz et al.*, 2017; *Alemayehu et al.*, 2019, 2020).

Not only is milk production influenced by the quantity of silage determined in the ration, but the nutrient content and fermentation of the silage can also affect the performance and health of the animals (*Fulgueira et al.,* 2007). Since current systems of dairy production demand exact knowledge of the production processes, it is important to monitor feeds during preparation and consumption. Regular monitoring of the quality of fermentation is particularly important in larger dairy farms, where silage silos may contain large quantities of potentially inhomogeneous, fast-consumed feed.

Sensory evaluation of forage over the years has been traditionally evaluated

according to the physical parameters such as color, softness, palatability, odor etc. Notwithstanding the importance of these parameters in analyzing forage quality, there may be certain limitations regarding assessment, since they remain subjective and difficult to standardize (*Schroeder*, 2004). For feed professionals, the first information in the monitoring is provided by the sensory tests (*Fulgueira et al*., 2007). Besides the parameters mentioned before, the sensory evaluation may include the examination of the structure of the feed, its tact (moisture content), the quantity and distribution of components, the degree of crushing, chop length, the clarity from foreign matter, and the odor. However, this information may be subjective in view of the age, lifestyle, routine of the sampler, or other sampling conditions and environmental impact. The odor is highly informative when it comes to determining harmful fermentation processes and deterioration (*Kung et al.*, 2018).

A pleasant odor indicates silage was ensiled properly. However, moldy and musty odors may occur in hay or silage stored at moisture contents above 16 to 18%. Animals may react or respond to off-odors by refusing to eat or going off feed. Ensiling overdried forages may cause excessive heat (>55°C) that results in typical odor. Interestingly, hay or silage with a slightly caramelized odor is often quite palatable to livestock even though the quality is reduced. The odor of silage can indicate a good or bad fermentation; if it smells of butyric acid, similar to rancid butter, it may lack palatability, and low animal intake is likely (*Ball et al.,* 2001).

Therefore, volatile substances can be critical indicator parameters for assessing the animal health risk of silages. The odor determination should be improved with quick objective methods that can simultaneously analyze large amounts of samples, thereby, helping the daily work of the feed industry to achieve efficient production and to maintain good animal health.

In recent years, electronic nose (e-nose) technologies have provided a quick and objective method for quality control (*Jiang and Chen*, 2014). Among the different applications of the electronic nose technique, feedstuffs analysis is one of the most promising and also the most important (*Magan and Evans*, 2000; *Di Natale et al*., 2001), however, silage quality assessment using electronic nose has not been discussed in the literature, yet. The e-nose is an instrument that is composed of an array of electronic chemical sensors, with partial specificity and a suitable pattern recognition system, which is capable of recognizing simple or complex volatile organic compound (VOCs) profiles related to product odor (*Gardener and Philip*, 1994).

Volatile organic compounds can be used as quality markers and the VOCs' profile may represent a unique marker for monitoring feed quality and detecting changes during the process, storage and ageing. The non-specific sensor response by an e-nose can be used for classification and prediction purposes. Furthermore, e-nose analysis is rapid, user-friendly, precise, objective, non-destructive, and no or simple sample pre-treatment is required. Therefore, e-nose constitutes a diagnostic device useful for real time monitoring, control of products and industrial processes, and for decision-making in the area of product quality and safety (*Di Rosa et al.,* 2017), in this regard, electronic nose could be used for rapid determination of silage quality.

The objectives of this preliminary study were to describe the chemical composition and odor profile of alfalfa and rye silages and to further reveal the relationship between the compositional data and the electronically measured odor in order to

establish a quality control system for forages based on machine sensing or olfaction.

MATERIALS AND METHODS

Samples

Alfalfa and rye silage and haylage routine samples (n = 22 and 38, respectively) derived from large scale commercial dairy farms and analyzed by Livestock Performance Testing Ltd., Gödöllő, Hungary, in 2017. As the samples included in this study were taken and sent to the laboratory by the farms, no further information is available about the harvest and ensiling technological parameters. Alfalfa silages were prepared for ensiling with and without wilting (n = 10 and 12, respectively), while rye silages were harvested before heading and in heading (n = 17 and 21, respectively). The fresh samples were dried at 70°C for 8 hours, then homogenized with a laboratory mill (Peppink Mills, Netherlands) and analyzed for dry matter, crude protein, ether extract, crude fiber, crude ash content, pH, acetic acid and

Table 1.

Chemical composition of the fermented forages determined with NIR spectroscopy

	Alfalfa silages and haylages (12) (n = 22)				Rye silage (13) (n = 38)			
	Mean (14)	SD (15)	Min.	Max.	Mean (14)	SD (15)	Min.	Max.
Dry matter (1) [g/kg]	404.48	102.72	228.0	613.0	302.59	74.61	187.0	469.0
Crude protein (2) [g/kg DM]	199.17	22.54	148.0	251.0	143.73	30.99	75.0	217.0
Ether extract (3) [g/kg DM]	27.00	6.28	18.0	44.0	34.30	5.50	22.0	44.0
Crude fiber (4) [g/kg DM]	261.00	30.22	180.0	318.0	268.54	48.09	165.0	381.0
Crude ash (5) [g/kg DM]	124.87	29.49	86.0	228.0	117.16	40.66	63.0	242.0
Total sugar (6) [g/kg DM]	21.65	11.49	10.0	52.0	41.03	34.57	10.0	135.0
NDF (7) [g/kg DM]	399.52	33.81	329.0	459.0	509.89	82.78	314.0	704.0
ADF (8) [g/kg DM]	309.43	29.08	228.0	351.0	300.64	61.64	193.0	448.0
ADL (9) [g/kg DM]	55.57	9.99	31.0	72.0	26.39	9.71	15.0	64.0
Lactic acid (10) [g/kg DM]	50.02	22.51	1.5	77.0	55.22	26.11	6.0	113.0
Acetic acid (11) [g/kg DM]	15.70	9.28	0.5	35.0	6.96	6.73	0.5	28.0
pH	4.69	0.38	4.1	5.5	4.26	0.25	3.9	5.0

NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin

1. táblázat Az erjesztett tömegtakarmányok NIR spektroszkópiás mérésre alapozott kémiai összetétele

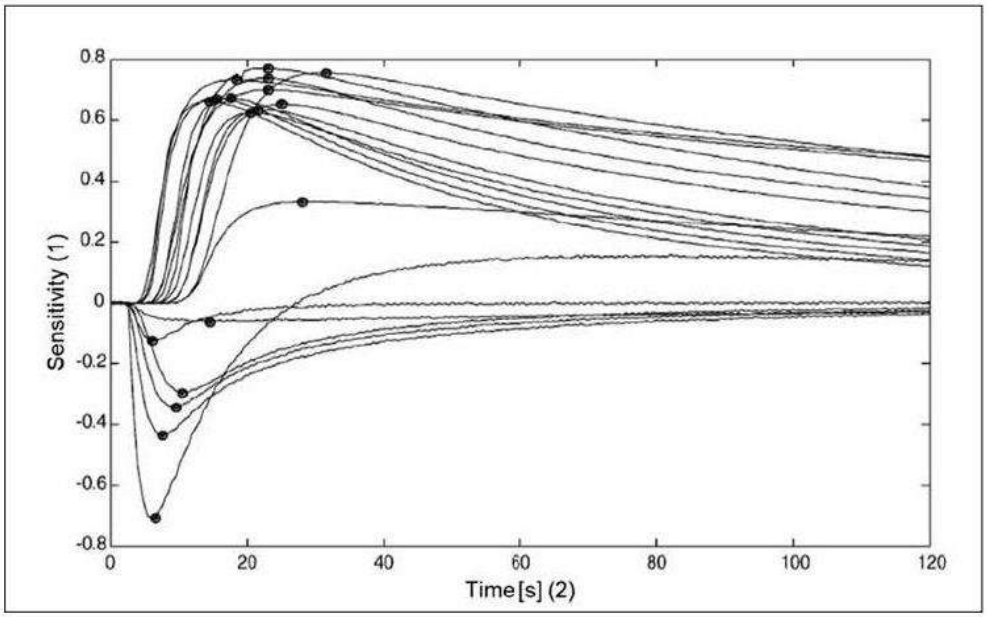
szárazanyag (1); nyersfehérje (2); nyerszsír (3); nyersrost (4); nyershamu (5); összcukor (6); neutrális detergens rost (7); savdetergens rost (8); savdetergens lignin (9); tejsav (10); ecetsav (11); lucernasziláz és -szenázs (12); rozsszilázs (13); átlag (14); szórás (15)

lactic acid concentrations by means of near-infrared (NIR) spectroscopy using a Quant FT-NIR spectrometer (Q-Interline, Denmark) and an internationally recognized calibration database (Samplinq®, Eurofins Agro Inc., Wageningen, Netherlands) at Livestock Performance Testing Ltd, Gödöllő, Hungary. Based on the chemical composition and overall quality, there were no extremities in the sample set (*Table 1*). The quality of samples was evaluated based on the pH and the lactic acid/acetic acid ratio. After NIR measurement, each sample was equally distributed into six plastic bags. The prepared sub-samples were stored at -20°C in sealed bags under vacuum, until the odor measurements. Frozen storage of any sample did not last longer than 30 days.

Odor measurement by means of electronic nose

The odor measurement was performed at Kaposvár University (now Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Kaposvár, Hungary) for six days where, the six sets of sub-samples were analyzed daily (n=60 per day). The odor of each sample was examined with an Alpha MOS FOX4000 electronic nose based on the metal oxide semiconductor (MOS) sensor array technology (Alpha M.O.S., Toulouse, France) within 30 days after sampling. Two

Figure 1. *Relative resistance changes (sensitivity) of the 18 MOS sensors of the electronic nose during one measurement cycle, with dots indicating the maximal deviation of the sensors' sensitivities forming the 18 variables describing the multidimensional odor fingerprint of one silage sample*



1. ábra Az elektronikus orr 18 MOS szenzorán mért relatív ellenállásváltozások (érzékenység) egy mérési ciklus során, pontokkal jelölve a szenzorok érzékenységeinek maximális változásait, melyekkel a vizsgált szilázsminta többdimenziós illatmintázata leírásra kerül

érzékenység (1); idő (2)

g of the sub-samples (n=360) were filled into 20 mL glass vials then sealed with polytetrafluoroethylene septa. The headspace samples containing the volatiles of the silages were generated above the solid sample during three-minute incubation at 40°C. Five mL of the headspace was injected into the continuous flow of synthetic air. The relative resistance changes (ΔR/R0) of 18 MOS sensors caused by the injected volatiles were measured after each injection and saved as sensitivity values. One measurement cycle took 20 minutes, including two minutes data acquisition and 18 minutes cleaning phase when pure synthetic air was used to rinse the sensors. The maximal sensitivity experienced on each sensor was saved in each measurement cycle (*Figure 1*), thus, all sub-samples were described with 18 variables.

Multivariate data analysis

The recorded data of the electronic nose were analyzed with multivariate classification methods using AlphaSoft v12 (Alpha M.O.S., Toulouse, France). The non-supervised principal component analysis (PCA) was used to select outliers and to describe the multivariate structure of the dataset. The supervised discriminant factor analysis (DFA) was applied to test the possibility of group identification based on the odor properties (*Naes et al.*, 2002). Cross-validation was used to test the supervised classification models when sub-samples of a single sample were left out of the modelling iteratively and were used for testing the classification capability of the model. The classification models were evaluated based on the confusion matrices where hit rates and misclassifications were indicated.

RESULTS AND DISCUSSION

The differences in the odor patterns of the different plant materials (alfalfa *vs.* rye) caused no general separation because the effect of the various technological processing was considerably stronger and caused significant variation within the species. Both the alfalfa and rye silages separated according to the different harvest technologies of ensiling. In the case of alfalfa, the application of wilting before ensiling caused significant odor variations, while in the case of rye, the maturity of the crop upon ensiling resulted in detectable characteristic variations of the odor profile (*Table 2*). In average, more than 65% of samples were classified correctly in the cross-validations in both types of silages.

Samples were ranked according to their quality based on the pH or the lactic acid/acetic acid ratio. The range of pH was narrow, and 4.4 seemed to be median value dividing the sample set into two quality parts. Thus, two classes were defined according to the pH of the samples: samples having pH lower than 4.4 (n=32) and samples having pH equal to or higher than 4.4 (n=28). The acid ratio had wide range over the 60 samples, therefore, three classes were defined according to the lactic acid / acetic acid ratio: acid ratio equal to or higher than 10 (n=25), acid ratio lower than 10 and higher than or equal to 5 (n=14), acid ratio lower than 5 (n=21).

Supervised DFA classifications were performed to evaluate the possibility of identifying the different quality groups based on the odor profiles. *Table 3* and *Table 4* show the cross-validation results of the developed models for the pH and

Table 2.

Modelling and cross-validation results of the odor-based classification of silages and haylages according to the applied harvest technologies

	Alfalfa silages and haylages (3)			Rye silages (7)		
	Processing groups (4)	(A)	(B)	Phenological phase groups (8)	(C)	(D)
Model (1)	(A) Direct-cut (5)	68.1%	31.9%	(C) Before heading (9)	78.0%	22.0%
	(B) Wilted (6)	28.3%	71.7%	(D) Heading (10)	41.7%	58.3%
Cross-validation (2)	(A) Direct-cut (5)	66.7%	33.3%	(C) Before heading (9)	77.4%	22.6%
	(B) Wilted (6)	30.0%	70.0%	(D) Heading (10)	41.7%	58.3%

2. táblázat Szilázsok és szenázsok illat alapján végzett, betakarítási mód szerinti osztályozásának eredménye a modellkészítés és a keresztvalidáció során

modell (1); keresztvalidáció (2); lucernaszilázs és -szenázs (3); előkezelés szerinti csoport (4); fonnyasztás nélkül (5); fonnyasztott (6); rozsszilázs (7); fenológiai fázis szerinti csoport (8); kalászhányás előtt (9); kalászhányáskor (10)

acid ratio, respectively. In the case of rye silages, 64.5% of the cross-validation (CV) samples were classified correctly according to the pH groups, while the classification of alfalfa silages was less accurate (average hit rate in CV: 60.3%). The overall average performance of the classifications based on the acid ratio was weaker, but the identification of the low-quality group (ratio < 5) of rye silages was highly successful (75% hit rate in CV). Results of alfalfa samples were less accurate, but good identification (75% hit rate in CV) of the group with high (≥ 10) lactic acid / acetic acid ratio was experienced.

Table 3.

Modelling and cross-validation results of the odor-based classification of silages and haylages according to the quality groups defined by the pH values

		Alfalfa silages and haylages (4)		Rye silages (5)	
	pH groups (3)	< 4.4 (n=6)	≥ 4.4 (n=16)	< 4.4 (n=26)	≥ 4.4 (n=12)
Model (1)	< 4.4	73.3%	26.7%	67.3%	32.7%
	≥ 4.4	42.2%	57.8%	35.2%	64.8%
Cross-validation (2)	< 4.4	66.7%	33.3%	66.0%	34.0%
	≥ 4.4	46.1%	53.9%	37.0%	63.0%

3. táblázat Szilázsok és szenázsok illat alapján végzett, pH-alapú minősítés szerinti osztályozásának eredménye a modellkészítés és a keresztvalidáció során

modell (1); keresztvalidáció (2); pH csoportok (3); lucernaszilázs és -szenázs (4); rozsszilázs (5)

Table 4.

Modelling and cross-validation results of the odor-based classification of silages and haylages according to the quality groups defined by the lactic acid/acetic acid ratios

	Lactic acid/ acetic acid groups (3)	Alfalfa silages and haylages (4)			Rye silages (5)		
		≥ 10 (n=4)	≥ 5; < 10 (n=4)	< 5 (n=14)	≥ 10 (n=21)	≥ 5; < 10 (n=10)	< 5 (n=7)
Model (1)	≥ 10	75.0%	20.8%	4.2%	38.9%	20.6%	40.5%
	≥ 5; < 10	12.5%	37.5%	50.0%	30.0%	45.0%	25.0%
	< 5	16.7%	27.4%	56.0%	3.3%	16.7%	80.0%
Cross-validation (2)	≥ 10	75.0%	20.8%	4.2%	37.3%	21.4%	41.3%
	≥ 5; < 10	25.0%	25.0%	50.0%	33.3%	41.7%	25.0%
	< 5	17.9%	32.1%	50.0%	6.7%	16.7%	76.7%

4. táblázat Szilázsok és szenázsok illat alapján végzett, tejsav/ecetsav arányra alapozott minősítés szerinti osztályozásának eredménye a modellkészítés és a keresztvalidáció során

modell (1); keresztvalidáció (2); tejsav/ecetsav csoportok (3); lucernaszilázs és -szenázs (4); rozsszilázs (5)

CONCLUSIONS

The time of harvesting (phenological phase of the crop) and the applied harvest technology (inclusion of wilting) causes significant odor changes in the case of rye silages and alfalfa silages, respectively. The quality groups defined by pH or acid ratio are distinguishable based on the measured odor patterns. On average, 60.3% of alfalfa and 64.5% of rye silage samples were classified in the correct pH groups. The average hit rates of alfalfa and rye silages were 50% and 51.9%, when classification was performed by the lactic acid / acetic acid ratios. In the case of rye silages, the identification of the low-quality samples was the most successful (76.7% hit rate) while in the case of alfalfa silages, the identification of the good-quality samples was suitable (75% hit rate). According to our results, the electronic nose technology proved to be applicable to classify the rye and alfalfa silage samples by their quality, even when quality groups are defined by NIR predicted values, causing double prediction error. The accuracy may be increased with the expansion of the sample set and application of wet chemical reference data.

REFERENCES

Alemayehu, W. – Tóthi, R. – Orosz, S. – Kacsala, L. – Bâzâr, G. – Tóth, T. (2019): Contents of nutrition and fermentation characteristics of ensiled Italian ryegrass and winter cereal mixtures for dairy cows. Krmiva: Review for animal feeding, production and feed technology. 61. https://doi.org/10.33128/k.61.1.6

Alemayehu, W. – Tóthi, R. – Orosz, Sz. – Fébel, H. – Tossenberger, J. – Húth, B. – Tóth, T. (2020): Nutritive value of ensiled Italian ryegrass and winter cereal mixture. Acta Fytotechnica et Zootechnica. Scientific Journal for phytotechnics and zootechnics. Future perspectives in animal production. 23. https://doi.org/10.15414/afz.2020.23.mi-fpap.7-14

Avasi, Z. – Szűcsné, P. J. – Seale, D. (2008): Ensilage of wilted lucerne treated with different types of biological preservatives. 13th ICFC. 138-139.

B. Kissné Kelemen, G. – Bana, B. (2002): Zöldlucerna silózása enzimtartalmú biológiai tartósítószerekkel. Állattenyésztés és Takarmányozás, 51. 6. 635-345.

Baintner, F. – B. Kissné Kelemen, G. (1989): A celluláz (Phylacell) kezelés hatása a szilázsok minőségére és emészthetőségére. Acta Ovariensis, XXXI. 5. 3-11.

Ball, D. M. – Collins, M. – Lacefield, G. D. – Martin, N. P. – Mertens, D. A. – Olson, K. E. – Putnam, D. H. – Undersander, D. J. – Wolf, M. (2001): Understanding forage quality. American Farm Bureau Federation Publication, IL, USA, 17.

Cheli, F. – Campagnoli, A. – Dell'Orto, V. (2013): Fungal populations and mycotoxins in silages: From occurrence to analysis. Anim. Feed Sci. Technol., 183. 1–16.

Di Natale, C. – Macagnano, A. – D'amico, A. – Paolesse, R. (2001): Artificial olfaction systems: Principles and applications to food analysis. Biotechnology, Agronomy, Society Environ., 5. 159–165.

*Di Rosa, A. R. – Leone, F. – Cheli, F. – Chiofalo, V. (*2017): Fusion of electronic nose, electronic tongue and computer vision for animal source food authentication and quality assessment – A review. J. Food Eng., 210. 62–75.

Fulgueira, C. – Amigot, S. – Gaggiotti, M. – Romero, L. – Basílico, J. (2007): Forage quality: Techniques for testing. Fresh Produce, 1. 121–131.

Gardener, J. W. – Philip, N. B. (1994): A brief history of electronic noses. Sensors and Actuators B, 18. 211–220.

Jiang, H. – Chen, Q. (2014): Development of electronic nose and near infrared spectroscopy analysis techniques to monitor the critical time in SSF process of feed protein. Sensors, 14. 19441–19456.

Keady, T. W. J. – Marley, C. M. – Scollan, N. D. (2012): Grass and Alternative Forage Silages for Beef Cattle and Sheep: Effects on Animal Performance. In: Proceedings XVI International Silage Conference. Ed.: Kuoppala, K., Rinne, M., Vanhatalo, A.; MTT Agrifood Research Finland and University of Helsinki Press, Helsinki, Finland, 152–165.

Kung, L. – Shaver, R. D. – Grant, R. J. – Schmidt, R. J. (2018): Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci., 101. 4020–4033.

Lehel, L. – Orosz, S. – Tóthné Polner, A. – Sümeghy, L. – Hajda, Z. – Sipiczki, B. – Várhegyi, J. – Fébel, H. (2011): The rye grass silage apparent digestibility, rumen protein degradability, content of metabolizable protein and net energy. Animal welfare etiology and health. AWETH, 7. 149-157.

Magan, N. – Evans, P. (2000): Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. J. Stored Prod. Res., 36. 319–340.

Næs, T. – Isaksson, T. – Fearn, T. – Davies, T. (2002): Multivariate Calibration and Classification. NIR Publictions, Chichester, UK, 344.

Orosz, Sz. (2009): A szilázsfüvek új generációja: az „édes füvek". Takarmányozás, 12. 4-10.

Orosz, Sz. – Horváthné, K. B. – Kruppa, J. – Kruppa, J. – Iván, F. – Hoffmann, R. (2017): Húshasznú tehenek, növendék- és hízó marhák hazai tömegtakarmány-ellátása a klímaváltozás tükrében. Állattenyésztés és Takarmányozás, 66. 365-382.

Reynolds, S. G. – Frame, J. (2006): Grasslands: developments, opportunities, perspectives. CRC Press, FL, USA, 539.

Schmidt, J. (2001): Korszerű módszerek a zöldlucerna erjesztéses tartósítására. Takarmányozás. 4, 12-16.

Schmidt, J. – Szakács, Gy. – Cenkvári, É. – Sipőcz, J. – Urbánszky, K. – Tengerdy, R. P. (2001): Enzyme assisted ensiling of alfalfa with enzymes by solid substrate fermentation. Biores. Technol. 76. 207-212.

Schroeder, J. W. (2004): Forage nutrition for ruminants. North Dakota State University. USA, 15.

Szűcsné, P. J. – Avasi, Z. – Kovács, T. (2005): Biológiai tartósítószerek hatása a lucerna erjedésdinamikájára és a szenázs aerob stabilitására. Holstein Magazin, 26-28.

Érkezett: 2020. október

Szerzők címe: *Yakubu H. G. – Bázár Gy. – Radó-Nyiczky É.*
Magyar Agrár- és Élettudományi Egyetem, Élettani és Takarmányozástani Intézet, Élettani és Állategészségügyi Tanszék

Authors' address: Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Animal Nutrition, Department of Physiology and Animal Health
H-7400 Kaposvár, Guba Sándor út 40.
bazar@agrilab.hu

Orosz Sz.
Állattenyésztési Teljesítményvizsgáló Kft.
Livestock Performance Testing Ltd.
H-2100 Gödöllő, Dózsa György út. 58.

Tóth T.
Adexgo Kft.
Széchenyi István Egyetem, Agrár és Élelmiszeripari Kutató Központ
H-9026 Győr, Egyetem tér 1.
Széchenyi István University, Agricultural and Food Research Centre
H-9026 Győr, Egyetem sqr. 1.
H-8230 Balatonfüred, Lapostelki út 13.

5.3. Feeding mixture silages of winter cereals and Italian ryegrass can modify the fatty acid and odor profile of bovine milk.

Title	Feeding mixture silages of winter cereals and Italian ryegrass can modify the fatty acid and odor profile of bovine milk
Authors	Yakubu Haruna Gado, Omeralfaroug Ali, András Szabó, Tamás Tóth, George Bazar
Journal	Agriculture
Year Volume Issue	2023 13 381
Pages Quartile IF	1-15 Q1 3.600
Doi	10.3390/agriculture13020381

agriculture

MDPI

Article

Feeding Mixed Silages of Winter Cereals and Italian Ryegrass Can Modify the Fatty Acid and Odor Profile of Bovine Milk

Haruna Gado Yakubu [1,*], Omeralfaroug Ali [1], András Szabó [1], Tamás Tóth [2,3] and George Bazar [3]

[1] Agriobiotechnology and Precision Breeding for Food Security National Laboratory, Institute of Physiology and Animal Nutrition, Department of Animal Physiology and Health, Hungarian University of Agriculture and Life Sciences, 40 Guba Sándor Street, 7400 Kaposvár, Hungary
[2] Agricultural and Food Research Centre, Széchenyi István University, 1 Egyetem Square, 9026 Győr, Hungary
[3] ADEXGO Kft., 13 Lapostelki Street, 8230 Balatonfüred, Hungary
* Correspondence: harunagadoyakubu@agrilab.hu; Tel.: +36-(233)-209027715

Abstract: The utilization of corn silage in animal diets is becoming a challenge, due to the crop's reduced yield as a result of climate change. Alternative silage types, such as mixtures of Italian ryegrass and winter cereals, may be a good complement to corn silage in diet formulation. Therefore, it is important to investigate how these alternative sources influence milk fatty acid and odor profile, as well as how these quality parameters could be efficiently evaluated. In this study, a corn silage-based control (CTR) and four experimental (EXP) diets—which contained winter cereals (WC), as well as WC with Italian ryegrass (IRG) silages in different proportions—were fed to Holstein-Friesian cows (n = 32) in a single-blinded efficacy study during a series of 4-week periods, with 2 weeks of adaption to each feed before the main trial. Milk from each trial was subjected to fatty acid (FA) analysis and odor profiling through the utilization of gas chromatography and an electronic nose, respectively. The results show that milk FAs in the EXP-3 and EXP-4 groups (which contained mixed silages using WC) changed the most when compared with other groups. Moreover, with a 7 kg/day inclusion rate of WC + IRG and of the WC silages in the diets of the EXP-2 and EXP-3 groups, respectively, the milk from these groups had their n6:n3 ratio reduced, thus indicating possible health benefits to consumers. The odor variation between the milk of the WC + IRG and WC groups was greater than the variation between the milk of the CTR and EXP groups. The main volatile compound responsible for the odor of the CTR milk was ethyl-butyrate, whereas 2-propanol and butan-2-one dominated the WC milk; the milk samples of the WC + IRG groups were influenced largely by ethanol. The study proved that with a 7 kg/day inclusion of mixed silages including winter cereals plus Italian ryegrass, the FA and odor profile of bovine milk could be modified.

Keywords: dairy feeding; machine olfaction; fatty acids; milk odor

Citation: Yakubu, H.G.; Ali, O.; Szabó, A.; Tóth, T.; Bazar, G. Feeding Mixed Silages of Winter Cereals and Italian Ryegrass Can Modify the Fatty Acid and Odor Profile of Bovine Milk. *Agriculture* **2023**, *13*, 381. https://doi.org/10.3390/agriculture13020381

Academic Editor: Andrea Summer

Received: 19 December 2022
Revised: 30 January 2023
Accepted: 2 February 2023
Published: 5 February 2023

1. Introduction

Milk is a major delicacy in many homes around the world, the consumption of which may be linked to the socio-economic status of the consumer [1]. Notwithstanding the socio-economic status of the consumer, the nutritional and sensory properties of milk may influence the final decision of an individual when purchasing it [2]. The cow's health status, milking environment, quality of feed, and feeding regime used significantly influence the overall quality of milk [3,4]. The influence of these variables may include chemical or nutritional alterations, as well as sensory properties, such as the aroma or odor profile [5–8]. Certain feeding strategies significantly affect the concentration of fat, fatty acid (FA) composition, and aromatic properties of milk. For instance, the use of dry olive pomace [9] and microalgae oil [10] as energy sources in feed was reported to have modified the FA and sensory properties of bovine milk. Moreover, the addition of extruded linseed, linseed oil, or fish oil to the cows' diet markedly modified the FA of the milk produced by

the cows in the experiment [11,12]. In addition, forage, fresh pasture, silage, and hay have also been found to influence the fatty acid composition and aromatic properties [13–16]. The ability of such feed resources to alter milk fat composition and concentration levels is mainly due to the high fiber levels [17]. Furthermore, mixed silages provide the required fiber level for this purpose. Therefore, in order to maintain the concentration level of milk fat—due to the fact that the major precursor of FA synthesis within the mammary gland is acetate—forage with high digestible fiber content (i.e., a minimum NDF of 19–21%) are particularly important [18]. Physiologically, during the biochemical process of lipolysis, free FAs or their precursors are released into the milk, which influences the aroma or odor profile of this milk, as well as other dairy products [19]. For example, hexanoic, octanoic, and decanoic acids have been found to be responsible for milk's characteristic aroma [20].

In the midst of various climate-change-based threats, new silage mixtures have been under development in Europe in order to serve as a corn silage substitute or as a complement to it. Indeed, corn silage remains the most widely used energy-dense diet in European dairy farms [21]. Moreover, some of the forage types undergoing trials are winter cereals and Italian ryegrass mixtures [22–24]. Worku et al. investigated various factors affecting forage suitability: aroma profile, microbial and chemical qualities of the mixtures [22]; the in situ ruminal degradability and fermentation characteristics of novel mixtures [23]; the influence of ensiling protocols on the nutritional composition, rumen microbial counts, and ruminal degradability of the mixtures [24]. However, the effect of feeding these mixtures in a total mixed ration (TMR) in terms of milk fatty acids and odor profile has not yet been studied.

The sensory properties of milk over the years have been determined by conventional means, such as the human sensory test [25]. This test is mostly associated with challenges, such as the time and cost required to undertake sensory analysis, especially when huge samples are involved. The challenge, mainly, is found in an inability to recruit large numbers of professional sensory panelists in order to evaluate a large number of products when needed [26]. This is in addition to the fatigue that is involved in the evaluation process, due to the fact that a small number of panelists may not provide an accurate evaluation when compared with machine olfaction. Additionally, subjectivity on the part of unprofessional panelists may lead to a false evaluation, due to differences in the sensitivities of smell receptors [27]. For the past few decades, the electronic nose (e-nose) approach, as a correlative analytical technology, has been employed in order to rapidly analyze the sensory properties of milk, with little or no sample preparation [28]. The instrumental method of the e-nose mimics the human olfaction system by utilizing sensors that interact with the volatile compounds within a headspace [28,29].

As such, the aim of this study was to evaluate the effects of total mixed rations (TMRs)—which were formulated based on a differing mixture of winter cereals and Italian ryegrass from mixed silages—on the fatty acid profile of raw bovine milk. A further aim was also to understand how the application of the e-nose approach, as a correlative analytical method, is conducted in order to evaluate the odor profiles of raw bovine milk.

2. Materials and Methods

2.1. Experimental Design

This study was carried out at the dairy farm (Fészerlak) of the Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár Campus. The experimental design used was a single-blinded randomized efficacy study, divided into 5 periods (Figure 1), and conducted between August 2019 and March 2020.

The dairy cows used for the study were 32 multiparous Holstein-Friesian cows (>150 days in milking and an average milk production of <25 kg/day). The cows were fed with 4 experimental diets and a control feed (CTR).

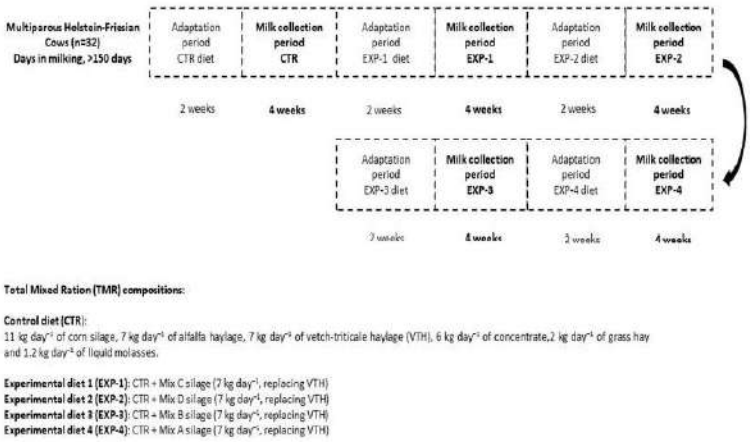


Figure 1. Experimental design of the study and the inclusion rates of the feed ingredients.

The CTR diet was mainly composed of corn silage + alfalfa haylage + vetch-triticale + grass haylage. Experimental diets 1 and 2 (i.e., the EXP-1 and EXP-2 groups) were diets containing a mixture of silages with winter cereals (WC) and Italian ryegrass (IRG); these diets will henceforth be referred to as WC + IRG, whereas experimental diets 3 and 4 (EXP-3 and EXP-4) were diets containing mixed silage with winter cereals (WC) (commercial product producer: Agroteam S.p.a., Torrimpietra (RM), Via di Granaretto, 26, 00054 Rome, Italy). The selection of these experimental mixtures was based on affordability, the availability of seed materials, nutritive value, and the ease with which one can cultivate the forage.

The compositions of the silage mixtures used in the experimental diets are presented as follows:

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat.
Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat.
Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat.
Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

Regarding the experimental design and ingredient inclusion rate of the diets, see Figure 1.

2.2. *Milk Collection and Storage*

Milk collection started after 2 weeks of dairy cow adaptation to each feeding trial (Figure 1). The study was performed taking into consideration the general principles of single-blinded efficacy studies. During this adaptation period, cows were fed their assigned diet and then studied for two weeks in order to facilitate the cows' physiological adjustment to the feed [30], as well as to avoid the influence of a preceding trial on a succeeding one. The adaptation period was implemented into the study design before the main feeding trial commenced for each diet. The cows' udder health was continually monitored with Mastatest (Mastaplex Ltd., Dunedin, New Zealand) in order to rule out mastitis. The milk of unhealthy cows was not included in the test sample. For each trial or period (5 trials in total), 8 separate collections of homogenized milk from each group were sampled into 0.5 L bottles ($n = 40$), which were then stored frozen (−20 °C) for subsequent e-nose measurement and lipid analysis.

2.3. Chemical Analysis of Total Mixed Ration

The crude protein (CP), ether extract (EE), crude ash, and total sugar contents were determined using the respective Association of Official Analytical Chemist AOAC protocols [31]. In addition, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to the procedure developed by Van Soest et al. [32].

2.4. Lipid Analysis (Milk and TMR)

Regarding milk, each sample ($n = 40$) was homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in a 20-fold volume of chloroform:methanol (2:1 v/v). Furthermore, the total lipid content was extracted [33]. The solvents were of ultrapure grade (Sigma-Aldrich, St. Louis, MO, USA); moreover, 0.01% w/v butylated hydroxytoluene was added in order to prevent FA oxidation. The samples were then evaporated to dryness under a nitrogen stream and transmethylated through a base-catalyzed $NaOCH_3$ method [34]. The dried TMR samples were hydrolyzed at 80 °C for 60 min in a methanolic (40 mL) NaOH 50 w/v% (6 mL) solution. After cooling, the total lipid was extracted with chloroform and was then trans-methylated via an acid-catalyzed method [35], using H_2SO_4 (1 v/v%) in methanol as a methyl donor and toluene as a solvent at 50 °C, storing the results overnight.

Fatty acid (FA) methyl-esters were extracted into 300 µL of ultrapure n-hexane for gas chromatography (AOC 20i automatic injector (Shimadzu 2030, Kyoto, Japan), equipped with a Phenomenex Zebron ZB-WAXplus capillary GC column (30 m × 0.25 mm ID, 0.25 µm film, Phenomenex Inc., Torrance, CA, USA), and a flame ionization detector (FID). The characteristic operating conditions were as follows—injector temperature: 220 °C; detector temperature: 250 °C; helium flow: 28 cm/s.

The oven temperature was graded from 60 (2 min of holding) to 150 °C, then from 150 to 180 °C, 2 °C/min and 10 min at 180 °C, from 180 to 220 °C, 2 °C/min and 16 min at 220 °C. The makeup gas used was nitrogen. Calculations were performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration. The TMR and milk FA results were expressed as the weight percentage of the total FA methyl esters, as shown in Tables 1 and 2 respectively.

Table 1. The calculated nutrients and analyzed fatty acid profile of the control (CTR) and experimental diets (EXP-1, 2, 3, and 4).

Calculated Nutrients, % DM	CTR	EXP-1	EXP-2	EXP-3	EXP-4
Crude protein, %	13.5	13.2	13.0	13.4	13.7
Neutral detergent fiber, %	32.5	31.6	31.9	31.5	31.1
Acid detergent fiber, %	19.2	20.2	20.1	19.7	19.8
Acid detergent lignin, %	4.35	4.25	4.22	4.36	4.35
Ether extract, %	1.95	1.98	1.97	1.98	1.92
Starch, %	21.5	20.5	20.9	21.3	21.0
Sugar, %	6.40	6.85	6.42	6.50	6.59
Calcium, %	1.30	1.18	1.14	1.13	1.22
Phosphorus, %	0.40	0.38	0.37	0.39	0.38
Sodium, %	0.22	0.22	0.22	0.22	0.22
Dry matter intake (kg)	19.1	19.1	19.2	18.9	18.7
Net energy for lactation (MJ kg^{-1}, dry matter)	6.32	6.29	6.30	6.32	6.31
Analyzed fatty acid (% of total)					
C12:0	0.51	0.47	0.61	0.55	0.75
C14:0	1.07	1.05	1.25	1.52	1.71
C15:0	0.63	0.58	0.73	0.63	0.80
C16:0	21.1	21.3	21.3	23.1	20.6
C17:0	0.65	0.61	0.76	0.68	0.84

Table 1. *Cont.*

Calculated Nutrients, % DM	CTR	EXP-1	EXP-2	EXP-3	EXP-4
C18:0	4.72	4.39	4.75	5.41	4.84
C20:0	1.54	1.45	1.70	1.52	1.75
C22:0	1.27	1.27	1.45	1.33	1.50
C24:0	1.17	1.05	1.19	1.08	1.27
C16:1n7	0.63	0.60	0.66	0.62	0.73
C18:1n9	16.0	14.7	14.4	13.0	12.6
C18:1n7	1.85	1.83	2.21	1.93	2.24
C20:1n9	0.92	0.83	0.96	0.86	1.05
C22:1n9	1.58	1.54	2.10	1.58	1.65
C18:2n6	35.8	34.8	32.8	32.3	31.6
C18:3n3	10.6	13.7	12.9	13.9	16.1
n6:n3	3.38	2.54	2.55	2.33	1.96

Table 2. Fatty acid profile (% (*w*/*w*) of total fatty acid methyl esters (mean ± standard deviation)) of raw milk in the control and experimental groups of the cows ($n = 40$).

Fatty Acid	CTR	EXP-1	EXP-2	EXP-3	EXP-4
Total SFA [1]	68.9 ± 0.70 ^{ab}	68.1 ± 0.84 ^{b}	69.4 ± 1.28 ^{ab}	70.3 ± 1.30 ^{a}	70.3 ± 2.31 ^{a}
C4:0	0.07 ± 0.06	0.18 ± 0.30	0.06 ± 0.06	0.06 ± 0.03	0.08 ± 0.05
C6:0	0.54 ± 0.24	0.59 ± 0.31	0.39 ± 0.25	0.45 ± 0.19	0.53 ± 0.23
C8:0	0.84 ± 0.10	0.83 ± 0.09	0.78 ± 0.11	0.85 ± 0.08	0.90 ± 0.13
C10:0	2.57 ± 0.14 ^{c}	2.46 ± 0.14 ^{c}	2.65 ± 0.19 ^{bc}	2.92 ± 0.26 ^{ab}	3.12 ± 0.26 ^{a}
C11:0	0.06 ± 0.01 ^{bc}	0.04 ± 0.01 ^{c}	0.05 ± 0.01 ^{c}	0.07 ± 0.02 ^{ab}	0.08 ± 0.02 ^{a}
C12:0	3.39 ± 0.10 ^{c}	3.29 ± 0.21 ^{c}	3.61 ± 0.3 ^{bc}	4.02 ± 0.40 ^{ab}	4.37 ± 0.39 ^{a}
C13:0	0.12 ± 0.01 ^{bc}	0.11 ± 0.01 ^{c}	0.11 ± 0.01 ^{bc}	0.14 ± 0.02 ^{ab}	0.15 ± 0.02 ^{a}
C14:0	12.5 ± 0.17 ^{c}	12.6 ± 0.26 ^{c}	13.1 ± 0.23 ^{bc}	13.4 ± 0.49 ^{ab}	14.0 ± 0.78 ^{a}
C15:0	1.36 ± 0.09 ^{ab}	1.28 ± 0.06 ^{b}	1.31 ± 0.06 ^{b}	1.36 ± 0.09 ^{ab}	1.43 ± 0.10 ^{a}
C16:0	36.9 ± 1.10	36.7 ± 0.83	36.6 ± 1.99	35.6 ± 2.40	35.4 ± 1.33
C17:0	0.61 ± 0.04	0.57 ± 0.02	0.60 ± 0.02	0.60 ± 0.03	0.58 ± 0.04
C18:0	9.77 ± 0.85 ^{ab}	9.33 ± 0.64 ^{b}	9.98 ± 1.04 ^{ab}	10.72 ± 0.92 ^{a}	9.54 ± 0.80 ^{ab}
C20:0	0.13 ± 0.05	0.09 ± 0.03	0.08 ± 0.01	0.06 ± -	- ± -
C22:0	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	- ± -
Total MUFA [2]	27.7 ± 0.84 ^{ab}	28.4 ± 0.84 ^{a}	27.2 ± 1.17 ^{ab}	26.0 ± 10.0 ^{b}	26.0 ± 2.12 ^{b}
C14:1n5	1.09 ± 0.17 ^{b}	1.38 ± 0.10 ^{a}	1.12 ± 0.16 ^{b}	1.03 ± 0.12 ^{b}	1.09 ± 0.09 ^{b}
C16:1n7	1.87 ± 0.16 ^{ab}	2.11 ± 0.17 ^{a}	1.75 ± 0.17 ^{bc}	1.54 ± 0.18 ^{c}	1.62 ± 0.11 ^{c}
C18:1n9	23.3 ± 0.76 ^{ab}	23.5 ± 0.83 ^{a}	22.9 ± 1.19 ^{ab}	21.9 ± 0.90 ^{ab}	21.6 ± 2.05 ^{b}
C18:1n7	1.44 ± 0.13	1.42 ± 0.05	1.48 ± 0.19	1.57 ± 0.25	1.64 ± 0.18
Total PUFA [3]	3.34 ± 0.15	3.52 ± 0.15	3.41 ± 0.40	3.69 ± 0.58	3.78 ± 0.30
C18:2n6	2.09 ± 0.12 ^{b}	2.12 ± 0.16 ^{ab}	2.07 ± 0.29 ^{b}	2.27 ± 0.38 ^{ab}	2.47 ± 0.23 ^{a}
CLA c9, *t*11	0.53 ± 0.03	0.59 ± 0.03	0.54 ± 0.04	0.54 ± 0.08	0.53 ± 0.05
C18:3n3	0.40 ± 0.04 ^{b}	0.46 ± 0.03 ^{ab}	0.47 ± 0.09 ^{ab}	0.53 ± 0.14 ^{ab}	0.55 ± 0.09 ^{a}
C20:3n6	0.11 ± 0.02 ^{ab}	0.13 ± 0.01 ^{ab}	0.12 ± 0.02 ^{ab}	0.15 ± 0.07 ^{a}	0.09 ± 0.02 ^{b}
C20:4n6	0.18 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}	0.17 ± 0.01 ^{ab}	0.16 ± 0.06 ^{ab}	0.13 ± 0.01 ^{b}
C20:5n3	0.03 ± 0.01 ^{a}	0.04 ± 0.01 ^{a}	0.04 ± 0.01 ^{a}	0.04 ± 0.01 ^{a}	0.01 ± 0.00 ^{b}
Total n3 FA	0.43 ± 0.04	0.50 ± 0.03	0.51 ± 0.09	0.57 ± 0.15	0.56 ± 0.09
Total n6 FA	2.91 ± 0.12	3.02 ± 0.13	2.90 ± 0.32	3.12 ± 0.46	3.22 ± 0.26
n6:n3	6.77 ± 0.36 ^{a}	6.04 ± 0.36 ^{ab}	5.69 ± 0.50 ^{b}	5.47 ± 1.15 ^{b}	5.75 ± 0.92 ^{ab}

^{a,b,c} values with different superscript letters refer to a significant difference among the treatments (p-value < 0.05). [1] The total of saturated fatty acids. [2] The total of monounsaturated fatty acids. [3] The total of polyunsaturated fatty acids.

2.5. E-Nose Measurement of Milk

Frozen milk samples were thawed at 4 °C overnight, as well as homogenized by shaking and perfusing through a syringe, in order to obtain a well-represented sample for the e-nose measurement. An AlphaMOS Heracles Neo electronic nose (Alpha MOS,

Toulouse, France), based on flash gas chromatography with two columns (column-1: MXT-5, column-2: MXT-1701, Restek, Bellefonte, PA, USA) was equipped with an HS100 autosampler (PAL Systems, Switzerland) and was used for measurement. The AlphaSoft v12 (Alpha MOS, Toulouse, France) software was used to operate the electronic nose. The hydrogen that was used as a carrier gas during the measurement process was FID-grade, with an ultra-high purity of 99.999%. Furthermore, 1 mL of the individual milk samples was transferred into 20 mL headspace vials, which were then sealed with PTFE + silicon septa and kept at 10 °C until the incubation, prior to measurement. After 5 min of incubation at 50 °C (500 rpm agitation), 5 mL of the generated headspace was injected into the analyzer unit (carrier gas: hydrogen, the flow of carrier gas: 30 mL/min, trapping temperature: 30 °C, initial oven temperature: 50 °C, the endpoint of the oven temperature: 250 °C, heating rate: 2 °C/s, acquisition duration: 110 s, acquisition period: 0.01 s, injection speed: 125 µL/s, and cleaning phase: 480 s). A total of 5 measurements were conducted for each sample in a randomized order.

The general principle that underpins the Heracles analyzer as an electronic nose is found in the fact that the recorded chromatographic peaks are considered to be the response of a sensor that measures a particular volatile component of the milk sample. In addition, as a result, the position (i.e., retention time) of each peak that is recorded for the milk sample can be interpreted as a certain variable for that particular milk. Furthermore, the area under a peak can be regarded as the intensity value of that particular variable. The retention time of each peak, defined as the amount of time that the relevant compound spends in the column after it has been injected [36] was converted to a Kováts retention index (RI) [37]. As there are many peaks in the chromatogram for a batch of milk samples, a matrix of results was obtained, with m rows (m = the number of samples) and n columns (n = the number of variables/peaks measured and identified). This matrix, considered to represent the smell fingerprint of the samples, can then be evaluated via multivariate data analysis approaches.

2.6. Multivariate Data Analyses for E-Nose

The chromatogram peaks were used as sensor data, thus representing the smell fingerprints of the milk samples [22]. The AlphaSoft v12 (Alpha MOS, Toulouse, France) software was applied for the purposes of multivariate data analysis of the smell fingerprints. Moreover, principal component analysis (PCA) was used as an unsupervised classification method to describe the general multidimensional patterns of the e-nose data. In addition, discriminant factor analysis (DFA), a supervised classification method, was used in order to establish the possibility of group identification, based on the odor properties of the milk samples [38]. The most abundant compounds were selected based on the optimization of the DFA classification; furthermore, those compounds were identified based on the retention indices via the AroChemBase v7 database (Alpha MOS, Toulouse, France). The identification, which was based on both of the GC columns, confirmed the validity of the approach. In this study, the retention indices identified for column 1 were indexed with "-1-A", while those identified for column 2 were indexed with "-2-A"—where A refers to the method of calculating the intensity value of the variables (i.e., the area under the peak).

2.7. Statistical Analysis

The SPSS version 26 software (IBM, Armonk, NY, USA) was used to evaluate the milk FA data. The effect of feeding on the FA profile of the milk groups was also evaluated with a one-way analysis of variance (ANOVA), followed by a post hoc test (Tukey's) for a comparison of the various means (p-value < 0.05).

3. Results

3.1. Fatty Acid Profile of TMR

The major FAs present in all diets were linoleic acid (C18:2n6, LA), palmitic acid (C16:0), oleic acid (C18:1n9), and α-linolenic acid (C18:3n3, ALA). These accounted for

up to 83.5, 84.3, 81.6, 82.3, and 80.9% of the total FAs in the CTR, EXP-1, 2, 3, and 4 diets, respectively. Furthermore, the EXP-3 and -4 diets were shown to contain high proportions of capric acid (C10:0), lauric acid (C12:0), and myristic acid (C14:0). Moreover, the EXP-3 group possessed the highest proportion of stearic acid, whereas it was lowest in the EXP-1 group. In comparison with the control, all experimental diets showed low levels of oleic acid. In contrast, the ALA proportion was high in all mixtures, resulting in a drop in their n6:n3 ratios. Lastly, the EXP-4 group possessed the lowest LA level (Table 1).

3.2. Fatty Acid Profile of Milk

The different feed combinations affected the milk's fatty acid (FA) composition, as shown in Table 2. In contrast to the CTR and EXP-1 groups, the proportions of FAs changed the most in the EXP-3 and EXP-4 groups. In addition, the EXP-3 and -4 groups increased the level of total FA saturation in milk fat when compared to the CTR and EXP-2 groups. Further, the EXP-1 group increased the level of total FA monounsaturation when compared to EXP-3 and EXP-4. Indeed, higher amounts of medium- and long-chain saturated FAs (FAs with carbon chains between 6 and 21 carbons) were primarily responsible for the enhanced overall saturation levels observed, whereas no alteration was detected in the proportion of short-chain FAs (FAs with carbon chains of < 5). Feeding in the EXP-4 group increased the proportions of capric acid, lauric acid, and myristic acid in the milk when compared to the CTR, EXP-1, and EXP-2 groups. In addition, the EXP-3 milk showed higher levels of stearic acid (C18:0) than the EXP-1 milk. Notably, most monounsaturated FAs were more abundant in the EXP-1 group than those found in the EXP-3 and EXP-4 groups. Despite alterations in polyunsaturated fatty acids (PUFAs) between the EXP-4 and CTR diets, the overall levels of n3 and n6 were unaffected. However, the n6 to n3 FA ratios in the EXP-2 and EXP-3 milk samples were significantly lower than those found in the CTR milk. Compared to the CTR diet, the EXP-4 diet induced the most significant alterations in milk PUFAs. It also increased the levels of LA and ALA in milk but reduced the proportions of arachidonic (C20:4n6) and eicosapentaenoic (C20:5n3, EPA) acids, respectively.

3.3. E-Nose Results

Figure 2 shows the chromatograms of a milk sample, as measured using the two GC columns of an AlphaMOS Heracles Neo (one is polar, MXT-5, and the other, MXT-1701, has medium polarity), which were used for the sensor data representing the smell fingerprint.

PCA was used to describe the general multidimensional patterns of the acquired e-nose sensor data. A result of 95.985% of the total variance was described for the first and second principal components (PCs). However, the scores of the samples did not form any clusters, according to the original feeding groups. The CTR milk formed one cluster, while the EXP milk samples were largely overlapping. Since the PCA failed to reveal group differences, discriminant factor analysis (DFA), a supervised classification method, was carried out. Through this analysis, we achieved group identification based on the odor properties of the milk samples obtained from dairy cows that were fed the five diets; the results are presented in Figures 3 and 4.

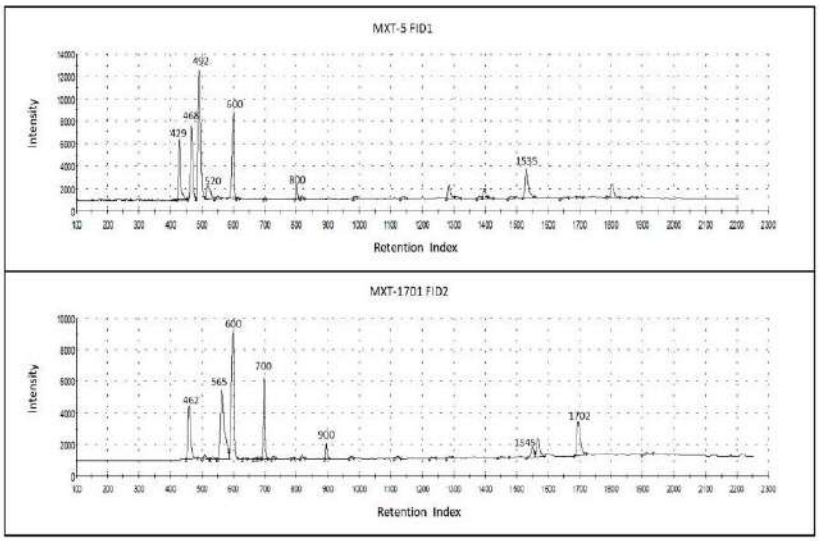


Figure 2. Chromatograms of a milk sample, as measured in the two GC columns of AlphaMOS Heracles Neo, MXT-5 (column #1), and the MXT-1701 (column #2), thereby indicating the most prominent volatile compounds of odor importance that were identified, with retention indices (Ris), acetaldehyde (429-1, 462-2), ethanol (468-1, 565-2), 2-propanol (492-1, 600-2), 2-methyl-propanol (520-1), butan-2-one (600-1, 700-2), ethyl-butyrate (800-1, 900-2), isopentyl salicylate (1535-1, 1702-2), and indole (1545-2).

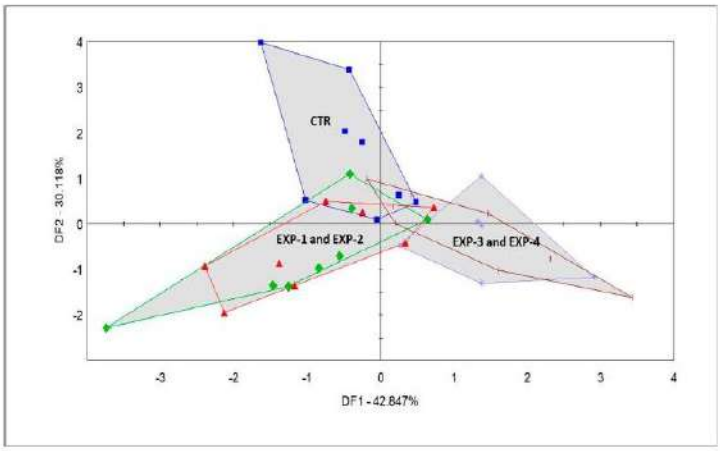


Figure 3. Discriminant factor analysis (DFA) score plot of the milk samples from the CTR group (blue square): corn silage + alfalfa haylage + vetch-triticale (VTH); EXP-1 (green diamond): CTR + Mix C; 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat (replacing VTH); EXP-2 (red triangle): CTR + Mix D; 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat (replacing VTH); EXP-3 (sea blue asterisk): CTR + Mix B; 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat (replacing VTH); EXP-4 (short brown vertical pipe): CTR + Mix A; 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat (replacing VTH).

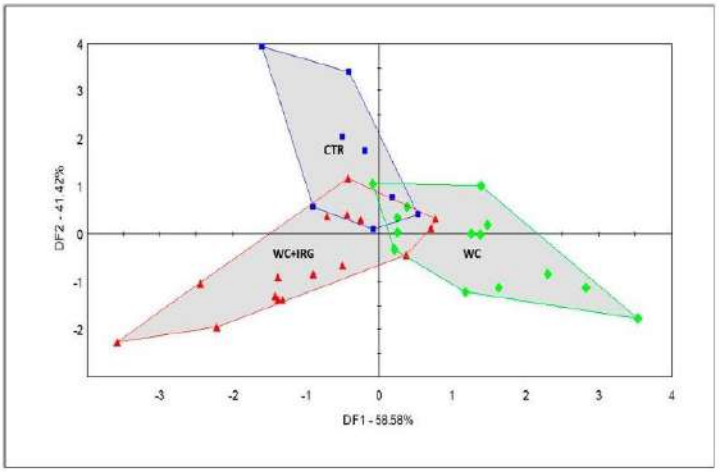


Figure 4. Discriminant factor analysis (DFA) score plot of the milk samples collected from cows fed with the five diets containing the different forage sources, as performed via the selected sensors (CTR (blue square): control diet; WC + IRG (red triangle): diets containing winter cereals and Italian ryegrass; WC (green diamond): diets containing winter cereals).

The DFA that was conducted with the five groups (Figure 3) shows the classification of the smell fingerprints of the milk samples, which was based on the various diet groups fed to the dairy cows. From the completion of the DFA, an overlapping of EXP-1 and EXP-2 milk samples and that of the EXP-3 and EXP-4 milk samples could be observed alongside DF 1, which explains 42.84% of the variation between the milk of these groups. The CTR group also separates from the rest (i.e., EXP-1 and EXP-2, as well as EXP-3 and EXP-4) alongside DF2, which comprises 30.118% of the variance.

The DFA (Figure 4) that was conducted with the three groups (CTR, WC, and WC + IRG) revealed the odor differences in the milk samples, which were caused by the feeds of different forage compositions. In addition, DF1 and DF2 helped to explain 58.58% and 41.42% of the total variance, respectively, with the milk of groups associated with feeding diets containing winter cereals and Italian ryegrass (WC + IRG), which make up the EXP-1 and EXP-2 groups, separating from the milk of groups associated with feeding diets containing winter cereals (WC), which make up EXP-3 and EXP-4, alongside DF1. The milk of the CTR group separates from that of the WC + IRG and WC groups, alongside DF2. This reveals that the odor variation between the milk samples of the WC + IRG and WC groups was bigger than the variation that existed between the milk samples of the CTR group and the milk samples of the experimental groups. Furthermore, the CTR and WC + IRG milk samples entirely overlap in the plane of the most dominant discriminating factor (DF1), while WC shows little overlap with the aforementioned samples. Thus, the WC milk samples were proven to possess the most different odor when compared with the other two main classes (i.e., WC + IRG and CTR).

Odor-based discrimination in the context of milk from the CTR group was largely influenced by sensor 800-1-A (ethyl-butyrate), whereas the milk of the WC groups was influenced largely by sensors 492-1-A (2-propanol) and 600-2-A (butan-2-one); furthermore, the milk of the WC + IRG groups was influenced largely by sensors 468-1-A and 565-2-A (ethanol).

Figure 5, in columns 1 and 2, show the most significant odor compounds that were detected. Furthermore, as based on the RIs, acetaldehyde (429-1-A, 462-2-A), ethanol (468-1-A, 565-2-A), 2-propanol (492-1A, 600-2-A), butan-2-one (600-1-A, 700-2-A), ethyl-butyrate (800-1-A, 900-2-A), and isopentyl salicylate (1535-1-A, 1702-2-A) were identified. Figure 5 also confirms that the WC milk samples possessed the most specific odor. The reason for

this is that the sensor signals (odor intensities) of 2-propanol and butan-2-one were more prominent within these samples.

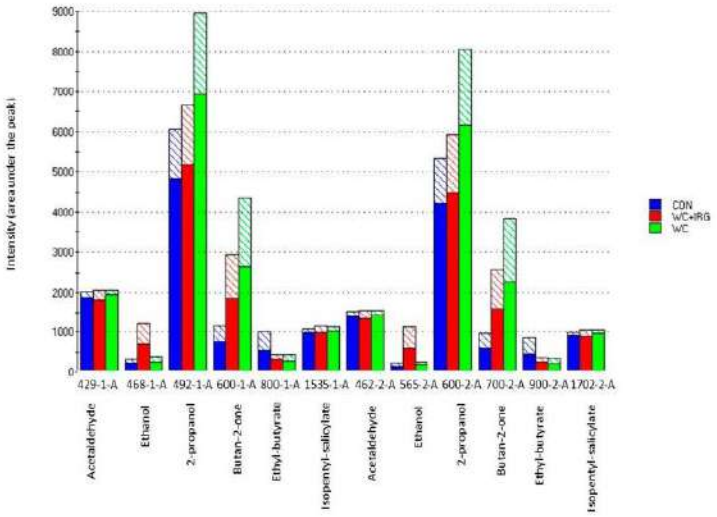


Figure 5. Bar graph of detection intensities, as measured with the electronic nose at retention indices (RI) that corresponded to certain identified odors, which produced volatile compounds, as detailed in column 1 ("-1-A") and 2 ("-2-A"), showing the milk samples obtained from dairy cows that were fed with the control (CTR) diet, as well as the diets containing winter cereal and Italian ryegrass (WC + IRG) or winter cereals only (WC). Solid fill areas of the graphs indicate the mean detection intensities, while the pattern fill indicate the standard deviation.

4. Discussion

4.1. Fatty Acids of Milk

Milk FAs are mainly derived from diet, adipose tissue, and de novo biosynthesis. Thus, such data must be assessed with caution due to the various factors altering the FA composition of whole milk. Lipolysis is the limiting factor behind the adipose tissue's contribution to milk FAs, which depends on adipocyte size, lactation stage, milk production, and energy demand [39]. Once milk production and energy level were equivalent across treatments, we assumed that the lipolysis of adipose tissue contributed minimally to our substantial findings regarding milk FAs.

All the analyzed milk samples' FA profiles were consistent with the comprehensive data obtained on bovine milk lipids [40]. Moreover, the short-chain FAs are mainly absorbed via the rumen wall with simple diffusion; thus, their concentration in the rumen greatly contributes to their levels in milk fat. In a recent study using similar silage mixtures, neither the content of short-chain FAs in the rumen nor its pH changed after 6 h of feeding mixed silage [23], thereby suggesting that there are no marked variations in short-chain FA concentrations in milk fat, which is in agreement with our findings.

When compared to the EXP-3 and EXP-4 (winter cereal mixtures) diets, the overall saturation of milk FAs increased (ca. + 2% surplus) in those cows fed on diets of Italian ryegrass mixtures (i.e., EXP-1 and EXP-2), which finding was in parallel with the high proportions of capric, lauric, myristic, and stearic acids in their respective milk samples. Moreover, the medium- and long-chain FAs are absorbed into the bloodstream through the intestinal wall. Unlike non-ruminants, ruminants possess a microbiome that biohydrogenates (poly) unsaturated FAs in the rumen, thus converting LA and ALA to saturated stearic acid. Furthermore, the up-regulation of de novo biosynthesis regarding saturated fatty acids (SFAs) is strongly associated with the increased consumption of starch,

thus leading to an increase in the milk's total FA saturation level [41]. We observed no substantial difference in the dry matter or starch content of various diets, whereas the LA and ALA levels were greatest in the milk from cows fed diets of winter cereal mixtures. Those findings indicate no change in the PUFA biohydrogenation process in the rumen, or in the de novo biosynthesis of SFAs. There is an association between the FA composition of feed and milk fat [42]. This is partially inconsistent with our present findings, as the total saturation level in the diets of winter cereal mixtures was not markedly different from that of the EXP-1 group. Thus, the diet's FA content is not necessary to reflect the FA profile of the milk, which is in agreement with the finding of Colín-Navarro et al. [43]. A possible explanation regarding the high total saturation levels in the milk of the EXP-3 and EXP-4 groups may be based on their conversion rate to unsaturated carbon chains.

Italian ryegrass mixtures' feeding led to higher FA monounsaturation levels in milk than those found when feeding the winter cereal mixtures. The lowest values in diets were found in the EXP-3 and EXP-4 groups, respectively, thereby indicating that levels of monounsaturated FAs in milk depend on their amount in the feed. Furthermore, our findings may be attributed to the de novo biosynthesis of these FAs by the liver and mammary gland, in which the alteration of stearoyl-CoA desaturase activity is hypothesized. This enzyme's activity was not assessed; furthermore, its indicator FAs in the plasma stream or liver was not determined. However, we can indirectly rule out the high activity of desaturase enzymes, as C14:1/C14:0, C16:1/C16:0 and C18:1/C18:0 were markedly highest in the EXP-1 group when compared to the EXP-3 and EXP-4 groups.

The proportion of PUFA in milk fat is affected by feedstuff conditions (i.e., fresh, dry, haylage, and silage) and the management system (i.e., grazing or zero grazing) [44]. Under the current design, cows were fully fed on the allotted rations, with no grazing activity permitted. Notably, the EXP-4 milk comprised the highest LA and ALA proportions. The elevated ALA in milk may be attributed to its level in the feed; however, this cannot be applied to LA. PUFAs' variation in milk may occur from the effect of forage on the rates of lipolysis and biohydrogenation in the rumen [45]. Indeed, our saturation results indirectly suggest no alteration in lipolysis or biohydrogenation activities, thus postulating an alternate route underlying these increases in LA and ALA. Furthermore, mammals lack the enzymes (delta-12 and delta-15 desaturases) that produce LA and ALA, [46]. We suspect that a probable down-regulation in the activity of delta-5 and/or delta-6 desaturases contributes to the accumulation of ALA and LA. This proposal seems feasible as C20:4(n6)/C20:3(n6) and C20:5(n3)/C18:3(n3) were low in the EXP-4 milk, for which a 30 and 72% reduction was identified, respectively, when compared to the control. We lack information on the particular nutrient that could potentially alter enzyme activities. Therefore, further investigations are essential to substantiate our hypothesis.

The most remarkable impact of the EXP-2 and EXP-3 diets was found in the substantial decline of the n6:n3 ratio, even though no grazing was permitted. Though there is no worldwide consensus on the defined-intake guidelines for the ratio between omega classes, lower values of n6:n3 (possibly, below 4) are certainly favored [47].

4.2. *Odor Profiles of Milk*

Generally, volatile compounds belonging to the acetaldehyde, alcohol, ketone, and ester groups form that part of the aroma or odor matrix that most influences the acceptability or rejection of dairy products by consumers [48,49]. For instance, at low levels, acetaldehyde can contribute a fruity odor to food. However, at higher levels, the odor can be very unpleasant to consumers [48]. In this current study, the intensity of acetaldehyde in all the milk samples analyzed was quite similar, but not at high enough levels that might cause an unpleasant odor [49]. In addition, ethanol was identified more often in the milk of the WC + IRG group for both columns, although only at small intensities. Herein, a study reported the possible transmission of silage flavors, including the alcohol produced during forage fermentation into bovine milk [50]. Diets that contain silage and are classified as well-fermented and having a pleasant aroma may still contain some level of ethanol,

which can, in turn, impart milk odor [51]. The most abundant volatile compound measured in all the milk samples, especially in the WC group, was 2-propanol, also known as isopropyl alcohol, which is a water-soluble aliphatic alcohol with a sweet odor (this was also reported by Sympoura et al. [52]), as observed in milk obtained from dairy cows that are fed with supplemented extruded linseed and α-tocopherol [52]. Though this study did not use similar feed supplements, the utilized forage sources—especially winter cereals—contain significant levels of naturally occurring α-tocopherol [53]. Additionally, 2-butanone, butan-2-one, or methyl ethyl ketone—an organic compound with a sharp-sweet odor—were reported to be present in the milk obtained from cows fed with mycorrhizal ensiled forage [54]. In the present study, the intensity of butan-2-one was higher in the WC milk. The identified odor that was determined to be the producing compound in the CTR milk was ethyl butyrate, which is also referred to as ethyl butanoate or butyric ether and is an ester that possesses a fruity odor [55]. According to Marina et al., esters are the most important odorants in dairy food products, due to their ability to influence the general odor or aroma matrix of such products [56].

5. Conclusions

The current study revealed how the feeding of dairy cows with different experimental total mixed rations, i.e., the inclusion of Italian ryegrasses (IRG) and winter cereals (WC), affected the fatty acid and odor profiles of raw milk. The proportions of fatty acids changed, most notably in the milk samples of cows fed winter cereal diets. Replacing vetch-triticale with WC increased the total FA saturation levels in the milk, whereas WC + IRG increased the total FA monounsaturation levels. However, a decrease in the n6:n3 FA ratio was observed in the WC + IRG and WC milk samples. The use of WC and WC + IRG mixed silages in the feed ration of dairy cows enabled the production of milk with a more favorable fatty acid profile, the human-health benefits of which have been widely documented. With respect to the odor profiling of the milk groups via an e-nose, the odor variation between the milk samples from the experimental groups was larger than the variation that existed between the milk of the CTR group and that of the experimental groups. An inclusion of WC or WC + IRG silages, as was applied in this study, resulted in beneficial changes in the milk's FA composition (the n6:n3 FA ratio was lowered). Based on the e-nose results, the inclusion of WC + IRG proved to be more beneficial than that of WC as it causes a less prominent odor alteration in milk when compared to the CTR. The e-nose is a highly useful and necessary element of supportive or complementary technology in chemical analysis to identify and quantify the organoleptic changes in dairy products that are caused by novel feed sources.

Author Contributions: H.G.Y.: conceptualization, acquisition, analysis, and interpretation of e-nose data, as well as drafting and editing the original manuscript; O.A.: curation and interpretation of GC-FID data, as well as drafting and editing the original manuscript; A.S.: curation and interpretation of GC-FID data, as well as drafting and editing the original manuscript; T.T.: conceptualization, design, acquisition, analysis, interpretation of data, drafting, editing the original manuscript, and funding acquisition; G.B.: conceptualization, design, analysis, acquisition, curation, and interpretation of data, as well as drafting and editing the original manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work was supported by the EFOP-3.6.3-VEKOP-16-2017-00005, "support of scientific research teams'" project. The support of the Hungarian National Laboratory project, RRF-2.3.1-21-2022-00007, is also gratefully acknowledged.

Institutional Review Board Statement: The animals were treated following the European Communities Council Directives (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII).

Data Availability Statement: The data are available from the corresponding author.

Conflicts of Interest: Authors George Bazar and Tamás Tóth were employed by the company ADEXGO Kft. The company was not involved in the study design, collection, analysis, or interpretation of data, the writing of this article, or the decision to submit it for publication. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Sanchez-Villegas, A.; Martínez, J.A.; Prättälä, R.; Toledo, E.; Roos, G.; Martínez-González, M.A. A Systematic Review of Socioeconomic Differences in Food Habits in Europe: Consumption of Cheese and Milk. *Eur. J. Clin. Nutr.* **2003**, *57*, 917–929. [CrossRef] [PubMed]
2. Zakaria, Z.; Yun, W.S.; Alias, N.; Noor, S.N.M.; Zakaria, A.J.; Mustapha, Z.; Hussin, N.; Wan Taib, W.R.; Ahmad, A.; Yusoff, N.A.M. Physicochemical Composition, Microbiological Quality and Consumers' Acceptability of Raw and Pasteurized Locally Produced Goat Milk. *Malays. J. Fundam. Appl. Sci.* **2020**, *16*, 475–482. [CrossRef]
3. Stanton, C.; Mills, S.; Ryan, A.; Di Gioia, D.; Ross, R.P. Influence of Pasture Feeding on Milk and Meat Products in Terms of Human Health and Product Quality. *Ir. J. Agric. Food Res.* **2021**, *59*, 292–302. [CrossRef]
4. Olika, C.D. Review on Effect of Nutrition on Milk Composition and Yield of Dairy Cows. *Eur. J. Sci. Innov. Technol.* **2021**, *1*, 24–31.
5. Martin, B.; Verdier-Metz, I.; Buchin, S.; Hurtaud, C.; Coulon, J.B. How Do the Nature of Forages and Pasture Diversity Influence the Sensory Quality of Dairy Livestock Products? *Anim. Sci.* **2005**, *81*, 205–212. [CrossRef]
6. Morand-Fehr, P.; Fedele, V.; Decandia, M.; Le Frileux, Y. Influence of Farming and Feeding Systems on Composition and Quality of Goat and Sheep Milk. *Small Rumin. Res.* **2007**, *68*, 20–34. [CrossRef]
7. Park, Y.; Jeanjulien, C.; Siddique, A. Factors Affecting Sensory Quality of Goat Milk Cheeses: A Review. *Adv. Dairy Res.* **2017**, *05*, 2. [CrossRef]
8. Bennato, F.; Ianni, A.; Innosa, D.; Martino, C.; Grotta, L.; Pomilio, F.; Verna, M.; Martino, G. Influence of Licorice Root Feeding on Chemical-Nutritional Quality of Cow Milk and Stracciata Cheese, an Italian Traditional Fresh Dairy Product. *Animals.* **2019**, *9*, 1153. [CrossRef]
9. Castellani, F.; Vitali, A.; Bernardi, N.; Marone, E.; Palazzo, F.; Grotta, L.; Martino, G. Dietary Supplementation with Dried Olive Pomace in Dairy Cows Modifies the Composition of Fatty Acids and the Aromatic Profile in Milk and Related Cheese. *J. Dairy Sci.* **2017**, *100*, 8658–8669. [CrossRef] [PubMed]
10. Till, B.E.; Huntington, J.A.; Posri, W.; Early, R.; Taylor-Pickard, J.; Sinclair, L.A. Influence of Rate of Inclusion of Microalgae on the Sensory Characteristics and Fatty Acid Composition of Cheese and Performance of Dairy Cows. *J. Dairy Sci.* **2019**, *102*, 10934–10946. [CrossRef] [PubMed]
11. Tóth, T.; Mwau, P.J.; Bázár, G.; Andrássy-Baka, G.; Hingyi, H.; Csavajda, É.; Varga, L. Effect of Feed Supplementation Based on Extruded Linseed Meal and Fish Oil on Composition and Sensory Properties of Raw Milk and Ultra-High Temperature Treated Milk. *Int. Dairy J.* **2019**, *99*, 104552. [CrossRef]
12. Hurtaud, C.; Faucon, F.; Couvreur, S.; Peyraud, J.L. Linear Relationship between Increasing Amounts of Extruded Linseed in Dairy Cow Diet and Milk Fatty Acid Composition and Butter Properties. *J. Dairy Sci.* **2010**, *93*, 1429–1443. [CrossRef] [PubMed]
13. Angeles-Hernandez, J.C.; Vieyra Alberto, R.; Kebreab, E.; Appuhamy, J.A.D.R.N.; Dougherty, H.C.; Castelan-Ortega, O.; Gonzalez-Ronquillo, M. Effect of Forage to Concentrate Ratio and Fat Supplementation on Milk Composition in Dairy Sheep: A Meta-Analysis. *Livest. Sci.* **2020**, *238*, 104069. [CrossRef]
14. Renes, E.; Gómez-Cortés, P.; de la Fuente, M.A.; Fernández, D.; Tornadijo, M.E.; Fresno, J.M. Effect of Forage Type in the Ovine Diet on the Nutritional Profile of Sheep Milk Cheese Fat. *J. Dairy Sci.* **2020**, *103*, 63–71. [CrossRef] [PubMed]
15. Serrapica, F.; Uzun, P.; Masucci, F.; Napolitano, F.; Braghieri, A.; Genovese, A.; Sacchi, R.; Romano, R.; Barone, C.M.A.; Di Francia, A. Hay or Silage? How the Forage Preservation Method Changes the Volatile Compounds and Sensory Properties of Caciocavallo Cheese. *J. Dairy Sci.* **2020**, *103*, 1391–1403. [CrossRef] [PubMed]
16. Renes, E.; Fernández, D.; Abarquero, D.; Ladero, V.; Álvarez, M.A.; Tornadijo, M.E.; Fresno, J.M. Effect of Forage Type, Season, and Ripening Time on Selected Quality Properties of Sheep Milk Cheese. *J. Dairy Sci.* **2021**, *104*, 2539–2552. [CrossRef]
17. Palmquist, D.L.; Denise Beaulieu, A.; Barbano, D.M. Feed and Animal Factors Influencing Milk Fat Composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771. [CrossRef]
18. Ashes, J.R.; Gulati, S.K.; Scott, T.W. Potential to Alter the Content and Composition of Milk Fat Through Nutrition. *J. Dairy Sci.* **1997**, *80*, 2204–2212. [CrossRef]
19. Poveda, J.M.; Cabezas, L. Free Fatty Acid Composition of Regionally-Produced Spanish Goat Cheese and Relationship with Sensory Characteristics. *Food Chem.* **2006**, *95*, 307–311. [CrossRef]
20. Le Quéré, J.-L.; Pierre, A.; Riaublanc, A.; Demaizières, D. Characterization of Aroma Compounds in the Volatile Fraction of Soft Goat Cheese during Ripening. *Lait* **1998**, *78*, 279–290. [CrossRef]
21. Bernardes, T.F.; Daniel, J.L.P.; Adesogan, A.T.; McAllister, T.A.; Drouin, P.; Nussio, L.G.; Huhtanen, P.; Tremblay, G.F.; Bélanger, G.; Cai, Y. Silage Review: Unique Challenges of Silages Made in Hot and Cold Regions. *J. Dairy Sci.* **2018**, *101*, 4001–4019. [CrossRef] [PubMed]

22. Worku, A.; Tóth, T.; Orosz, S.; Fébel, H.; Kacsala, L.; Húth, B.; Hoffmann, R.; Yakubu, H.G.; Bazar, G.; Tóthi, R. Aroma Profile, Microbial and Chemical Quality of Ensiled Green Forages Mixtures of Winter Cereals and Italian Ryegrass. *Agriculture* **2021**, *11*, 512. [CrossRef]
23. Worku, A.; Tóthi, R.; Orosz, S.; Fébel, H.; Kacsala, L.; Húth, B.; Hoffmann, R.; Tóth, T. In Situ Ruminal Degradability and Fermentation Characteristics of Novel Mixtures of Winter Cereal and Italian Ryegrass plus Winter Cereal Silages. *Czech J. Anim. Sci.* **2021**, *66*, 302–314. [CrossRef]
24. Worku, A.; Tóthi, R.; Orosz, S.; Fébel, H.; Kacsala, L.; Vermeire, D.; Tóth, T. Novel Mixtures of Italian Ryegrass and Winter Cereals: Influence of Ensiling on Nutritional Composition, Fermentation Characteristics, Microbial Counts and Ruminal Degradability. *Ital. J. Anim. Sci.* **2021**, *20*, 749–761. [CrossRef]
25. Clark, S.; Costello, M.C.; Drake, M.D.; Bodyfelt, F. *The Sensory Evaluation of Dairy Products*, 2nd ed.; Springer Science and Business Media: New York, NY, USA, 2009. [CrossRef]
26. Andrewes, P.; Bullock, S.; Turnbull, R.; Coolbear, T. Chemical Instrumental Analysis versus Human Evaluation to Measure Sensory Properties of Dairy Products: What Is Fit for Purpose? *Int. Dairy J.* **2021**, *121*, 105098. [CrossRef]
27. Bliss, P.J.; Schulz, T.J.; Senger, T.; Kaye, R.B. Odour Measurement—Factors Affecting Olfactometry Panel Performance. *Water Sci. Technol.* **1996**, *34*, 549–556. [CrossRef]
28. Yakubu, H.G.; Kovacs, Z.; Toth, T.; Bazar, G. Trends in Artificial Aroma Sensing by Means of Electronic Nose Technologies to Advance Dairy Production—A Review. *Crit. Rev. Food Sci. Nutr.* **2021**, *63*, 234–248. [CrossRef]
29. Di Natale, C.; Macagnano, A.; D'amico, A.; Paolesse, R. Artificial Olfaction Systems: Principles and Applications to Food Analysis. *Biotechnol. Agron. Soc. Environ.* **2001**, *5*, 159–165.
30. Malekkhahi, M.; Tahmasbi, A.M.; Naserian, A.A.; Danesh-Mesgaran, M.; Kleen, J.L.; AlZahal, O.; Ghaffari, M.H. Effects of Supplementation of Active Dried Yeast and Malate during Sub-Acute Ruminal Acidosis on Rumen Fermentation, Microbial Population, Selected Blood Metabolites, and Milk Production in Dairy Cows. *Anim. Feed Sci. Technol.* **2016**, *213*, 29–43. [CrossRef]
31. Association of Official Analytical Chemists. *Official Methods of Analysis*, 18th ed.; AOAC International: Washington, DC, USA, 2006.
32. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-Starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
33. Folch, J.; Lees, M.; Sloane-Stanley, G.H. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef] [PubMed]
34. Christie, W.W. A Simple Procedure for Rapid Transmethylation of Glycerolipids and Cholesteryl Esters. *J. Lipid Res.* **1982**, *23*, 1072–1075. [CrossRef] [PubMed]
35. Christie, W.W. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*, 3rd ed.; Oily Press: Bridgwater, UK, 2003.
36. Burian, C.; Brezmes, J.; Vinaixa, M.; Cañellas, N.; Llobet, E.; Vilanova, X.; Correig, X. MS-Electronic Nose Performance Improvement Using the Retention Time Dimension and Two-Way and Three-Way Data Processing Methods. *Sens. Actuators B Chem.* **2010**, *143*, 759–768. [CrossRef]
37. Guardino, X.; Albaigés, J.; Firpo, G.; Rodríguez-Viñals, R.; Gassiot, M. Accuracy in the Determination of the Kováts Retention Index. Mathematical Dead Time. *J. Chromatogr. A* **1976**, *118*, 13–22. [CrossRef]
38. Næs, T.; Isaksson, T.; Fearn, T.; Davies, T. *A User Friendly Guide to Multivariate Calibration and Classification*; NIR Publications: Chichester, UK, 2002.
39. Contreras, G.A.; Strieder-Barboza, C.; Raphael, W. Adipose Tissue Lipolysis and Remodeling during the Transition Period of Dairy Cows. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 1–12. [CrossRef] [PubMed]
40. Jensen, R.G. The Composition of Bovine Milk Lipids: January 1995 to December 2000. *J. Dairy Sci.* **2002**, *85*, 295–350. [CrossRef]
41. Walker, G.P.; Dunshea, F.R.; Doyle, P.T. Effects of Nutrition and Management on the Production and Composition of Milk Fat and Protein: A Review. *Aust. J. Agric. Res.* **2004**, *55*, 1009–1028. [CrossRef]
42. Morales-Almaráz, E.; De La Roza-Delgado, B.; González, A.; Soldado, A.; Rodríguez, M.L.; Peláez, M.; Vicente, F. Effect of Feeding System on Unsaturated Fatty Acid Level in Milk of Dairy Cows. *Renew. Agric. Food Syst.* **2011**, *26*, 224–229. [CrossRef]
43. Colín-navarro, V.; López-gonzález, F.; Morales-almaráz, E.; González-alcántara, F.D.J.; Estrada-flores, J.G.; Arriaga-jordán, M.C. Fatty Acid Profile in Milk of Cows Fed Triticale Silage in Small-Scale Dairy Systems in the Highlands of Central Mexico. *J. Appl. Anim. Res.* **2021**, *49*, 75–82. [CrossRef]
44. Kalač, P.; Samková, E. The Effects of Feeding Various Forages on Fatty Acid Composition of Bovine Milk Fat: A Review. *Czech J. Anim. Sci.* **2010**, *55*, 521–537. [CrossRef]
45. Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.F.; Scollan, N.D. Increasing the Concentrations of Beneficial Polyunsaturated Fatty Acids in Milk Produced by Dairy Cows in High-Forage Systems. *Anim. Feed Sci. Technol.* **2006**, *131*, 168–206. [CrossRef]
46. Lee, J.M.; Lee, H.; Kang, S.B.; Park, W.J. Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. *Nutrients* **2016**, *8*, 23. [CrossRef] [PubMed]
47. Simopoulos, A.P. The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids. *Biomed. Pharmacother.* **2002**, *56*, 365–379. [CrossRef] [PubMed]
48. Giberti, A.; Carotta, M.C.; Fabbri, B.; Gherardi, S.; Guidi, V.; Malagù, C. High-Sensitivity Detection of Acetaldehyde. *Sens. Actuators B Chem.* **2012**, *174*, 402–405. [CrossRef]

49. Van Aardt, M.; Duncan, S.E.; Bourne, D.; Marcy, J.E.; Long, T.E.; Hackney, C.R.; Heisey, C. Flavor Threshold for Acetaldehyde in Milk, Chocolate Milk, and Spring Water Using Solid Phase Microextraction Gas Chromatography for Quantification. *J. Agric. Food Chem.* **2001**, *49*, 1377–1381. [CrossRef] [PubMed]
50. Shipe, W.F.; Ledford, R.A.; Peterson, R.D.; Scanlan, R.A.; Geerken, H.F.; Dougherty, R.W.; Morgan, M.E. Physiological Mechanisms Involved in Transmitting Flavors and Odors to Milk. II. Transmission of Some Flavor Components of Silage. *J. Dairy Sci.* **1962**, *45*, 477–480. [CrossRef]
51. Randby, A.T.; Selmer-Olsen, I.; Baevre, L. Effect of Ethanol in Feed on Milk Flavor and Chemical Composition. *J. Dairy Sci.* **1999**, *82*, 420–428. [CrossRef] [PubMed]
52. Sympoura, F.; Cornu, A.; Tournayre, P.; Massouras, T.; Berdagué, J.L.; Martin, B. Odor Compounds in Cheese Made from the Milk of Cows Supplemented with Extruded Linseed and α-Tocopherol. *J. Dairy Sci.* **2009**, *92*, 3040–3048. [CrossRef]
53. Lachman, J.; Hejtmánková, A.; Orsák, M.; Popov, M.; Martinek, P. Tocotrienols and Tocopherols in Colored-Grain Wheat, Tritordeum and Barley. *Food Chem.* **2018**, *240*, 725–735. [CrossRef] [PubMed]
54. Genovese, A.; Marrazzo, A.; De Luca, L.; Romano, R.; Manzo, N.; Masucci, F.; Di Francia, A.; Sacchi, R. Volatile Organic Compound and Fatty Acid Profile of Milk from Cows and Buffaloes Fed Mycorrhizal or Nonmycorrhizal Ensiled Forage. *Molecules* **2019**, *24*, 1616. [CrossRef]
55. Nagata, Y. Measurement of Odor Threshold by Triangle Odor Bag Method. *Meas. Rev.* **2003**, *118*, 118–127.
56. Marina, R.; Paola, D.; Mondello, L. *Hand Book of Dairy Food Analysis*, 2nd ed.; Toldrá, F., Nollet, L.M.L., Eds.; CRC Press: Boca Raton, FL, USA, 2021. [CrossRef]

5.4. Micro-encapsulated microalgae oil supplementation has no systematic effect on the odor of vanilla shake-test of an electronic nose

Title	Micro-encapsulated microalgae oil supplementation has no systematic effect on the odor of vanilla shake– test of an electronic nose
Authors	Yakubu Haruna Gado, Omeralfaroug Ali, Imre Ilyés, Dorottya Vigyázó, Brigitta Bóta, George Bazar, Tamás Tóth, András Szabó
Journal	Food
Year Volume Issue	2023 11 3452
Pages Quartile IF	1-19 Q1 5.200
Doi	10.3390/foods11213452

foods

MDPI

Article

Micro-Encapsulated Microalgae Oil Supplementation Has No Systematic Effect on the Odor of Vanilla Shake-Test of an Electronic Nose

Haruna Gado Yakubu [1], Omeralfaroug Ali [1], Imre Ilyés [2], Dorottya Vigyázó [2], Brigitta Bóta [3], George Bazar [4,*], Tamás Tóth [4] and András Szabó [1]

[1] Agribiotechnology and Precision Breeding for Food Security National Laboratory, Institute of Physiology and Nutrition, Department of Physiology and Animal Health, Hungarian University of Agriculture and Life Sciences, Guba Sándor utca 40, 7400 Kaposvár, Hungary
[2] HuBakers Trade Ltd., ÁTI-Sziget Ipari Park 11, 2310 Szigetszentmiklós, Hungary
[3] ELKH-MATE Mycotoxins in the Food Chain Research Group, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences, Guba Sándor utca 40, 7400 Kaposvár, Hungary
[4] ADEXGO Kft., Lapostelki utca 13, 8230 Balatonfüred, Hungary
* Correspondence: bazar@agrilab.hu; Tel.: +36-302248059

Abstract: In this study, we aimed to carry out the efficient fortification of vanilla milkshakes with micro-encapsulated microalgae oil (brand: S17-P100) without distorting the product's odor. A 10-step oil-enrichment protocol was developed using an inclusion rate of 0.2 to 2 $w/w\%$. Fatty acid (FA) profile analysis was performed using methyl esters with the GC-MS technique, and the recovery of docosahexaenoic acid (C22:6 n3, DHA) was robust ($r = 0.97$, $p < 0.001$). The enrichment process increased the DHA level to 412 mg/100 g. Based on this finding, a flash-GC-based electronic nose (e-nose) was used to describe the product's odor. Applying principal component (PC) analysis to the acquired sensor data revealed that for the first four PCs, only PC3 (6.5%) showed a difference between the control and the supplemented products. However, no systematic pattern of odor profiles corresponding to the percentages of supplementation was observed within the PC planes. Similarly, when discriminant factor analysis (DFA) was applied, though a classification of the control and supplemented products, we obtained a validation score of 98%, and the classification pattern of the odor profiles did not follow a systematic format. Again, when a more targeted approach such as the partial least square regression (PLSR) was used on the most dominant sensors, a weak relationship ($R^2 = 0.50$) was observed, indicating that there was no linear combination of the qualitative sensors' signals that could accurately describe the supplemented concentration variation. It can therefore be inferred that no detectable off-odor was present as a side effect of the increase in the oil concentration. Some volatile compounds of importance in regard to the odor, such as ethylacetate, ethyl-isobutarate, pentanal and pentyl butanoate, were found in the supplemented product. Although the presence of yeasts and molds was excluded from the product, ethanol was detected in all samples, but with an intensity that was insufficient to cause an off-odor.

Keywords: fortification; odor profiling; machine olfaction; docosahexaenoic acid; food enrichment; functional food

Citation: Yakubu, H.G.; Ali, O.; Ilyés, I.; Vigyázó, D.; Bóta, B.; Bazar, G.; Tóth, T.; Szabó, A. Micro-Encapsulated Microalgae Oil Supplementation Has No Systematic Effect on the Odor of Vanilla Shake-Test of an Electronic Nose. *Foods* **2022**, *11*, 3452. https://doi.org/10.3390/foods11213452

Academic Editor: Diana De Santis

Received: 26 September 2022
Accepted: 25 October 2022
Published: 31 October 2022

1. Introduction

Micro-encapsulation is a widely used and accepted form of flavor and aroma preservation in the food industry, and it has been an important technique for a long time. Micro-encapsulation is not only successful in preserving or masking flavor and aroma compounds in foods; it has also been found to enhance the thermal and oxidative stability of aromatic compounds in foods [1].

The application of micro-encapsulation in food systems has also been carried out with the aim of overcoming the challenges of high volatility, or to control the fast release and to

improve the poor bioavailability of bioactive compounds [1–3]. Many techniques, such as spray-drying (SD), freeze-drying (FD), coacervation, spray granulation (SG), emulsification, the use of supercritical fluids (SCFs), and electrospraying [4–8], have been reported as successful encapsulation methods of docosahexaenoic acid (C22:6n3, DHA) in foods. The success of DHA encapsulation may also depend on the composition of the encapsulation wall material [9,10]. Due to the rapid growth of the food industry sector, there have been possibilities to improve the nutritional quality of products through the development of so-called "functional foods" [11,12]. Microalgae biomass and oil have been widely incorporated into foods and beverages [13] during the past decade. Microalgae oil has been successfully incorporated into foods such as ice cream, milk drinks [14,15], sausages [16], yoghurt [17], and cheeses [18,19], increasing the omega 3 fatty acid (n3 FA) content of these products without altering their odor.

The addition of microalgae oil to a vanilla milkshake can improve its fatty acid (FA) composition through increasing the bioactive n3 FA proportions, such as eicosapentaenoic acid (C20:5n3, EPA) and DHA. These FAs have been documented to reduce the risk of coronary heart disease and inflammatory disorders [20,21].

Ideally, fish and microalgae oils, which are widely known to be rich in DHA, may also lead to an unpleasant odor and flavor, which is a product of their poor oxidative stability [22], and may affect or limit their use as nutraceuticals or as functional food agents. Interestingly, some methods have been applied over the years to stabilize highly polyunsaturated oils in foods, such as the addition of antioxidants to the bulk oil. However, this does not allow the successful removal of all the unpleasant flavors or odors [6,9], and encapsulation has been very widely used to achieve this purpose.

The sensory characteristics of foods enriched with DHA, such as their odor and taste, can vary according to the levels of supplementation. In the case of odor, the electronic nose (e-nose) is currently one of the most widely used technologies for rapid aroma profiling of foods and has proven to be an efficient sensory technology [23,24]. The e-nose, in some instances, is used as the best complementary technique to validate conventional or traditional methods of odor profiling and can even substitute those when technical conditions are required [25]. As a rapid analytical system or method, the e-nose is made up of three major parts: the sample delivery, detection, and computing systems [26].

To date, numerous technological solutions have been made available, using various materials, such as sensor arrays with metal oxide semiconductor sensors (MOS), the metal-oxide-semiconductor field-effect transistor (MOSFET), conducting polymer composites and intrinsically conducting polymers [27–29], and the gas chromatography (GC)-based electronic nose. Hence, applying chemometrics to e-nose sensory data helps to discriminate between various identified volatile compounds [30].

The milkshake is a very common dairy product that is consumed by both young people and adults due to its easy/rapid method of preparation and its desirable flavors. On the other hand, S17-P100 is a micro-encapsulated microalgae oil product that is known to be rich in EPA and DHA, which is also commonly available on the European market. Though the fortification of food products using microalgae oil is not new, limited information is available concerning its use in milkshake fortification and, more importantly, concerning how the e-nose could be applied to verify the success of this fortification process and to ensure the final product's quality.

In this study we therefore aimed to ascertain the efficacy of the addition of the n3 FA fortification brand (micro-encapsulated microalgae oil) in fulfilling the dietary recommendations for n3-FA-enriched food products, and to test the application of the e-nose in profiling the odor of n3-FA-enriched vanilla milkshakes. The basic hypothesis to be tested was whether or not the characteristically oxidative or fishy odor associated with micro-encapsulated microalgae oil had a detectable systematic distorting effect on the odor profile of the fortified vanilla shake product.

2. Materials and Methods

2.1. Experimental Shake Powder

The vanilla shake powder used in this study was a product available on the market, of which the ingredients are given in Table 1.

Table 1. Constituents of the commercial vanilla shake powder used in the experiment.

Ingredients	%
milk whey concentrate	69.2
hydrolyzed collagen	10
vegetable fat (coconut fat)	8
branched chain amino acids	4
freeze dried gelatin	4
Na-carboxy-methyl-cellulose	3
vitamin premix	1
vanilla aroma	0.3
beta-carotene	0.3
Sucralose	0.2

2.2. Determination of the Product's Fatty Acid Profile

Samples (shake powder, FA additive, and complemented shake powder) were homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in a 20-fold volume of chloroform:methanol (2:1 *v:v*) and total lipid content was extracted according to the method of Folch et al. [31]. Solvents were ultrapure-grade (Carl Roth, Karlsruhe, Germany) and 0.01% *w/v* butylated hydroxytoluene was added to prevent FA oxidation. Directly to the raw, dry sample, C19:0 internal standard was added (Merck cat. No.: 72332). The internal standard used was a solution of 1 mg/mL in chloroform:methanol (2:1 *v:v*). The total amount added was ca. 1/20 mass of the extracted fat, i.e., to 1 g raw sample (ca. 100 mg crude extract), 5 mg C19:0 was added.

The total lipid extract (also including the internal standard) was dried fully on a rotary evaporator under nitrogen stream and was trans-methylated via the acid-catalyzed method [32], using H_2SO_4 (1 *v/v*%) in methanol as a methyl donor, and toluene was used as a solvent. For the quantitative analysis, C19:0 methyl ester standard calibration was used at 6 points (Merck cat. No.: 74208) to assess the detector response, and the concentration of analyte in the calibration was between 5 and 500 µg/mL. The correlation coefficient was not less than 0.999, proving the linearity of the analysis. Fatty acid methyl-esters were extracted into ultrapure n-hexane for gas chromatography. This was performed on a Shimadzu GCMS-QP2010 apparatus (AOC 20i automatic injector), equipped with a Phenomenex Zebron ZB-WAX Capillary GC column (30 m × 0.25 mm ID, 0.25 µm film, Phenomenex Inc., Torrance, CA, USA). The characteristic operating conditions were: injector temperature: 270 °C, detector temperature: 300 °C, helium flow: 28 cm/s. The oven temperature was graded from 80 °C to 205 °C: 2.5 °C/min, 5 min at 205 °C, from 205 °C to 250 °C 10 °C/min and 5 min at 210 °C. FA results are expressed as mg/g of raw sample mass, as well as a weight% of the total FAs. All samples were analyzed in duplicate, and results are means of 2 analyses. The limit of detection was determined as three times the signal-to-noise ratio (3S/N), whereas the limit of quantification was 10S/N. The range of the LOD was between 0.1 and 0.5 µg/mL for the FAs (C4:0 to C24:0).

2.3. Omega-3 Fatty Acid Enrichment Protocol

To perform a graded n3 FA fortification protocol (primarily docosahexaenoic acid enrichment), a micro-encapsulated marketed algae-oil-based product was chosen, with the brand name S17-P100 (Life's DHA, DSM Nutritional Products Inc., Heerlen, The Netherlands).

The original shake mixture and the food additive product were subjected to FA analysis, as shown in Tables 2 and 3, respectively. The graded enrichment protocol for the vanilla shake powder with the FA based product is shown in Table 4.

Table 2. Quantitative and qualitative fatty acid composition of the S17-P100 food additive.

Fatty Acid	mg FA/g Sample	Weight % of FA
C4:0	0.24	0.05
C8:0	2.88	0.65
C10:0	3.38	0.77
C12:0	0.72	0.16
C14:0	24.2	5.50
C14:1n5	0.28	0.06
C15:0	1.81	0.41
C16:0	85.2	19.3
C16:1n7	0.61	0.14
C17:0	0.46	0.10
C18:0	5.60	1.27
C18:1n9c	48.1	10.9
C18:2n6c	5.72	1.30
C18:3n3	0.56	0.13
C20:0	0.74	0.17
C20:3n6	1.99	0.45
C20:4n6	3.19	0.72
C20:3n3	0.08	0.02
C22:0	0.72	0.16
C20:5n3 (EPA)	7.37	1.67
C24:0	0.68	0.15
C22:6n3 (DHA)	245.8	55.8
Total FA mg/g sample	440.3	
n3	253.8	57.6
n5	0.28	0.06
n6	10.9	2.48
n7	0.61	0.14
n9	48.1	10.9
n6/n3	0.04	
saturated	126.6	28.8
monounsaturated	49.0	11.1
polyunsaturated	264.7	60.1
EPA + DHA mg in 100 g	253.17	57.5

n3, omega-3; n5, omega-5; n6, omega-6; n7, omega-7; n9, omega-9; n6/n3, omega-6 to omega-3 ratio; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 3. Quantitative and qualitative fatty acid profiles of the original vanilla shake powder.

Fatty Acid	mg FA/g Sample	Weight % of FA
C4:0	0.02	0.02
C6:0	0.03	0.03
C8:0	2.86	3.07
C10:0	4.32	4.64
C11:0	0.03	0.03
C12:0	28.6	30.7
C13:0	0.04	0.04
C14:0	15.3	16.4
C14:1n5	0.23	0.25
C15:0	0.41	0.44
C16:0	17.2	18.5
C16:1n7	0.40	0.43
C17:0	0.21	0.23
C17:1n7	0.06	0.06
C18:0	5.99	6.44

Table 3. *Cont.*

Fatty Acid	mg FA/g Sample	Weight % of FA
C18:1n9t	0.05	0.05
C18:1n7t	0.03	0.03
C18:1n9c	12.1	13.0
C18:2n6t	0.07	0.08
C18:2n6c	4.12	4.43
CLA(9c,11t)	0.18	0.19
CLA(10t,12c)	0.02	0.02
C18:3n3	0.21	0.23
C20:0	0.14	0.15
C20:1n9	0.05	0.05
C20:2n6	0.01	0.01
C20:3n6	0.12	0.13
C20:4n6	0.09	0.10
C22:0	0.08	0.09
C20:5n-3 (EPA)	0.04	0.04
C24:0	0.06	0.06
Total FA mg/g sample	93.1	
n3	0.25	0.27
n5	0.23	0.25
n6	4.61	4.95
n7	0.49	0.53
n9	12.2	13.1
n6/n3	18.4	
saturated	75.3	80.9
monounsaturated	12.9	13.9
polyunsaturated	4.86	5.20

n3, omega-3; n5, omega-5; n6, omega-6; n7, omega-7; n9, omega-9; n6/n3, omega-6 to omega-3 ratio; EPA, eicosapentaenoic acid.

Table 4. The graded food additive enrichment protocol (product composition).

No.	Shake Powder	S17-P100	Total Mass	Calc. DHA	Calc. EPA	Calc. EPA + DHA
	mg in 10 g					
1	9980	20	10,000	4.9	1.5	6.4
2	9960	40	10,000	9.8	3.0	12.8
3	9940	60	10,000	14.7	4.4	19.1
4	9920	80	10,000	19.6	5.9	25.5
5	9900	100	10,000	24.5	7.4	31.9
6	9880	120	10,000	29.4	8.9	38.3
7	9860	140	10,000	34.3	10.4	44.7
8	9840	160	10,000	39.2	11.8	51.0
9	9820	180	10,000	44.1	13.3	57.4
10	9800	200	10,000	49.0	14.8	63.8

S17-P100, brand name of the primarily docosahexaenoic acid enrichment used; EPA, eicosapentaenoic acid (C20:5 n3); DHA, docosahexaenoic acid (C22:6 n3).

2.4. Aroma Analysis with the Electronic Nose

The odors of vanilla shake powders were measured in four replicates, 10 days after the enrichment protocol presented in Table 4. Before the odor measurement, three 1 g aliquots of each sample were placed into three 20 mL headspace vials, sealed with a magnetic cap and an UltraCleanTM polytetrafluoroethylene/silicone septum (Supelco, Inc., Merck KGaA, Darmstadt, Germany). The e-nose measurement was performed with a Heracles Neo 300 ultra-fast GC analyzer (Alpha MOS, Toulouse, France), equipped with a PAL-RSI autosampler unit (CTC Analytics AG, Zwingen, Switzerland) for standard handling of the samples. We generated the headspace and injected the headspace into the Heracles analyzer

unit, including an odor concentrator trap and two metal capillary columns: (1) Restek MXT-5: length: 10 m, ID: 0.18 mm, thickness: 0.40 µm, low-polarity stationary phase composed of Crossbond 5% diphenyl/95% dimethyl polysiloxane (Restek, Co., Bellefonte, PA, USA); (2) Restek MXT-1701: length: 10 m, ID: 0.18 mm, thickness: 0.40 µm, mid-polarity stationary phase composed of Crossbond 14% cyanopropylphenyl/86% dimethyl polysiloxane (Restek, Co., Bellefonte, PA, USA). The volatile compounds were separated by both columns simultaneously and detected using two flame ionization detectors (FIDs). The autosampler and analyzer were operated using AlphaSoft ver. 16 (Alpha MOS, Toulouse, France). The same software was used for the data acquisition and transformation. The retention times of the volatiles were recorded at both FIDs, followed by a conversion to retention indices (RIs). The Kovats RIs relate the retention times of the detected volatile molecules of a sample to the retention times of n-alkanes under the same conditions [33].

The RIs characterize the volatile compounds on the specific columns and can be assigned to specific aromas recorded in AroChemBase v7 in the AlphaSoft software. Throughout this manuscript, "1-A" is used as an identifier after RI to refer to column MXT-5, and "2-A" is used to refer to column MXT-1701. Before the analysis, a method was developed with the following parameters of the PAL-RSI Autosampler and Heracles GC analyzer: (1) autosampler: incubation at 80 °C for 10 min with 500 rpm agitation to generate headspace, 5 mL of headspace injected into the Heracles analyzer, flushing time between injections: 90 s; (2) analyzer: carrier gas: hydrogen, the flow of carrier gas: 30 mL/min, trapping temperature: 60 °C, initial oven temperature: 50 °C, the endpoint of oven temperature: 250 °C, heating rate: 2 °C/s, acquisition duration: 110 s, acquisition period: 0.01 s, injection speed: 125 µL/s, cleaning phase: 8 min.

2.5. Microbiological Testing

To elucidate the possible origins of ethanol in the sample, the enumeration of viable osmophilic yeasts and xerophilic molds was performed with conventional agar plate testing [34] using Dichloran-Glycerol (DG18) agar.

2.6. Statistical Evaluation

The recovery, i.e., the relationship between the calculated and measured amount of DHA, was implemented as a linear relationship between the two variables. The linear regression model was calculated with QtiPlot (version 1.0.0., 2020).

The multivariate data of the e-nose measurements describing the odor profiles of the S17-P100-enriched vanilla shake samples were analyzed using AlphaSoft (ver. 16) software. The chromatograms were transformed into a series of variables called sensors based on the identified chromatogram peaks [35]. The name of a sensor originating from the location of the peak within the chromatogram and was identical to the respective RI. The sensor intensity was calculated based on the area under the respective chromatogram peaks.

Principal component analysis (PCA) [36] was performed using the sensor data to describe the unsupervised clustering of the samples within the multidimensional space defined by the sensor variables. Supervised classification models were built using discriminant factor analysis (DFA) [36] to identify the predefined groups of samples based on their odor signals. Partial least-squares regression (PLSR) [36] was used to fit calibration models describing the relationship between the odor signals and the concentration of the S17-P100 food additive.

The accuracy of the DFA and PLSR models was tested with leave-one-out cross-validation, in which a single record was left out of the modeling process and was used for testing by predicting its qualitative or quantitative properties; this process was repeated iteratively until all samples had been used for validation once [36]. The sensor selection function of AlphaSoft was used to identify the most distinctive variables during the qualitative and quantitative analyses. In addition, DFA and PLSR calculations based on the selected sensors were performed. The volatile compounds described by the selected sensors were identified using the AroChemBase database.

3. Results

3.1. Fatty Acid Profile of the Enriched Shake

The enriched vanilla shake powder demonstrated a gradual increase in the proportions (and concentrations) of the medium- and long-chain FAs (FAs with carbon chains > 16) that were abundant in the FA additive. Consequently, this was visible in the overall increasing level of polyunsaturation, as well as that of monounsaturation, whereas the n6/n3 FA proportion decreased. The detailed FA profile of the enriched vanilla shake is provided in Table 5.

Table 5. Fatty acid profile (quantitative (mg/g product) and qualitative (%)) of the enriched milkshake sample series.

Sample No.	1		2		3		4		5	
Fatty Acid	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
C4:0	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.02
C6:0		0.00	0.12	0.14	0.08	0.10	0.12	0.13	0.27	0.29
C8:0	1.07	1.36	3.20	3.72	2.92	3.48	3.28	3.85	3.95	4.22
C10:0	3.03	3.85	3.93	4.56	3.71	4.43	3.85	4.51	4.20	4.48
C11:0	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02
C12:0	24.6	31.3	27.6	32.0	25.5	30.5	24.7	29.0	26.6	28.4
C13:0	0.03	0.04	0.03	0.04	0.04	0.05	0.05	0.06	0.05	0.05
C14:0	13.2	16.8	14.1	16.4	13.6	16.2	13.6	16.0	14.6	15.5
C14:1n5	0.18	0.22	0.15	0.17	0.15	0.17	0.18	0.21	0.20	0.22
C15:0	0.26	0.33	0.32	0.37	0.37	0.44	0.40	0.47	0.40	0.43
C16:0	16.2	20.5	16.6	19.3	16.9	20.2	17.5	20.5	17.4	18.5
C16:1n7	0.27	0.35	0.33	0.38	0.33	0.39	0.30	0.35	0.43	0.46
C17:0	0.14	0.17	0.17	0.20	0.19	0.23	0.21	0.24	0.21	0.22
C17:1n7	0.05	0.06	0.04	0.05	0.04	0.05	0.05	0.06	0.06	0.07
C18:0	5.28	6.71	5.30	6.15	5.65	6.74	5.66	6.64	5.94	6.34
C18:1n9t	0.02	0.02	0.05	0.05	0.04	0.05	0.04	0.04	0.03	0.04
C18:1n7t	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.01
C18:1n9c	10.1	12.8	9.82	11.4	9.41	11.2	10.0	11.8	12.9	13.7
C18:2n6t	0.04	0.06	0.06	0.07	0.05	0.06	0.05	0.06	0.09	0.09
C18:2n6c	3.24	4.11	3.23	3.75	3.14	3.74	3.40	3.99	4.03	4.31
CLA(9c, 11t)	0.12	0.15	0.13	0.15	0.15	0.18	0.13	0.15	0.15	0.16
CLA(10t, 12c)	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:3n3	0.14	0.18	0.15	0.17	0.15	0.18	0.18	0.21	0.20	0.21
C20:0	0.09	0.11	0.11	0.13	0.11	0.13	0.11	0.13	0.13	0.13
C20:1n9	0.03	0.04	0.03	0.03	0.04	0.04	0.04	0.05	0.04	0.04
C20:2n6	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01
C20:3n6	0.08	0.10	0.07	0.08	0.09	0.10	0.10	0.12	0.10	0.11
C20:4n6	0.06	0.08	0.08	0.09	0.07	0.08	0.08	0.09	0.10	0.10
C22:0	0.05	0.07	0.07	0.08	0.07	0.09	0.08	0.09	0.08	0.09
C20:5n3 (EPA)	0.01	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.06
C24:0	0.02	0.03	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05
C22:5n3	0.07	0.08	0.08	0.09	0.07	0.08	0.09	0.10	0.15	0.16
C22:6n3 (DHA)	0.26	0.33	0.28	0.32	0.74	0.88	0.91	1.07	1.27	1.36
Total FA mg/g sample	78.7		86.1		83.8		85.3		93.7	
n3	0.48	0.61	0.52	0.61	1.00	1.19	1.23	1.44	1.68	1.79
n5	0.18	0.22	0.15	0.17	0.15	0.17	0.18	0.21	0.20	0.22
n6	3.56	4.53	3.58	4.16	3.51	4.19	3.78	4.43	4.50	4.80
n7	0.33	0.42	0.38	0.44	0.39	0.47	0.36	0.42	0.51	0.54
n9	10.1	12.9	9.9	11.5	9.5	11.3	10.1	11.9	12.9	13.8
n6/n3	7.44		6.84		3.52		3.08		2.68	
saturated	64.0	81.4	71.6	83.1	69.3	82.6	69.7	81.6	73.9	78.8
monounsaturated	10.6	13.5	10.4	12.1	10.0	12.0	10.7	12.5	13.7	14.6
polyunsaturated	4.04	5.13	4.10	4.76	4.51	5.38	5.00	5.86	6.18	6.59
EPA + DHA mg in 100 g	27.0	0.34	29.5	0.34	77.4	0.92	95.8	1.12	132.8	1.42

Table 5. *Cont.*

Sample No.	6		7		8		9		10	
Fatty Acid	**mg/g**	**%**	**mg/g**	**%**	**mg/g**	**%**	**mg/g**	**%**	**mg/g**	**%**
C4:0	0.04	0.05	0.02	0.02	0.02	0.02	0.04	0.04	0.05	0.05
C6:0	0.40	0.44	0.04	0.05	0.07	0.08	0.37	0.37	0.40	0.41
C8:0	4.08	4.40	2.77	3.10	2.57	2.98	4.18	4.27	3.98	4.13
C10:0	4.13	4.44	3.82	4.28	3.43	3.99	4.26	4.35	4.06	4.21
C11:0	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03
C12:0	25.3	27.2	25.1	28.0	21.8	25.4	26.4	27.0	25.2	26.2
C13:0	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.05
C14:0	13.9	15.0	14.0	15.7	12.5	14.5	14.7	15.0	13.9	14.4
C14:1n5	0.22	0.23	0.21	0.23	0.27	0.31	0.25	0.26	0.22	0.23
C15:0	0.41	0.45	0.42	0.47	0.43	0.50	0.43	0.44	0.44	0.46
C16:0	17.3	18.6	17.6	19.7	17.1	19.8	17.8	18.1	17.8	18.5
C16:1n7	0.46	0.50	0.40	0.45	0.49	0.57	0.50	0.51	0.52	0.54
C17:0	0.22	0.24	0.21	0.24	0.22	0.25	0.22	0.22	0.20	0.20
C17:1n7	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.08
C18:0	5.85	6.30	6.02	6.74	5.68	6.60	5.69	5.81	5.57	5.78
C18:1n9t	0.05	0.05	0.04	0.05	0.03	0.04	0.05	0.05	0.05	0.05
C18:1n7t	0.02	0.03	0.02	0.02	0.02	0.03	0.01	0.01	0.02	0.02
C18:1n9c	13.3	14.3	11.6	13.0	13.2	15.4	14.1	14.4	13.9	14.4
C18:2n6t	0.09	0.10	0.08	0.09	0.09	0.10	0.07	0.08	0.08	0.08
C18:2n6c	4.14	4.46	3.77	4.22	3.94	4.57	4.34	4.43	4.08	4.23
CLA(9c, 11t)	0.17	0.19	0.16	0.18	0.20	0.23	0.19	0.20	0.24	0.24
CLA(10t, 12c)	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
C18:3n3	0.21	0.22	0.19	0.21	0.24	0.27	0.24	0.24	0.24	0.25
C20:0	0.14	0.15	0.13	0.15	0.13	0.15	0.12	0.12	0.13	0.14
C20:1n9	0.05	0.05	0.04	0.05	0.05	0.06	0.06	0.06	0.06	0.06
C20:2n6	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02
C20:3n6	0.14	0.15	0.13	0.15	0.14	0.17	0.16	0.16	0.16	0.17
C20:4n6	0.11	0.11	0.11	0.13	0.13	0.15	0.14	0.14	0.16	0.16
C22:0	0.10	0.11	0.09	0.10	0.08	0.10	0.09	0.09	0.10	0.10
C20:5n3 (EPA)	0.07	0.08	0.08	0.08	0.12	0.14	0.13	0.13	0.16	0.16
C24:0	0.05	0.06	0.05	0.06	0.06	0.07	0.07	0.07	0.07	0.07
C22:5n3	0.16	0.17	0.14	0.16	0.18	0.21	0.21	0.22	0.24	0.24
C22:6n3 (DHA)	1.72	1.85	1.92	2.14	2.70	3.13	2.92	2.99	4.12	4.28
Total FA mg/g sample	92.9		89.3		86.1		97.9		96.4	
n3	2.16	2.32	2.32	2.60	3.23	3.75	3.50	3.57	4.76	4.93
n5	0.22	0.23	0.21	0.23	0.27	0.31	0.25	0.26	0.22	0.23
n6	4.67	5.02	4.29	4.80	4.52	5.25	4.93	5.04	4.74	4.92
n7	0.55	0.60	0.48	0.54	0.58	0.68	0.60	0.61	0.60	0.63
n9	13.4	14.4	11.7	13.1	13.3	15.5	14.2	14.5	14.0	14.5
n6/n3	2.16		1.85		1.40		1.41		1.00	
saturated	71.9	77.4	70.4	78.8	64.2	74.5	74.4	76.0	72.1	74.8
monounsaturated	14.2	15.3	12.4	13.8	14.2	16.5	15.1	15.4	14.8	15.4
polyunsaturated	6.82	7.34	6.61	7.40	7.75	9.01	8.43	8.61	9.49	9.85
EPA + DHA mg in 100 g	179.1	1.93	199.2	2.23	281.3	3.27	305.0	3.12	428.0	4.44

n3, omega-3; n5, omega-5; n6, omega-6; n7, omega-7; n9, omega-9; n6/n3, omega-6 to omega-3 ratio; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

3.2. Fatty Acid Recovery from the Enriched Shake

With the S17-P100-enriched vanilla shake powder, FA analysis was performed. To test the mixing accuracy, we analyzed the relationship between the estimated and the recovered amount of the largest additive component, docosahexaenoic acid (DHA, C22:6n3) (Figure 1).

The R^2 value was found to be relatively robust, but the intercept was found to be significantly different from zero ($p < 0.001$).

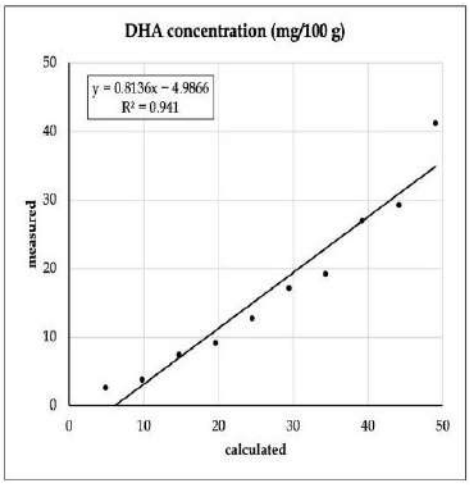


Figure 1. Recovery of docosahexaenoic acid (C22:6 n3, DHA) from the enriched vanilla shake product.

3.3. Microbiology

The counts of osmophilic yeasts and xerophilic molds both remained below 100 CFU/g of product, i.e., below the limit of detection.

3.4. Electronic Nose

The chromatograms recorded on the two GC columns during the analysis of the headspace of one vanilla shake sample are shown in Figure 2. Based on the retention indices, the main identified volatile compounds were ethanol (463-1-A; 560-2-A), propylene glycol (723-1-A; 950-2-A), limonene (1043-1-A; 1070-2-A), and vanillin (1437-1-A; 1706-2-A).

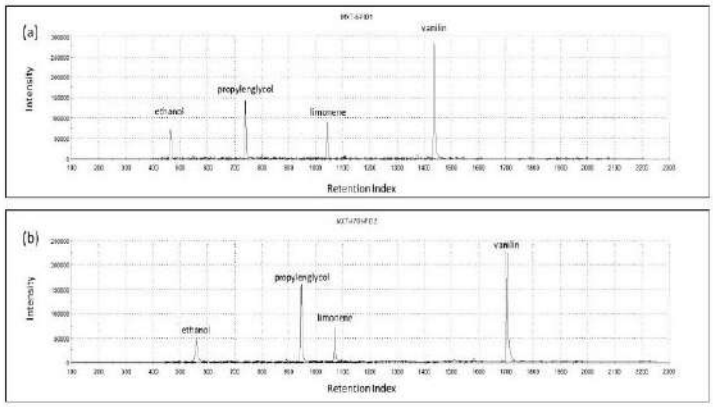


Figure 2. Chromatograms of columns MXT-5 (**a**) and MXT-1701 (**b**) for one vanilla shake sample, indicating the most prominent volatiles.

The main volatiles mentioned above were responsible for the dominant smell of the product, but a larger fraction of the variation in the multicomponent odor fingerprint data was influenced by the smaller chromatogram peaks. To describe the multivariate odor patterns, principal component analysis (PCA) was performed upon all the sensor signals that were derived from the chromatograms (as described in Section 2). The first (PC1) and second (PC2) principal components explained 72.2% and 19.4% of the e-nose data variation,

respectively, without any systematic (increased or decreased) pattern of odor differences between the supplemented samples and the control.

The odor difference between the control and supplemented samples appeared only in the 3rd principal component (PC3) (a higher PC) which described 6.5% of the total sensor signal variation (Figure 3). Even though this latent variable described the difference in odor between the control and supplemented samples, it had no relation with the level of S17-P100 supplementation, as no systematic arrangement among treatments could be identified. Though the variation in the odor profile may not have been negligible, this component did not describe the dominant odor pattern of the supplemented milkshakes that may affect consumer choice.

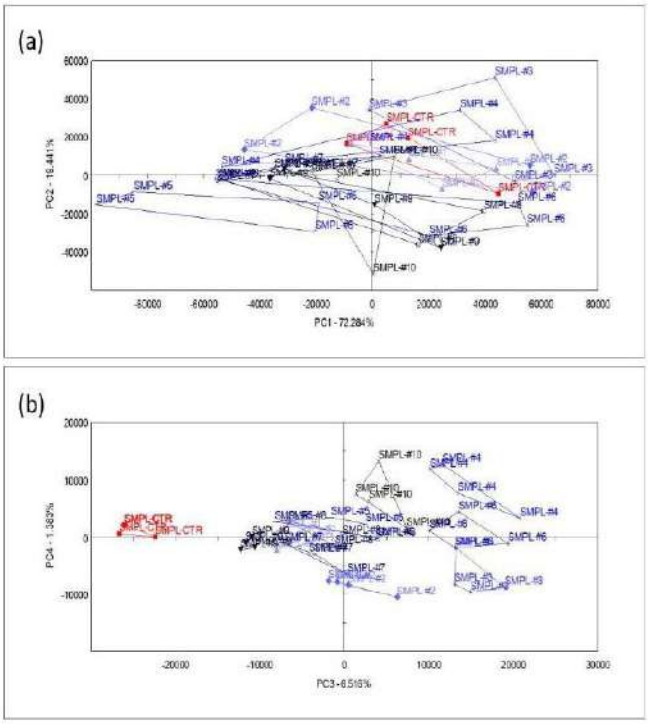


Figure 3. Score plots of PCA performed with data from all sensors, showing the planes of the 1st and 2nd principal components (**a**) and the 3rd and 4th principal components (**b**) (n = 4 replicates/sample, SMPL-CTR: milkshake with no supplementation (control); SMPL#1 to SMPL#10: milkshake samples with increasing levels of SP17-100 supplementation (0.2–2%).

The supervised classification of samples was performed with discriminant factor analysis (DFA), using the data from all sensors. The samples containing different concentrations of the supplementation could be identified with 98% accuracy during the cross-validation (Figure 4), but the arrangement of the groups within the plane of discriminant factors did not follow the concentration level of the supplementation (a similar observation to that of the unsupervised classification (PCA)). This demonstrates that the DFA model could find odor (or odor intensity) differences between sample groups, but these differences were not influenced by the level of supplementation due to the lack of a systematic effect on odor intensities in the DFA plane.

On the other hand, when the relation between the sensor data and the supplementation level was described with partial least-squares regression (PLSR), an accurate (R^2 = 0.833) calibration model was built (Figure 5), which indicated the possibility of finding

supplementation-dependent odor variations. To further investigate this seeming possibility, the specific sensors were selected that contributed the most to the successful classification of samples with different levels of supplementation. Figure 6 shows the mean sensor signals for each concentration group. It was noticeable that the supplemented samples had higher intensities at 547-1-A, 612-1-A, 756-1-A, 802-1-A, 996-1-A, and 778-2-A than the control samples. The possible volatile compounds identified for these retention indices in the AroChemBase are shown in Table 6. PLSR calibration and sensor selection based on PLSR calibration are targeted approaches to finding specific odor differences focusing on predefined parameters or constituents; thus, finding the relevant odor variations is possible even if the causative odorants are very weak. However, an approach that is non-targeted, such as PCA, is relevant when identifying general odor patterns, focusing on the major odor variations, which in this paper revealed non-dependent odor differences in relation to the increased levels of DHA supplementation.

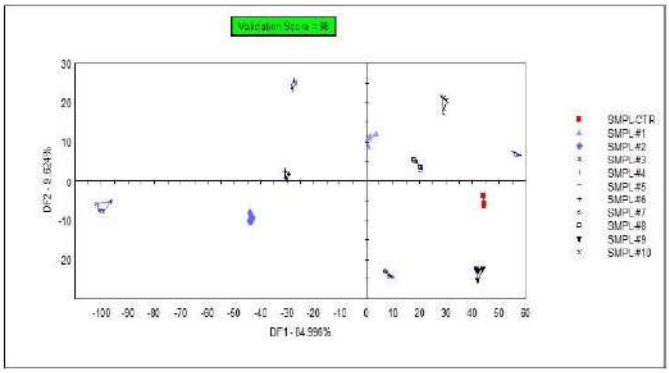


Figure 4. Graphical representation of the DFA classification of samples with different levels of S17-P100 supplementation obtained using data from all sensors (SMPL-CTR: milkshake with no supplementation (control); SMPL#1 to SMPL#10: milkshake samples with increasing levels of DHA supplementation (0.2–2%).

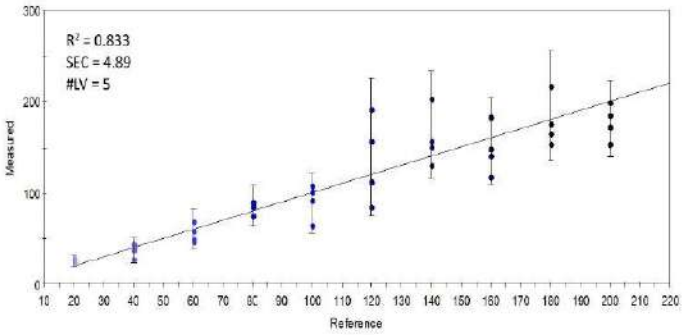


Figure 5. Y-fit of the PLSR calibration on the S17-P100 concentration (mg/in 10 g product) using data from all sensors (R^2: determination coefficient; SEC: standard error of calibration; #LV: number of latent variables); the intensity of the colors from light blue to black along the Y-fit line represents the increase in concentration levels of the DHA supplementation.

In a supervised DFA (Figure 7) classification of samples with different S17-P100 contents based on the data from the selected qualitative sensors (targeted modeling), the model was able to sort 98% of the samples into the correct classes. Again, no concentration-dependent arrangement of the classes could be recognized in this model.

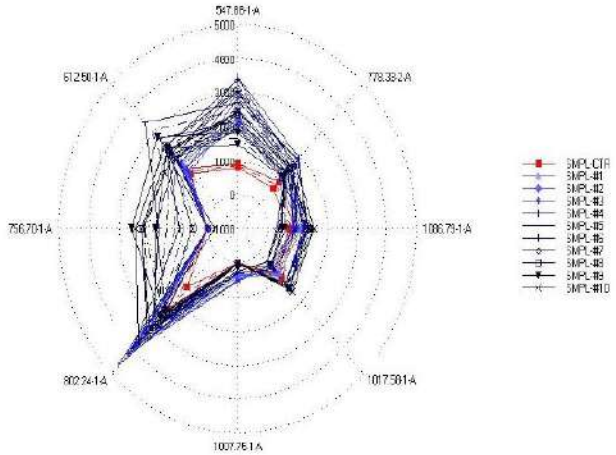


Figure 6. Spider plot of intensities (mean ± SD) of the sensors specifically selected to provide the best classification of the samples according to the different S17-P100 concentrations (SMPL-CTR: milkshake with no supplementation (control); SMPL#1 to SMPL#10: milkshakes with increasing levels of DHA supplementation (0.2–2%)).

Table 6. Assignment of possible volatile compounds to the retention indices of the selected qualitative sensors using the AroChemBase database (Alpha MOS, Toulouse, France).

Retention Index	Compound 1 *	Compound 2 *	Compound 3 *
547-1-A	tert-buthylmethylether	1-propanol	2-propanol
612-1-A	ethylacetate	acetic acid	–
756-1-A	ethyl isobutyrate	Pyrrole	–
802-1-A	ethyl butyrate	propyl propanoate	–
996-1-A	ethyl hexanoate	butyl butanoate	–
1017-1-A	alpha-terpinene	1,4-cinelole	acetylpyrazine
1086-1-A	pentyl butanoate	benzyl butanoate	–
778-2-A	pentanal	propyl acetate	pentan-2-one

* Compounds 1, 2, and 3, represent the likelihood of the identified compound being associated with the identified retention indexes (Compound 1: highest possibility of occurrence, Compound 2: the next highest to occur, and Compound 3: the lowest to occur), and "–" means no compound 3 was identified at that specific intensity.

Although the data from the selected qualitative sensors (targeted modeling) held enough information to differentiate the samples based on the DHA inclusion levels in the S17-P100 supplementation, this differentiation was not based on volatiles that had a direct relationship with the supplemented concentration. This is demonstrated in Figure 8, which shows the results of the PLSR calibration on the supplementation level using the data from the selected sensor set. The weak results (R^2 = 0.509) indicate that there was no linear combination of the qualitative sensors' signals that could accurately describe the variation in supplemented concentrations.

In a final attempt, sensors which gave an optimal linear combination for the PLSR calibration of the concentration of S17-P100 supplementation were selected. The largest variation and the variation that was proportional to the concentration of supplementation was found in sensor 756-1-A, referring to the retention index of ethyl isobutyrate (or that of pyrrole). The good results (R^2 = 0.813) of the PLSR calibration shown in Figure 9 demonstrate that the linear combinations of the limited number of selected quantitative sensors held enough information to describe the concentration-dependent odor variations

caused by S17-P100 supplementation. The calibration fitted onto the data from these few sensors was almost as good as that obtained with all the sensors (Figure 5). However, as explained earlier, this targeted method of classification or modeling using specific or selected signals is not good enough to correctly describe the major odor differences.

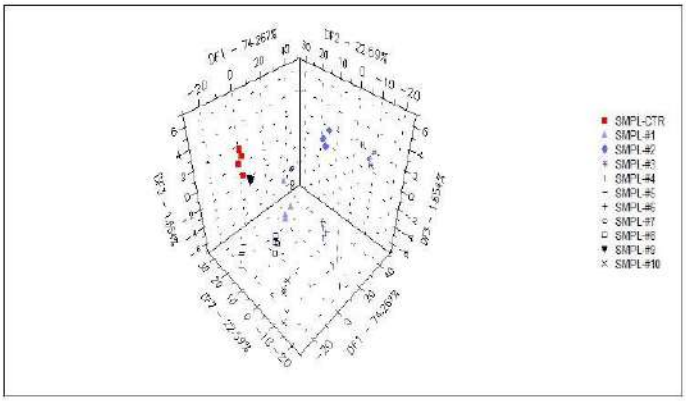


Figure 7. 3D plot of the DFA classification of samples with different S17-P100 inclusion levels based on the data of the selected qualitative sensors (cross-validation score = 98%) SMPL-CTR: milkshake with no supplementation (control); SMPL#1 to SMPL#10: milkshakes with increasing levels of DHA supplementation (0.2%–2%).

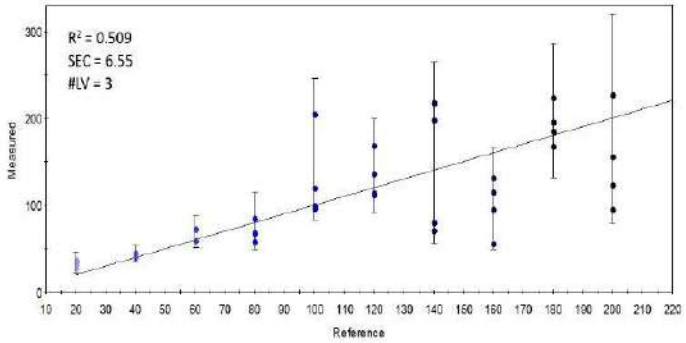


Figure 8. Y-fit of the PLSR calibration on the S17-P100 concentration (mg/10 g product) using data from the selected qualitative sensors (R^2: determination coefficient; SEC: standard error of calibration; #LV: number of latent variables); the intensity of the colors from light blue to black along the Y-fit line represents the increase in concentration levels of the DHA supplementation.

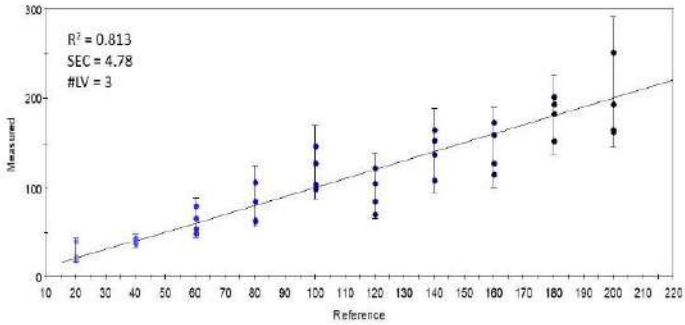


Figure 9. Y-fit of the PLSR calibration on the S17-P100 concentration (mg/10 g product) using data from the selected quantitative sensors (R^2: determination coefficient; SEC: standard error of calibration; #LV: number of latent variables); the intensity of the colors from light blue to black along the Y-fit line represents the increase in concentration levels of the DHA supplementation.

4. Discussion

4.1. Original Fatty Acid Profile of the Shake Powder

The original shake powder's FA profile typically resembled that of coconut fat, with a slight modification due to the effect of milk whey. The FA profile of the un-complemented (i.e., original) product definitely indicated the dominance of saturated FAs (~81%), with lauric (C12:0, ~30%), palmitic (C16:0, ~18%), and myristic (C14:0, ~16%) acids as the main components. The major monoenoic FA was oleic acid (C18:1 n9), with a share of 13%, and the most abundant polyunsaturated one was linolenic acid (C18:2n6, slightly over 4%) (Table 3). The n3 FAs were present only minimally, with α-linolenic acid (C18:3n3) providing 0.23% and eicosapentaenoic acid (C20:5 n3) accounting for 0.04%. The n3 FAs of the C22 chain length were absent from the original sample.

The dominant FA source ingredient was coconut oil. Coconut oil is available in three forms: refined oil, copra oil, and virgin coconut oil. The FA profiles of the different types were practically uniform [37]. The form used here was powdered coconut fat. According to [37,38], coconut oil is the highest natural source of lauric acid, and possesses high proportions of caprylic, capric and lauric acids; these were detected in our sample as well, in proportions of ~3%, ~4.5%, and ~30%, respectively.

In summary, we implemented the n3 FA enrichment of a food product that had a very strongly saturated FA profile and of which the medium-chain length acids were already quite abundant.

4.2. Meeting the Dietetic Recommendations Regarding Omega-3 Fatty Acids for Humans

Vanilla aroma shakes are not easy to classify within the wide varieties of milk products. This product is similar to most infant formula products (composed of 70% milk whey) and it is prepared in a ready-to-drink form with the addition of water. The basic rationale behind the addition of n3 long chain FAs from microalgae to a product that is originally poor in them (Table 3) is absolutely clear.

The globally recommended levels for EPA (C20:5n3) and DHA (C22:6n3) are generally handled together and are mostly specific for gender, health status, and age. In 2002, the WHO recommended a total daily amount of n3 FA of 1–2% of the total energy intake for adults, whereas for EPA + DHA, the recommended range is from 100–150 mg/day (2–4 years) and up to 300 mg/day (pregnant or lactating women) [39]. The European Food Safety Authority also recommends 250 mg day^{-1} DHA + EPA for adults [40]. The highest global recommendation is at least 500 mg EPA + DHA/day/capita [41]. The US [42] and NATO recommendations [43] are quite similar to that of the FAO [39], at 300–400 mg EPA + DHA/day, and slightly exceed the pregnant and lactating women's recommendations (Koletzko et al. suggested 200 mg EPA + DHA) [44]. Thus, in this study we aimed at

a range covering and possibly slightly exceeding most of the above-cited doses by using S17-P100 levels above 100 mg/10 g milkshake powder (with a theoretical maximum of 428 mg DHA and 16 mg EPA in 100 g).

In summary, in practice the recommended levels were nearly reached in this study, and we may add that the relatively high level of micro-encapsulated microalgae oil added to this product (from 0.2% to 2%, i.e., an increase of one order of magnitude) has been supposed to induce an off-odor corresponding systematically to the increase in the added levels of DHA.

4.3. The Possibly Negative Side-Effects of Omega-3 Enrichment

To fulfill consumer demands, microalgae n3 polyunsaturated FAs are present in an increasing number of newly marketed foods [45], including milk products and milky emulsions [46]. With a high degree of polyunsaturation, the *differentia specifica* contribution to the high nutritional/biological value of the fats of these food items makes them prone to lipid oxidation [47]. It is possible that the emerging oxidation products may not only have negative health effects (for a review, see [48]), but their positive nutritional effects may also be compromised by undesirable aromatic properties and thus reduced consumer acceptance [49]. Even if lipid peroxidation can be mostly inhibited with effective methods such as the application of one or more antioxidants [45], the enrichment of emulsified foods with n3 FAs gives rise to undesirable and rancid off-flavors, in most cases making some kind of masking approach necessary [50]. Herein, the e-nose analysis approach was performed to identify possible variations in the odor patterns related to the n3 FA enrichment levels. Based on the data obtained from e-nose sensors (for a detailed discussion, see Section 4.4), we speculate that the negative oxidative effect of n3 polyunsaturated fatty acids was minimal—at least, it did not compromise the odor patterns.

4.4. E-Nose Odor Profiling

With the application of the electronic nose system, our aim was to detect how the odor pattern was modified when different levels of micro-encapsulation of DHA containing microalgae oil (SP17-100) were added to the vanilla milkshakes.

When a more targeted approach, such as PLSR calibration using selected sensors, was used to analyze S17-P100 supplementation, a successful calibration could be achieved, which indicated that an odor pattern did exist that was related with the concentration of the additive, i.e., the concentration of S17-P100 had an impact on the odor. However, since the classification models that identified the groups of the different supplementation levels did not identify the concentration-dependent odor patterns, it can be stated that the major odor patterns were independent of S17-P100 supplementation, and these major odor patterns provided the possibility of identifying the sample groups. The minor odor patterns which were dependent on the S17-P100 supplementation concentration could be traced when specifically targeted, but they were generally hidden by the major (independent) odor patterns. Few studies have examined the application of the electronic nose to the odor profiles of milkshakes enriched with n3 FAs. However, some valuable information has been obtained on other related products of functional food value. For instance, our findings are consistent with those of [51], who reported that the addition of *Pavlova lutheri* (an algae) lipid extract to yoghurt increased the n3 FA content without altering the odor 28 days after manufacturing. However, contrary results were obtained in a study in which the odor stability of milk-based infant formula fortified with PUFAs (ARA (C20:4 n6) and DHA (C22:6 n3)) was measured after 30, 60, and 90 days of production [52]. The sixty- and ninety-day odor profiles significantly differed from that obtained at thirty days.

Though the odor profile of the supplemented product did not significantly differ from the controls, there were some volatile compounds of importance in regard to odor that were identified based on retention indices. As shown in Table 6, some of the associated volatile compounds of importance with regard to odor that were identified in the enriched samples were 1-propanol and 2-propanol (isomeric forms of propanol (an alcohol) molecule

with a strong odor), ethyl acetate or ethyl ethanoate (an ester formed between acetic acid and ethanol), benzyl butanoate and ethyl hexanoate (a fatty acid ethyl ester) with fruit-like odors [53–55], 1,4-cineole (an oxabicycloalkane with a minty lime-oil odor [56]), and pentanal (a saturated fatty aldehyde with an acrid odor [55]). In other studies in which other n3 sources were used for the enrichment process, other volatile compounds of importance with regard to odor were found. In a study in which the volatile compounds in milk enriched with 5% cod oil were measured, the major compounds of importance with regard to odor were trans-2-hexenal and cis-4-heptenal [57], which have a characteristic green leafy or oily odor. Hen et al. attempted to predict the fishy off-odor and identify the compounds of importance with regard to odor in dairy powders, reporting that volatile ketones, aldehydes and furans were the compounds that contributed the most to the identified fishy off-odor [58]. It has been widely reported that the main chemical process underlying the formation of an off-odor is oxidation/the development of rancidity. With respect to fish oil, the oxidation of fish oil is enhanced or increased when it is added to food products. For the quality control of omega-3 PUFA-fortified foods, efficient methods of oxidation assessment should be used, starting from the raw material and continuing during the processing, storage, and distribution of the products [59], and also employing preservation methods such as encapsulation, which may help to mask or hide off-odors. It is also important to always employ rapid quality-checks to detect possible volatile compounds of importance in relation to oxidation or off-odors [60], and the electronic nose could be one of the precise rapid analytical tools to be used for this purpose.

5. Conclusions

Adding micro-encapsulated microalgae oil into milkshakes enriched the concentration of n3 FAs, notably DHA (concentration > 400 mg/g), which may contribute to cardiovascular health benefits. When the e-nose was applied to detect the influence of this supplementation process on odor, the odor of the supplemented samples did not show any systematic concentration-dependent pattern as a result of supplementation. Since this observation was evident in most of the models generated, it can be inferred that no major off-odor occurred as a result of the increased level of supplementation. If any major off-odor was present, it must have been manifested in an increased odor pattern with the level of supplementation. However, when targeted signals were selected and used in the modeling process (PLSR), a possible concentration-dependent odor could be calibrated, but this may not represent the major odorants in the product. The presented e-nose approach has high potential in the evaluation of value-added functional foods fortified with health-promoting additives, and may serve as a supportive tool for the development of healthier foods with well-described sensory parameters. The demonstrated e-nose results provided evidence on the major odor profiles of the investigated products. However, human perception may be greatly influenced by several factors, such as but not limited to combinations of smells and mixed sensations of odor, taste and texture. Therefore, further studies, also including human sensory panels, are necessary in order to establish the effect of micro-encapsulated oil on other sensory qualities, such as flavor, color, and taste, to demonstrate consumer acceptance.

Author Contributions: H.G.Y.: conceptualization, design, acquisition, analysis and interpretation of e-nose data, drafting and editing the original manuscript; O.A.: curation and interpretation, drafting and editing the manuscript; I.I.: conceptualization and design; D.V.: conceptualization, design of the enrichment method; B.B.: microbiology analysis; G.B.: conceptualization and design, acquisition, curation and interpretation of e-nose data, drafting and editing the original manuscript; T.T.: drafting and editing the original manuscript; A.S.: conceptualization and design, acquisition, funding acquisition, supervision, acquisition, curation and interpretation of GC-MS data, drafting and editing the original manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work was supported by the projects 2018.-1.1.2-KFI-2018-00077 and EFOP-3.6.3-VEKOP-16-2017-00005. The support of the project 2020-1.1.2.-PIACI-KFI-2021-00245 and that of the Hungarian National Laboratory project RRF-2.3.1-21-2022-00007 is gratefully acknowledged. The publication was financed by the Hungarian National Research, Development and Innovation Office, "One Health" project, grant number 2020-4.1.1-TKP2020.

Data Availability Statement: The data are available from the corresponding author.

Conflicts of Interest: Authors Imre Ilyés and Dorottya Vigyázó were employed by the company HuBakers Trade Ltd. The company had the following involvement with the study: provision of vanilla shake powder and omega-3 fatty acid source. Authors George Bazar and Tamás Tóth were employed by the company ADEXGO Kft. The Company was not involved in the study design, collection, analysis, or interpretation of data; the writing of this article; or the decision to submit it for publication. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Saifullah, M.; Shishir, M.R.I.; Ferdowsi, R.; Rahman, M.R.T.; van Vuong, Q. Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: A critical review. *Trends Food Sci. Technol.* **2019**, *86*, 230–251. [CrossRef]
2. Ozdal, T.; Yolci-Omeroglu, P.; Tamer, E.C. *Role of Encapsulation in Functional Beverages*; Elsevier Inc.: Amsterdam, The Netherlands, 2019.
3. Lakkis, J.M. *Encapsulation and Controlled Release Technologies in Food Systems*, 2nd ed.; Blackwell Publishing: Oxford, UK, 2016.
4. Garcia-Moreno, P.J.; Özdemir, N.; Stephansen, K.; Mateiu, R.V.; Echegoyen, Y.; Lagaron, J.M.; Chronakis, I.S.; Jacobsen, C. Development of carbohydrate-based nano-microstructures loaded with fish oil by using electrohydrodynamic processing. *Food Hydrocoll.* **2017**, *69*, 273–285. [CrossRef]
5. Celli, G.B.; Ghanem, A.; Brooks, M.S.L. Bioactive Encapsulated Powders for Functional Foods—A Review of Methods and Current Limitations. *Food Bioprocess Technol.* **2015**, *8*, 1825–1837. [CrossRef]
6. Encina, C.; Vergara, C.; Giménez, B.; Oyarzún-Ampuero, F.; Robert, P. Conventional spray-drying and future trends for the microencapsulation of fish oil. *Trends Food Sci. Technol.* **2016**, *56*, 46–60. [CrossRef]
7. Barrow, C.J.; Wang, B.; Adhikari, B.; Liu, H. Spray drying and encapsulation of omega-3 oils. In *Food Enrichment with Omega-3 Fatty Acids*; Jacobsen, C., Nielsen, N.S., Horn, A.F., Moltke Sørensen, A., Eds.; Woodhead Publishing: Cambridge, UK, 2013; pp. 194–225. [CrossRef]
8. Anwar, S.H.; Weissbrodt, J.; Kunz, B. Microencapsulation of fish oil by spray granulation and fluid bed film coating. *J. Food Sci.* **2010**, *75*, E359–E371. [CrossRef]
9. Busolo, M.A.; Torres-Giner, S.; Prieto, C.; Lagaron, J.M. Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. *Innov. Food Sci. Emerg. Technol.* **2019**, *51*, 12–19. [CrossRef]
10. Pereira, D.M.; Valentão, P.; Andrade, P.B. Nano-and microdelivery systems for marine bioactive lipids. *Mar. Drugs* **2014**, *12*, 6014–6027. [CrossRef]
11. Henry, C. Functional foods. *Eur. J. Clin. Nutr.* **2010**, *64*, 657–659. [CrossRef]
12. Lang, T. Functional foods. *Br. Med. J.* **2007**, *334*, 1015–1016. [CrossRef]
13. Sidari, R.; Tofalo, R. A Comprehensive Overview on Microalgal-Fortified/Based Food and Beverages. *Food Rev. Int.* **2019**, *35*, 778–805. [CrossRef]
14. Gallaher, J.J.; Hollender, R.; Peterson, D.G.; Roberts, R.F.; Coupland, J.N. Effect of composition and antioxidants on the oxidative stability of fluid milk supplemented with an algae oil emulsion. *Int. Dairy J.* **2005**, *15*, 333–341. [CrossRef]
15. Chee, C.P.; Djordjevic, D.; Faraji, H.; Decker, E.A.; Hollender, R.; McClements, D.J.; Peterson, D.G.; Roberts, R.F.; Coupland, J.N. Sensory Properties of Vanilla and Strawberry Flavored Ice Cream Supplemented with Omega-3 Fatty Acids. *Milchwissenschaft* **2007**, *62*, 66–69.
16. Valencia, I.; Ansorena, D.; Astiasarán, I. Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium sp.*: Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chem.* **2007**, *104*, 1087–1096. [CrossRef]
17. Chee, C.P.; Gallaher, J.J.; Djordjevic, D.; Faraji, H.; McClements, D.J.; Decker, E.A.; Hollender, R.; Peterson, D.G.; Roberts, R.F.; Coupland, J.N. Chemical and sensory analysis of strawberry flavoured yogurt supplemented with an algae oil emulsion. *J. Dairy Res.* **2005**, *72*, 311–316. [CrossRef] [PubMed]
18. Agustini, T.W.; Ma'ruf, W.F.; Widayat; Suzery, M.; Hadiyanto; Benjakul, S. Application of Spirulina platensis on ice cream and soft cheese with respect to their nutritional and sensory perspectives. *J. Teknol.* **2016**, *2*, 245–251. [CrossRef]
19. de Medeiros, V.P.B.; da Costa, W.K.A.; da Silva, R.T.; Pimentel, T.C.; Magnani, M. Microalgae as source of functional ingredients in new-generation foods: Challenges, technological effects, biological activity, and regulatory issues. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 4929–4950. [CrossRef]
20. Mohebi-Nejad, A.; Bikdeli, B. Omega-3 supplements and cardiovascular diseases. *Tanaffos* **2014**, *13*, 6–14.

21. Simopoulos, A.P. Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* **2002**, *21*, 495–505. [CrossRef]
22. Wang, J.L.; Dong, X.Y.; Wei, F.; Zhong, J.; Liu, B.; Yao, M.H.; Yang, M.; Zheng, C.; Quek, S.Y.; Chen, H. Preparation and characterization of novel lipid carriers containing microalgae oil for food applications. *J. Food Sci.* **2014**, *79*, E169-77. [CrossRef]
23. Cheng, H. Volatile flavor compounds in yogurt: A review. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 938–950. [CrossRef]
24. Mortazavian, A.M.; Rezaei, K.; Sohrabvandi, S. Application of advanced instrumental methods for yogurt analysis. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 153–163. [CrossRef] [PubMed]
25. Yakubu, H.G.; Kovacs, Z.; Toth, T.; Bazar, G. Trends in artificial aroma sensing by means of electronic nose technologies to advance dairy production—A review. *Crit. Rev. Food Sci. Nutr.* **2021**, 1–15. [CrossRef] [PubMed]
26. Hines, E.L.; Boilot, P.; Gardner, J.W.; Gongora, M.A. Patter Analysis for Electronic Noses. In *Handbook of Machine Olfaction*; Pearce, T.C., Schiffman, S.S., Nagle, H.T., Gardner, J.W., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006.
27. Scott, S.M.; James, D.; Ali, Z. Data analysis for electronic nose systems. *Microchim. Acta* **2006**, *156*, 183–207. [CrossRef]
28. Gardener, J.; Bartlett, P. *Electronic Noses, Principles and Application*; Oxford University Press: New York, NY, USA, 1999.
29. Loutfi, A.; Coradeschi, S.; Mani, G.K.; Shankar, P.; Rayappan, J.B.B. Electronic noses for food quality: A review. *J. Food Eng.* **2015**, *144*, 103–111. [CrossRef]
30. Ampuero, S.; Bosset, J.O. The electronic nose applied to dairy products: A review. *Sens. Actuators B Chem.* **2003**, *94*, 1–12. [CrossRef]
31. Folch, J.; Lees, M.; Stanley, G.H.S. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
32. Christie, W.W. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*, 3rd ed.; Oily Press: Bridgwater, UK, 2003.
33. Roszkos, R.; Bazar, G.; Tóth, T.; Kovacs, Z.; Febel, H.; Mezes, M. Effect of n-3 polyunsaturated fatty acid feeding on the fatty acid profile and odor of milk in danbred sows. *J. Appl. Anim. Res.* **2021**, *49*, 447–459. [CrossRef]
34. *MSZ ISO 21527-2*; Microbiology of Food and Animal Fedding Stuffs. Horizontal Method for the Enumeration of Yeasts and Moulds. Part 2: Colony Count Technique in Products with Water Activity Less than of Equal to 0.95. Hungarian Standards Institution: Budapest, Hungary, 2013.
35. Kovacs, Z.; Bodor, Z.; Zinia Zaukuu, J.L.; Kaszab, T.; Bazar, G.; Tóth, T.; Mohácsi-Farkas, C. Electronic nose for monitoring odor changes of Lactobacillus species during milk fermentation and rapid selection of probiotic candidates. *Foods* **2020**, *9*, 1539. [CrossRef]
36. Næs, T.; Isaksson, T.; Fearn, T.; Davies, T. *Multivariate Calibration and Classification*; NIR Publictions: Chichester, UK, 2002.
37. Hewlings, S. Coconuts and Health: Different Chain Lengths of Saturated Fats Require Different Consideration. *J. Cardiovasc. Dev. Dis.* **2020**, *7*, 59. [CrossRef]
38. Gunstone, F.D. *Vegetable Oils in Food Technology Composition, Properties and Uses*, 2nd ed.; John Wiley & Sons: New York, NY, USA, 2011.
39. FAO. *Fats and Fatty Acids in Human Nutrition: Report of an Expert Consultation*; FAO Food and Nutrition: Rome, Italy, 2010; Volume 91.
40. EFSA. European Food Standard Agency Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids. *EFSA J.* **2010**, *8*, 1–107.
41. ISSFAL. Report of the Sub-Committee on Recommendations for Intake of Polyunsaturated Fatty Acids in Healthy Adults. 2014. Available online: https://www.issfal.org/assets/globalrecommendationssummary19nov2014landscape_-3-.pdf%0A%0A (accessed on 5 August 2022).
42. Simopoulos, A.P.; Leaf, A.; Salem, N. Workshop Statement on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids. *Prostaglandins Leukot. Essent. Fat. Acids* **2000**, *63*, 119–121. [CrossRef] [PubMed]
43. Simopoulos, A.P. Summary of the NATO Advanced Research Workshop on Dietary w3 and w6 Fatty Acids: Biological Effects and Nutritional Essentiality. *J. Nutr.* **1989**, *119*, 521–528. [CrossRef] [PubMed]
44. Koletzko, B.; Cetin, I.; Brenna, J.T. Perinatal Lipid Intake Working Group, Child Health Foundation; Diabetic Pregnancy Study Group; European Association of Perinatal Medicine; European Association of Perinatal Medicine; European Society for Clinical Nutrition Metabolism; European Society for Paediatric Gastroenterology. Dietary fat intakes for pregnant and lactating women. *Br. J. Nutr.* **2007**, *98*, 873–877. [CrossRef] [PubMed]
45. Let, M.B.; Jacobsen, C.; Meyer, A.S. Lipid oxidation in milk, yoghurt, and salad dressing enriched with neat fish oil or pre-emulsified fish oil. *J. Agric. Food Chem.* **2007**, *55*, 7802–7809. [CrossRef]
46. Lafarga, T.; Mayre, E.; Echeverria, G.; Viñas, I.; Villaró, S.; Acién-Fernández, F.G.; Castellari, M.; Aguiló-Aguayo, I. Potential of the microalgae Nannochloropsis and Tetraselmis for being used as innovative ingredients in baked goods. *Lwt* **2019**, *115*, 108439. [CrossRef]
47. Holman, R.T. Autoxidation of fats and related substances. *Prog. Chem. Fats Other Lipids* **1954**, *2*, 51–98. [CrossRef]
48. Albert, B.B.; Cameron-Smith, D.; Hofman, P.L.; Cutfield, W.S. Oxidation of marine omega-3 supplements and human health. *Biomed Res. Int.* **2013**, *2013*, 464921. [CrossRef]
49. Clark, S.; Costello, M.; Drake, M.A.; Bodyfelt, F. *The Sensory Evaluation of Dairy Products*, 2nd ed.; Springer Science+Business Media, LLC: New York, NY, USA, 2009. [CrossRef]

50. Murage, M.W.; Muge, E.K.; Mbatia, B.N.; Mwaniki, M.W. Sensory Evaluation of Omega-3-Rich Nile Perch Fish Oil-Fortified Yogurt. *Int. J. Food Sci.* **2021**, *2021*, 8838043. [CrossRef]
51. Robertson, R.C.; Mateo, M.R.G.; O'Grady, M.N.; Guihéneuf, F.; Stengel, D.B.; Ross, R.P.; Fitzgerald, G.F.; Kerry, J.P.; Stanton, C. An assessment of the techno-functional and sensory properties of yoghurt fortified with a lipid extract from the microalga Pavlova lutheri. *Innov. Food Sci. Emerg. Technol.* **2016**, *37*, 237–246. [CrossRef]
52. Jia, H.-X.; Chen, W.-L.; Qi, X.-Y.; Su, M.-Y. CyTA-Journal of Food The stability of milk-based infant formulas during accelerated storage the stability of milk-based infant formulas during accelerated storage. *CyTA-J. Food* **2019**, *17*, 96–104. [CrossRef]
53. Pérez-Silva, A.; Odoux, E.; Brat, P.; Ribeyre, F.; Rodriguez-Jimenes, G.; Robles-Olvera, V.; García-Alvarado, M.A.; Günata, Z. GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chem.* **2006**, *99*, 728–735. [CrossRef]
54. Galetto, W.G.; Hoffman, P.G. Some benzyl ethers present in the extract of vanilla (*Vanilla planifolia*). *J. Agric. Food Chem.* **1978**, *26*, 195–197. [CrossRef]
55. Fenaroli, G.; Bellanca, N.; Furia, T.E. *Fenaroli's Handbook of Flavor Ingredients*, 2nd ed.; CRC Press, Inc.: Cleveland, OH, USA, 1975.
56. Furia, T. *Handbook of Food Additives*, 2nd ed.; N.W. 24th Street, CRC Press, Inc.: Boca Raton, FL, USA, 1980.
57. Jimenez-Alvarez, D.; Giuffrida, F.; Golay, P.; Cotting, C.; Destaillats, F.; Dionisi, F.; Keely, B. Profiles of volatile compounds in milk containing fish oil analyzed by HS-SPME-GC/MS. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 277–283. [CrossRef]
58. Chen, C.; Husny, J.; Rabe, S. Predicting fishiness off-flavour and identifying compounds of lipid oxidation in dairy powders by SPME-GC/MS and machine learning. *Int. Dairy J.* **2018**, *77*, 19–28. [CrossRef]
59. Kolanowski, W.; Jaworska, D.; Weißbrodt, J. Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. *J. Sci. Food Agric.* **2007**, *87*, 181–191. [CrossRef]
60. Let, M.B.; Jacobsen, C.; Meyer, A.S. Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. *Int. Dairy J.* **2005**, *15*, 173–182. [CrossRef]

6. GENERAL DISCUSSION

In the first study in which NIR spectroscopic method was used to evaluate the quality differences of mixture forages and silages, the application of spectral pre-treatments in the form of SNV and 2nd derivative on the raw forage spectra revealed important absorption bands in the NIR region of the spectrum. 1450 nm and 1930 nm related to the O-H (moisture) stretch first overtone and combination bands, respectively (Yang et al., 2017). 1725 nm related to the C-H (fiber fraction) stretch first overtone (Yang et al., 2017) and 2300 nm related to C-H combination band (Workman and Weyer, 2012). The N-H (protein) absorption occurred at 1590 nm and 2100 nm (Yang et al., 2017; Cozzolino et al, , 2001; Murray, 1986). Prior knowledge of these absorption bands is important in the accurate qualitative and quantitative evaluation of the forages measured with the NIR spectrometer. This explains why the 1100-2500 nm range was chosen for the quantitative modelling of the forage constituents.

The qualitative evaluation of the forages using the unsupervised PCA classification showed group dependent variations in both harvesting groups and forage mixture type. In the case of the harvesting or cutting time influence on forage quality, cuts 1 and 2, defined as the early harvesting phases, showed similar quality characteristics, same as cuts 4 and 5 (last phases), with cut 3 the middle phase not showing distinct or specific quality thread. This was found to be related to the stage of maturity and chemical composition of the plants at this phase of growth. The 3rd cut in mixtures A and B (mixture forages of winter cereals) was closer to a less mature stage, while in mixtures C and D (mixture forages of winter cereals and Italian ryegrasses) the 3rd cut was closer to a more mature stage in the NIR classifications, and this was also observed by Worku et al. (2021a) in an

earlier study involving the nutritional composition of these forage mixtures at different phenological phases.

Protein-related N-H absorptions (1450-1650 nm, 1900-2200 nm) were found to have influenced the quality similarities of cut 1 and 2, whereas fiber (C-H) (1600-1800 nm, 2200-2400 nm), as described by Yang et al. (2017), Workman and Weyer (2012) and Murray (1986) explained the similarities of cut 3 and 4. Similar absorption bands explained the separation between the two major forage mixtures; A and B vs. D and C, made up of winter cereals vs. winter cereals and Italian ryegrass, respectively.

The qualitative evaluation of the ensiled forages (silages) also revealed group dependent variations in the days of fermentation (day 0, 7, 14 and 90) when samples were dried and measured with the NIR spectroscopy, and analyzed using PCA. In order to evaluate the quality characteristics of the silages from the early phase of fermentation to the last phase, day 0 and day 90 silages were compared. It was observed that, on day 0, all silage mixtures were similar, that is, all the silage mixtures did not show distinct classification trend, since the fermentation was at the initial stage. However, at day 90, the last phase of the fermentation process, mixtures A and C had similar characteristics, while mixture B and D were different. For most winter cereals and Italian ryegrasses, complete fermentation could be achieved on day 60, from first fermentation (Mohd-Setapar et al., 2012), this perhaps explains why different silage mixtures qualities were observed on the day 90 of the fermentation. Through the PCA loadings, it was observed that water (O-H) absorbance peaks at 1450 nm and 1950 nm (Murray, 1986) were pronounced in both day 0 and 90, explaining a stable moisture levels of the silages from the initial phase to final phase of fermentation.

The quantitative PLS models which explain the relationship between the NIR data and the chemical laboratory data (reference data) of the forage mixtures were built by applying a 2^{nd} derivative math treatment to the NIR

data. The least precise model was built for the total sugar content.. The coefficients of determination for calibration and cross-validation (R^2 and $R^2_{CV)}$ for the other constituents were greater than 0.9, except the R^2_{CV} for EE (0.87). The RMSECV ranged between 0.2-2.4, lower than the standard deviations (SD) of the reference chemical data and with the number of latent variables lower than $1/10^{th}$ of the total sample number (3-7) used in the study, representing robust models. These results were similar to what Acosta et al. (2020) and Alomar et al. (2009) reported for CP, García & Cozzolino (2006) for CF, and Cozzolino et al. (2001) for ash in NIR silage quality prediction studies. The NIR approach was feasible in the evaluation of the new-type forage and silage mixtures qualities.

In the second study, which involved the application of a MOS based e-nose to profile the qualities of alfalfa and rye silages, the results show no variation in the odor patterns based on the plant material type or specie (alfalfa vs rye silages). However, the effect of the various processing technologies (wilting vs direct cut) caused significant variation within the alfalfa silages. It was observed that the application of wilting to alfalfa forages before ensiling caused significant odor variations as compared to ensiling direct cut alfalfa forages. With regards to the rye silages, the silages were identifiable based on the different phenological phases at harvest (before heading vs. heading). It was observed that harvesting the rye crop before heading could cause detectable characteristic variations of the odor profile upon ensiling. Overall, when cross-validation was applied on the acquired e-nose data, on average, more than 65% of samples were classified correctly in both types of silages, with varying hit rates based on the processing type and phenological phase of harvest.

In an attempt to classify the silages based on quality parameters such as pH and lactic acid/acetic acid ratio, silages were ranked according to the pH and lactic acid/acetic acid ratio to obtain some classes within the larger

range defining the silage qualities. With regards to the pH, the range was narrow with 4.4 (the median value) dividing the sample set into two quality parts. Thus, two classes were defined according to the pH of the samples: samples having pH lower than 4.4 (n=32) and samples having pH equal to or higher than 4.4 (n=28). The acid ratio however had a wide range over the 60 samples. From this range, three classes were defined according to the lactic acid / acetic acid ratio. These classes had acid ratio equal to or higher than 10 (n=25), acid ratio lower than 10 and higher than or equal to 5 (n=14), acid ratio lower than 5 (n=21).

A supervised classification (DFA) performed to evaluate the various different quality groups of the silages (pH and lactic acid/acetic ratio) based on the odor profiles, showed that, in the case of rye silages, 64.5% of the cross-validation (CV) samples were classified correctly according to the pH groups, with the classification of alfalfa silages being the less accurate (average hit rate in CV: 60.3%). Whereas the average hit rates of alfalfa and rye silages were 50% and 51.9% respectively, when classification was performed by the lactic acid / acetic acid ratios. The overall average performance of the classifications based on the acid ratio was weaker, but the identification of the low-quality group (ratio < 5) of rye silages was highly successful (75% hit rate in CV). Results of alfalfa samples were less accurate, but good identification (75% hit rate in CV) of the group with high (≥ 10) lactic acid/acetic acid ratio was experienced. According to the above preliminary results, the electronic nose technology proved to be applicable to classify the rye and alfalfa silage samples by their quality, even when quality groups are defined by NIR predicted values which could cause double prediction error.

In the third study an ultra-fast flash GC-FID e-nose was used to analyze milk produced by dairy cows fed on five different diets (see section 4.3.1 for diet composition), profiling of the milks revealed group

identification based on the odor properties of the milk from the five feeding regimes. A supervised classification (DFA) of the acquired e-nose-data of the five groups of milk revealed an overlap of EXP-1 and 2 milks and EXP-3 and 4 milks along DF 1, which also explains 42.84% variation between the milks of these groups. Along the DF2, however, CTR group separated from the rest (EXP-1 and 2, and EXP-3 and 4), which comprises 30.118% of variance.

When a DFA was done based on the main forage compositions of the silage mixtures, three groups; control (CTR, corn and alfalfa based), winter cereals (WC) and winter cereals plus Italian ryegrasses (WC+IRG) milk groups were identified. The variance of DF1 and DF2 were 58.58% and 41.42% respectively, with milk of groups associated with feeding diets WC+IRG which make up EXP-1 and 2, separating from milk from groups associated with feeding diets containing WC, which make up EXP-3 and 4, along DF1. Milk of the CTR group separated from that of WC+IRG and WC groups along DF2, a similar trend of the DFA involving five groups.

The pattern of the DFA revealed that the odor variation between milk samples of WC+IRG and WC groups was larger than the variation that existed between the milk samples of the CTR group and that of the experimental groups. CTR and WC+IRG milks entirely overlap in the plane of the most dominant discriminating factor (DF1), while WC shows little overlap with the aforementioned. Thus, WC milks proved to have the most different odor when compared with the other two main classes (WC+IRG and CTR).

When milk from each feed group was subjected to FA analysis, the results show that the overall saturation of milk FAs decreased (ca. - 2% surplus) in the cows fed on diets containing WC+IRG (i.e., EXP-1 and EXP-2), which was in parallel with the high proportions of capric, lauric, myristic, and stearic acids in their respective milks than milk produced from WC (winter groups (i.e., EXP-3 and EXP-4). The high FA saturation levels in the

milk of the WC+IRG group, and low levels in the WC group, might have influenced the difference or variation in odor observed.

With regards to the sensor identification of odor, the odor-based discrimination of the milk of the CTR group was largely influenced by sensor 800-1-A (ethyl-butyrate), milk of WC groups was influenced largely by sensors 492-1-A (2-propanol) and 600-2-A (butan-2-one), whiles milk of WC+IRG groups was influenced largely by sensors 468-1-A and 565-2-A (ethanol). The identified odor producing compound in the CTR milk was ethyl butyrate, also referred to as ethyl butanoate, or butyric ether, an ester having a fruity odor (Nagata, 2003). According to Marina et al. (2021) esters are the most important odorants in dairy food products, because of their ability to influence the general odor or aroma matrix of such products.

The most abundant volatile compound measured in all the milk samples, especially in the WC group, was 2-propanol, also known as isopropyl alcohol, a water soluble aliphatic alcohol with a sweet odor, which was also reported by Sympoura et al. (2009) to be in milk obtained from dairy cows fed with supplemented extruded linseed and α-tocopherol. Though this study did not use similar feed supplements, the forage sources, especially winter cereals contain significant levels of naturally occurring α-tocopherol (Lachman et al., 2018).

Also, 2-butanone or butan-2-one or methyl ethyl ketone, an organic compound with sharp-sweet odor was reported to be present in milks obtained from cows fed with mycorrhizal ensiled forage (Genovese et al., 2019). In this present study, the intensity of butan-2-one was higher in WC milks. A study reported the possible transmission of silage flavors including ethanol produced during forage fermentation into bovine milk (Shipe et al., 1962). Diets that contain silages classified as well fermented with a pleasant aroma may still contain some level of ethanol in such amounts, which can impart milk odor (Randby et al., 1999).

In the fourth study, the ultra-fast GC-FID based e-nose was also employed to detect the odor pattern in vanilla milkshake powder fortified with different levels of micro-encapsulated DHA product of microalgae oil origin (S17-P100). Generally, milkshakes are not easy to classify within the wide varieties of milk products. However, this product is similar to most infant formula products (composed of 70% milk whey) and it is mostly prepared in a ready-to-drink form with the addition of water. The basic rationale behind the addition of n3 long chain FAs from microalgae to this product is because originally it's poor in n3 FAs, and microalgae oil is one of the most efficient sources of n3 FAs. The enrichment process (an inclusion rate of 0.2 to 2 w/w %) used for this study involved the use of S17-P100 levels above 100 mg/10g milkshake powder (with a theoretical maximum of 428 mg DHA and 16 mg EPA in 100 g) to fulfil and possibly slightly exceed most recommended levels, for example, the European Food Safety Authority recommendation of 250 mg/day DHA + EPA for adults (European Food Safety Authority, 2010), the US (Simopoulos et al., 2000) and NATO recommendations (Simopoulos, 1989) which are quite similar to that of the FAO (FAO, 2010) recommendations of 300–400 mg EPA+DHA/day. At the end of the fortification process, the DHA level was successfully increased to about 412 mg/100 g and EPA 16 mg/100 g in the milkshake product. The possibility of this fortification to cause off odor was then tested using the e-nose. The measurement was done on the milkshake powder and not ready shake (liquid form) because the objective was to test the smell effect of fats in the form in which fat can be smelled well. If it was in liquid form, many other smells from the multicomponent milkshake would much easier dominate the complex smell of the inclusion oils and fats, because fat is non-polar. So, the powder form was used, based on pre-test knowledge. And, from consumers' point of view, the smell in a powdered form is also important, because when the package of the product is opened, the initial

smell before mixing with water will likely form the choice of the product as well.

The modelling results of the e-nose sensor data (all and selected) did not show any odor dependent variation when both unsupervised (PCA) and supervised (DFA) classification techniques were used. Even though the odor differences between the non-supplemented (control) and the fortified milkshake samples could be identified, it can be inferred that no major off odor occurred as a results of the increased level of supplementation, since the PCA and DFA plots didn't show concentration dependent variation from the control product.

When a more targeted approach like a PLS calibration using selected sensors on the S17-P100 supplementation was used, a successful calibration was achieved, which indicated the existence of an odor pattern that is in relation with the concentration of the additive, S17-P100. However, since the classification models that identify the groups of the different supplementation levels did not pick the concentration-dependent odor patterns, it can be deduced that there are major odor patterns independent of S17-P100 supplementation and these major odor patterns provide the possibility to identify the sample groups. The minor odor patterns dependent on the S17-P100 supplementation concentration can be traced when specifically targeted, but they are generally hidden by the major (independent) odor patterns.

Though the odor profile of the supplemented product did not significantly differ from the control, there exist some volatile compounds of odor importance that were identified based on retention indices. These are 1-propanol and 2-propanol which are isomeric forms of propanol (an alcohol) molecule with strong odor, ethyl acetate or ethyl ethanoate (ester formed between acetic acid and ethanol), benzyl butanoate and ethyl hexanoate (a fatty acid ethyl ester) with a fruity like odors (Fenaroli et al., 1975; Galetto & Hoffman, 1978; Pérez-Silva et al., 2006), 1,4-cineole is an oxabicycloalkane

with minty lime oil odor (Furia, 1980), pentanal is a saturated fatty aldehyde with acrid odor (Fenaroli et al., 1975) present in samples with different levels of supplementation.

The above discussed results of the four experiments show how correlative analytical technologies such as NIR spectroscopy and e-nose could be employed in the quality evaluation of forage and silage mixtures, milk from different feeding regimes and fortified dairy product, such as DHA enriched milkshake.

7. CONCLUSIONS

Based on the results of the first experiment, it can be concluded that near-infrared (NIR) spectroscopic analysis could be helpful in the evaluation of the quality differences between mixture forages of winter cereals and Italian ryegrass, during harvesting especially at different phenological phases of the forages. Again, the fermentation quality of silages made from these novel forage mixtures could be monitored during the various days or phases of ensiling, to enable the production of good quality silages on time, to meet the preference or demand of dairy farms. Quantitatively, the predictive models developed could be used for quick analysis of some essential nutritive constituents such as crude protein, crude fiber, and ash contents with high accuracy and precision, at low cost while avoiding the laborious conventional laboratory methods. According to literature the applicability of these models in portable or handheld NIR devices is possible for on-farm or farm gate evaluation of the forage mixtures during production or when supplying to farmers. Though the models developed under this current research utilized a small sample size (100 samples), the models serve as good starters for future industrial or commercial NIR quality evaluation of these novel forage mixtures for efficient dairy cows feeding.

As a preliminary test, the use of an e-nose with metal oxide semiconductor(MOS) gas sensors in the evaluation of rye and alfalfa silages based on different harvest technologies, phenological phase of harvest, and the pH and the lactic acid/ acetic acid ratio proved promising in the second experiment. The application of the e-nose technology for quick screening of silages could ensure safe selection of silages with good characteristic smell or aroma to meet acceptable preference. The best harvesting and processing method could also be selected through the smell characteristics of the silages, thereby ensuring efficient good silage production. Based on this study, the application of wilting to alfalfa forages before ensiling caused odor variations

as compared to ensiling direct cut alfalfa forages, while harvesting rye crop before heading caused variations of the odor profile upon ensiling. Thus, these outcomes could provide silage producers a fair idea of which technology or processing method and phenological phase of harvest to depend on to ensure good silage production. This preliminary study affirms instrumental odor testing methodology to be promising in the field of quality testing of fermented forages. Studies of our workgroup conducted such as Worku et al. (2021a) which evaluated the ensiled novel forage mixtures, and a yet to be published study that sought to evaluate lupin (*Lupinus albus L.*) silages with e-noses other than the MOS e-nose used in this current study.

The application of the e-nose technology in the dairy product quality testing has fast gained enormous recognition in food science. In experiment three, the application of an ultra-fast flash GC-FID e-nose to evaluate bovine milk from different feed regimes proved effective. Based on the results, it can be concluded that milk odor and fatty acid composition can be altered through feeding dairy cows with feeds made from the novel mixtures of winter cereals and Italian ryegrass silages evaluated in experiment one while also the level of odor alteration can be monitored and the best option of mixed silages may be selected based on the odor pattern of the produced milk. In experiment four as well, the effectiveness of the e-nose technology to test the odor stability of a bioactive improved milkshake proved efficient. The e-nose could therefore serve as a rapid analytical tool to complement human sensory evaluation in the production and development of milk and milk-based products to ensure high quality standard, integrity, and to meet consumer preference or choice. The limitation of the technology is its inability to determine dairy product preference by the consumer, notwithstanding its efficient detection of aroma pattern of the products.

It is important to mention, that at this current stage, the e-nose methods investigated are not applicable in current daily routine, as there is no

version of these e-noses that could function well in routine practice (accurately with good repeatability at a reasonable price). However, the e-nose studies done in the thesis were necessary to investigate how these technologies with high precision could work. We decided not to focus on the actual cost effective technologies only, but investigate the needs and possible outputs of still expensive ways, so we can direct the developments on a way to create the cheaper technologies that are really needed. Now, based on the results, we noticed that these are technologies providing relevant results. As for predicting and projecting some ideas for the future, the next step shall be to select or build such instruments (e.g. hand held instruments) that will be cost effective and useful for daily routine.

8. NEW SCIENTIFIC RESULTS

From the four experiments that form the content of this PhD work, I declare the following scientific results.

1. The near-infrared (NIR) spectroscopic measurement coupled with principal component analysis (PCA) as a chemometric tool was able to qualitatively differentiate between the mixture forages of winter cereals only, and winter cereals plus Italian ryegrass, harvested at different phenological phases of growth, and ensiled at different days.
2. The partial least square (PLS) regression models developed for predicting the chemical constituents of the mixture forages of winter cereals and Italian ryegrass from their NIR spectra are robust with high accuracy (R^2 and $R^2_{CV} > 0.9$) with root mean square error of cross-validation ($RMSE_{CV}$ = 0.59%, 0.76% and 0.31%) and latent variables (LV= 3, 3, 6) for crude protein, crude fiber, and ash, respectively.
3. The applied MOS e-nose was capable of identifying the odor differences in alfalfa silages caused by wilting, and can therefore be used for the efficient monitoring of the quality changes that occur in silages due to wilting (75% hit rate in CV).
4. Based on the odor patterns measured, the MOS e-nose proved to be useful in the detection and description of the quality groups of alfalfa and rye silages objectively as defined by the pH of the silages (average hit rate in CV=60.3% and 64.5% respectively).
5. The applied ultra-fast flash GC-FID e-nose measurement revealed the odor differences in milks from the different feeding regimes. As detected by the e-nose, milk which originated from cows fed corn silage and alfalfa haylage based control diet was largely influenced by ethyl-butyrate, whereas milk associated with supplementation

with silage of winter cereals was influenced by 2-propanol and butan-2-one and that of winter cereals plus Italian ryegrass was influenced by ethanol.

6. The applied ultra-fast flash GC-FID e-nose revealed that to produce bovine milk with less organoleptic differences, the inclusion of winter cereals plus Italian ryegrass silage (7 kg/day) in cows' diet could be more beneficial over that of winter cereals only, as it caused less prominent odor alterations, but still caused changes in the milk fatty acid composition.
7. As detected by the applied ultra-fast flash GC-FID e-nose, an increased DHA level in vanilla milkshake up to 412 mg/100 g with the aim of improving the bioactive component of the product using micro-encapsulated algae oil would not cause off-odor in the product.

9. SUMMARY

Study 1

In this study, the near-infrared (NIR) spectroscopy was employed to determine the differences between forage mixtures and to also evaluate the fermentation characteristics of mixed silages from winter cereals and Italian ryegrasses origin. Harvesting of the forages by cutting at a 10 cm stubble height was carried out on different phases based on the existing extended BBCH scale: five phases (Cut 1-5), with one week interval (n = 100). The yield of the last harvest (Cut 5) was then ensiled and analysed on four different days (D0, D7, D14, and D90) (n = 80). The results of the qualitative analysis via principal component analysis based on the NIR data revealed differences according to the days of harvest, group differences between winter cereals and Italian ryegrass forages, and group differences in the fermentation phases of the silages. The quantitative analysis via partial least square regression models gave excellent determination coefficient in cross-validation ($R^2_{CV} > 0.9$) for crude protein (CP), crude fiber (CF), and ash gave, while models for ether extract (EE) and total sugar content were the least precise ($R^2_{CV} = 0.87$ and 0.74, respectively). The values of root mean square error of cross-validation (RMSECV) that show accuracy of models were 0.59, 0.76, 0.22, 0.31, and 2.36 %DM, for CP, CF, EE, ash, and total sugar, respectively. The application of NIR proved to be an efficient tool in evaluating the type and growth differences of the winter cereals and Italian ryegrass forage mixtures, and the quality changes that occur during ensiling.

Study 2

In this study, the odor profile of alfalfa and rye silages (n = 22 and 38, respectively) produced with different time of harvest (before heading vs. heading) and processing technologies (direct-cut vs. wilted), and covering a

wide range of quality based on pH and lactic acid/acetic acid ratio, was analyzed with metal oxide semiconductor sensor array technology. The odor patterns based on the harvesting, processing and quality categories based on the pH and the lactic acid / acetic acid ratio were compared. The applied e-nose was not suitable for distinguishing between samples made from alfalfa or rye with average compositional parameters, because the effect of species on the odor was less characteristic than that of the diverse processing conditions. However, on the basis of the odor profiles described by the measured sensor signals, alfalfa-based forages prepared with different processing (direct-cut vs. wilted) could be accurately identified, and rye samples were identifiable according to the different phenological phases at harvest (before heading vs. heading). In the supervised classification (discriminant analysis) of groups of samples based on pH and lactic acid / acetic acid ratio, 65-75% of the samples selected for validation were correctly identified, which indicates reliability of the method. Based on the results, the presented instrumental odor testing methodology proved to be promising in the field of quality testing of fermented forages.

Study 3

In this study, a corn silage based control (CTR) and four experimental (EXP) diets were fed to Holstein-Friesian cows (n = 32, >150 days in milking, average milk production: <25 kg/day) in a single-blinded efficacy study during a series of 4-week periods, with 2 weeks of adaptation to each feed before the main trial. EXP diets contained winter cereals (WC), as well as WC plus Italian ryegrass (IRG) silages in different proportions. Milk collected from each trial was subjected to fatty acid (FA) analysis and odor profiling through the utilization of gas chromatography and an electronic nose, respectively. The results show that milk FAs in the EXP-3 and EXP-4 groups (which contained mixed silages using WC) changed the most when compared with other groups. Moreover, the 7 kg/day inclusion rate of the

WC + IRG and of the WC silages in the diets of the EXP-2 and EXP-3 groups, respectively, reduced the n6:n3 ratio of the milk, which is a nutritional advantage for consumers. The odor variation between the milk of the WC + IRG and WC groups was greater than the variation between the milk of the CTR and EXP groups. The main volatile compound responsible for the identification of the odor of the CTR milk was ethyl-butyrate, whereas 2-propanol and butan-2-one dominated the WC milk; the milk samples of the WC + IRG groups were influenced largely by ethanol. The study proved that with a 7 kg/day inclusion of mixed silages of winter cereals plus Italian ryegrass, the FA and odor profile of bovine milk could be modified, but the odor was less altered than by including silages of winter cereals only.

Study 4

In this study, the aim was to verify whether efficient fortification of vanilla milkshakes with micro-encapsulated microalgae oil (brand: S17-P100) was performed without distorting the product's odor. A 10-step oil-enrichment protocol was developed using an inclusion rate of 0.2 to 2 w/w%. Fatty acid (FA) analysis was performed using methyl esters with the GC-MS technique, and the recovery of docosahexaenoic acid (C22:6 n3, DHA) was robust ($r = 0.97$, $p < 0.001$). The enrichment process increased the DHA level to 412 mg/100 g. Based on this finding, a flash-GC-based electronic nose (e-nose) was used to describe the product's odor. Applying principal component (PC) analysis to the acquired sensor data revealed that for the first four PCs, only PC3 (covering 6.5% of the total variance of odor profiles) showed a difference between the control and the supplemented products. However, no systematic pattern of odor profiles corresponding to the percentages of supplementation was observed within the PC planes. Similarly, when discriminant factor analysis (DFA) was applied, although a classification of the control and supplemented products was successful with a validation score

of 98%, the classification pattern of the odor profiles (arrangement of inclusion classes) did not follow a systematic format.

Again, when a more targeted approach such as the partial least square regression (PLSR) was used on the most dominant sensors, a weak relationship ($R^2 = 0.50$) of variations of odor and inclusion rate was observed, indicating that there was no linear combination of the qualitative sensors' signals that could accurately describe the inclusion-dependent odor variation.

It can therefore be inferred that no off-odor was present as a side effect of the increase in the oil concentration. Some volatile compounds of importance in regard to the odor, such as ethyl acetate, ethyl-isobutarate, pentanal and pentyl butanoate, were found in the supplemented product. Although the presence of yeasts and molds was excluded from the product, ethanol was detected in all samples, but with an intensity that was insufficient to cause off-odor.

10. ÖSSZEFOGLALÁS

1. vizsgálat

Ebben a vizsgálatban a közeli infravörös (NIR) spektroszkópiát alkalmaztam a fűkeverékek közötti különbségek meghatározására, valamint az őszi kalászos és olaszperje eredetű vegyes szilázsok fermentációs jellemzőinek értékelésére. A szálas takarmányok betakarítása 10 cm-es tarlómagasságban történő vágással, a kiterjesztett BBCH skála szerint öt különböző fázisban történt (1-5 vágás), egyhetes intervallumokban (n = 100). Az utolsó betakarítás hozamát (5. vágás) ezután silóztuk és négy különböző napon (D0, D7, D14 és D90) mintáztuk (n = 80). A NIR adatokon alapuló főkomponensanalízissel (PCA) végzett kvalitatív elemzés eredményei a betakarítási napok szerinti eltéréseket, az őszi kalászosok és az olasz perje szilázsok közötti csoportkülönbségeket, valamint a szilázsok erjedési fázisaiban mutatkozó csoportkülönbségeket mutattak ki. A részleges legkisebb négyzetes regressziós (PLSR) modellekkel végzett kvantitatív elemzés kiváló determinációs együtthatót adott a keresztvalidációban (R2CV > 0,9) a nyersfehérje- (NYF), a nyersrost- (NYR) és a hamutartalom esetében, míg a nyerszsír- (NYZS) és az összes cukortartalom modelljei voltak a legkevésbé pontosak (R2CV = 0,87 és 0,74). A kereszt-validáció négyzetes középhibájának (RMSECV) értékei, amelyek a modellek megbízhatóságát mutatják, 0,59, 0,76, 0,22, 0,31 és 2,36 %DM voltak a NYF, NYR, NYZS, hamu- és összes cukortartalom esetében. A NIR alkalmazása hatékony eszköznek bizonyult az őszi kalászosok és az olaszperje szálastakarmány keverékek fajtáinak és növekedési különbségeinek, valamint a silózás során bekövetkező minőségi változások értékelésében.

2. vizsgálat

Ebben a vizsgálatban a különböző időpontokban betakarított (kalászhányás előtti vs. utáni) és eltérő feldolgozási technológiával (közvetlen betakarítás vs. fonnyasztás) előállított, a pH és a tejsav/ecetsav arányon alapuló minőséget széles skálát lefedő lucerna- és rozsszilázsok (n = 22, illetve 38) illatprofilját vizsgáltuk fém-oxid félvezető (MOS) szenzorsoros technológiával. Összehasonlítottam a betakarítási, feldolgozási, valamint a pH és a tejsav/ecetsav arány szerinti minőségi kategóriák illatprofiljait. Az alkalmazott e-orr nem volt alkalmas az átlagos összetételű lucerna vagy rozs eredetű szilázsok megkülönböztetésére, mert a növényfajok illatra gyakorolt hatása kevésbé volt jellemző, mint a változatos feldolgozási körülmények. A mért szenzorjelekkel leírt illatprofilok alapján azonban a különböző feldolgozással (közvetlen betakarítás vs. fonnyasztás) előállított lucerna alapú takarmányok megbízhatóan azonosíthatók, a rozsminták a különböző betakarításkori fenológiai fázisok (kalászhányás előtti vs. utáni) szerint azonosíthatók voltak. A mintacsoportok pH és tejsav/ecetsav arány alapján történő felügyelt osztályozása (diszkriminanciaanalízis) során a validálásra kiválasztott minták 65-75%-a helyesen került azonosításra, ami a módszer megbízhatóságát jelzi. Az eredmények alapján a bemutatott műszeres illatvizsgálati módszertan ígéretesnek bizonyult az erjesztett tömegtakarmányok minőségvizsgálata terén.

3. vizsgálat

Ebben a vizsgálatban egy kukoricaszilázs alapú kontroll (CTR) és négy kísérleti (EXP) takarmányt etettek holstein-fríz tehenekkel (n = 32, fejésben >150 nap, átlagos tejtermelés: <25 kg/nap), négyhetes periódusokban végzett egyszerű vak (single blind) vizsgálatsorozat során, 2 hét adaptációs időszakkal minden vizsgálati szakasz előtt. Az EXP

takarmánykeverékek különböző arányban tartalmaztak őszi kalászosok (WC), valamint WC és olaszperje (IRG) keverékének szilázsát. Az egyes kísérletekből gyűjtött tejet zsírsavanalízisnek és illatprofilelemzésnek vetettük alá gázkromatográfia, illetve elektronikus orr segítségével. Az eredmények azt mutatják, hogy az EXP-3 és EXP-4 csoportokban (amelyek WC alapú szilázst tartalmaztak) változott a legjobban a tej zsírsavösszetétele a többi csoporthoz képest. Az EXP-2 és az EXP-3 csoport takarmányának WC+IRG és WC szilázsokkal történő 7 kg/nap dózisú kiegészítése csökkentette a tejzsír n6:n3 arányát, mely a fogyasztók számára táplálkozásélettani előnyt jelent. A WC+IRG és a WC csoportok teje közötti illatkülönbség nagyobb volt, mint a CTR és az EXP csoportok tejei közötti eltérés. A CTR tej illatának azonosíthatóságáért felelős fő illékony vegyület az etil-butirát volt, míg a WC-tejben a 2-propanol és a bután-2-on dominált; a WC+IRG csoportok tejmintáit nagymértékben befolyásolta az etanol. A vizsgálat bebizonyította, hogy őszi kalászosokból és olaszperjéből készült keverékszilázsok 7 kg/nap dózisú hozzáadásával a tehéntej zsírsavösszetétele és illatprofilja módosítható, de az illat kevésbé változik, mint őszi kalászosokból készült keverékszilázs alkalmazása esetén.

4. vizsgálat

Ennek a vizsgálatnak a célja annak ellenőrzése volt, hogy a vaníliás turmixokat mikrokapszulázott mikroalgaolajjal (márka: S17-P100) hatékonyan dúsították-e a termék illatának torzítása nélkül. Egy 10 lépésből álló olajdúsítási protokollt alkalmaztunk 0,2-2 tömeg%-os bekeverési aránnyal. A zsírsav-metilésztereket GC-MS technikával vizsgáltuk, és a dokozahexaénsav (C22:6 n3, DHA) visszanyerése robusztus volt ($r = 0,97$, $p < 0,001$). A dúsítási folyamat 412 mg/100 g-ra emelte a DHA-szintet. Ezen megállapítás alapján a termék illatának leírására flash-GC-alapú elektronikus orrot használtunk. A kapott szenzoradatok főkomponens (PC) elemzése azt

mutatta, hogy az első négy PC-nél csak a PC3 (mely az illatprofilok teljes varianciájának 6,5%-át fedi le) mutatott eltérést a kontroll és a kiegészített termékek között. A PC-síkon belül azonban nem volt megfigyelhető az illatprofilok olajkiegészítés százalékos arányának megfelelő szisztematikus mintázata. Hasonlóképpen, a diszkrimináns faktorelemzés (DFA) alkalmazásakor, bár a kontroll és a kiegészített termékek besorolása sikeres volt (98%-os validációs eredménnyel), az illatprofilok osztályozási mintája (csoportrendeződése) nem követett szisztematikus formátumot.

Hasonlóképpen, amikor egy célzottabb megközelítést, a részleges legkisebb négyzetes regressziót (PLSR) alkalmaztuk a legdominánsabb szenzorokkal, gyenge összefüggést (R^2 = 0,50) találtunk az illat és a dúsítási arány változása között, ami azt jelzi, hogy az illatprofilt leíró kvalitatív szenzoroknak nem voltak olyan lineáris kombinációi, amelyek pontosan leírhatják a kiegészítéstől függő illatváltozásokat.

Mindebből arra következtethetünk, hogy az olajkoncentráció növekedésének mellékhatásaként nem alakult ki torz illat. A kiegészített termékben néhány, az illat szempontjából fontos illékony vegyületet, például etil-acetátot, etil-izobutarátot, pentanál- és pentil-butanoátot találtunk. Bár igazoltuk az élesztők és penészgombák jelenlétének hiányát a termékben, etanolt minden mintában kimutattunk, de olyan intenzitással, amely nem volt elegendő a kellemetlen szag kialakulásához.

11. ACKNOWLEDGEMENTS

I would like to express my profound gratitude to Dr. György Bázár, for the enormous support he provided me throughout this academic journey. George, my friend and mentor, I sincerely acknowledge your kindness, and the special mentor-mentee relationship you created between us. You picked me up from a naïve level in the field of NIRS and e-nose, and mentored me to an appreciable level. I am eternally grateful to you, and, it is my hope, that the mentorship will continue beyond this PhD thesis.

I also single out the special support of Prof. Tamás Tóth, to the success of this thesis. I am very grateful! To Prof. Dr. Melinda Kovács, thank you very much for the support offered to me on this journey. I would like to thank Prof. Dr. András Szabó, Prof. Zoltán Kovács, my colleague, Omeralfaroug Ali, and all co-authors for well-coordinated collaborations. I sincerely acknowledge your support throughout this period.

I extend my profound gratitude to my uncle, Dr. Bakari Yusuf Dramanu, for his kind support throughout this journey. Dr. Bakari, may God reward you a thousandfold! To the Mabunwura Yakubu family of Kpembe, my mother, Mariama Alaja, and my lovely wife, Nafisah Issah, thank you for your support. I dedicate this work to the entire family. It is my hope and wish that this work motivates the next generation of the family, to work towards the highest academic excellence. At least, they have a reference point to start from, with no excuse. Though time reveals so many things, I sincerely believe that time will be kind to them!

And, finally, I would like to acknowledge the following grants: Hungarian government and EU under Project No.: GINOP-2.3.4.-15-2016-00005, Ministry for Innovation and Technology under GINOP-2.3.2-15-2016-00046; the Ministry of Human Capacities under EFOP-3.6.3-VEKOP-16-2017-00005; the Hungarian Academy of Sciences under MTA-KE-SZIE 13003.

12. REFERENCES

Abbasi, S., & Azari, S. (2011). Efficiency of novel iron microencapsulation techniques: Fortification of milk. *International Journal of Food Science and Technology*,*46*(9),1927–1933.https://doi.org/10.1111/j.13652621.2011.02703.x

AbuGhazaleh, A. A., & Holmes, L. D. (2007). Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. *Journal of Dairy Science*, *90*(6), 2897–2904. https://doi.org/10.3168/jds.2006-684

Acosta, J. J., Castillo, M. S., Hodge, G. R., & Resources, E. (2020). Comparison of Benchtop and Handheld Near Infrared Spectroscopy Devices to Determine Forage Nutritive Value. *Crop Science*. https://doi.org/10.1002/csc2.20264.This

Adinepour, F., Pouramin, S., Rashidinejad, A., & Jafari, S. M. (2022). Fortification/enrichment of milk and dairy products by encapsulated bioactive ingredients. *Food Research International*,*157*,*111212*. https://doi.org/10.1016/J.FOODRES.2022.111212

Alomar, D., Fuchslocher, R., Cuevas, J., Mardones, R., & Cuevas, E. (2009). Determination of quality parameter of pasture by NIR reflentance spectroscopy. *Chilean Journal of Agricultural Research*, *69*(2), 198–206. https://doi.org/10.4067/S0718-58392009000200009

Association of Official Analytical Chemist. (2006). *Official methods of analysis* (18th ed.). AOAC International.

Auldist, M. J., Marett, L. C., Greenwood, J. S., Hannah, M., J.L., J., & Wales, W. J. (2013). Effects of different strategies for feeding supplements on milk production responses in cows grazing a restricted pasture allowance. *Journal of Dairy Science*, *96*, 1218–1231.

Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *AppliedSpectroscopy*,*43*(5),772–777. https://doi.org/10.1366/0003702894202201

Butler, W. R. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science*,*60–61*,449–457. https://doi.org/10.1016/S0378-4320(00)00076-2

Capuano, E., Van Der Veer, G., Boerrigter-Eenling, R., Elgersma, A., Rademaker, J., Sterian, A., & Van Ruth, S. M. (2014). Verification of fresh grass feeding, pasture grazing and organic farming by cows farm milk fatty acid profile. *Food Chemistry*, *164*, 234–241. https://doi.org/10.1016/j.foodchem.2014.05.011

Carroll, S. M., DePeters, E. J., Taylor, S. J., Rosenberg, M., Perez-Monti, H., & Capps, V. A. (2006). Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. *Animal Feed Science and Technology*,*131*(3–4),451–473. https://doi.org/10.1016/J.ANIFEEDSCI.2006.06.019

Castillo, C., Pereira, V., Abuelo, Á., & Hernández, J. (2013). *Effect of Supplementation with Antioxidants on the Quality of Bovine Milk and Meat Production*. *2013*.

Christie, W. W. (1982). A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *Journal of Lipid Research*, *23*(7), 1072–1075. https://doi.org/10.1016/S0022-2275(20)38081-0

Christie, W. W. (2003). *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids* (3rd ed.). Oily Press.

Cowe, I. A., & McNicol, J. W. (1985). The use of principal components in the analysis of near-infrared spectra. *Applied Spectroscopy*, *39*(2), 257–266.

Cozzolino, D., Fassio, A., & Gimenez, A. (2001). The use of near-infrared reflectance spectroscopy (NIRS) to predict the composition of whole maize plants. *Journal of the Science of Food and Agriculture*, *81*(1), 142–146. https://doi.org/10.1002/1097-0010(20010101)81:1<142::AID-JSFA790>3.0.CO;2-I

Cronjé, P. B. (1990). Supplementary feeding in ruminants- A physiological approach. *South African Journal of Animal Science*, *20*(3), 110–117.

Darwin, A., Barnes, A., & Cord-Ruwisch, R. (2018). In vitro rumen fermentation of soluble and non-soluble polymeric carbohydrates in relation to ruminal acidosis. *Annals of Microbiology*, *68*, 1–8. https://doi.org/10.1007/s13213-017-1307-x

Douphrate, D. I., Hagevoort, G. R., Nonnenmann, M. W., Lunner Kolstrup, C., Reynolds, S. J., Jakob, M., & Kinsel, M. (2013). The Dairy Industry: A Brief Description of Production Practices, Trends, and Farm Characteristics Around the World. *Journal of Agromedicine*,*18*(3),187–197. https://doi.org/10.1080/1059924X.2013.796901

Edwards, J. S., Bartley, E. E., & Dayton, A. D. (1980). Effects of Dietary Protein Concentration on Lactating Cows. *Journal of Dairy Science*, *63*(2), 243–248. https://doi.org/10.3168/JDS.S0022-0302(80)82920-1

Ekin, C., Mohammad, S., & Gharibzahedi, T. (2021). Trends in Food Science & Technology Yogurts supplemented with lipid emulsions rich in omega-3 fatty acids : New insights into the fortification, microencapsulation, quality properties, and health-promoting effects. *Trends in Food Science & Technology*, *110*(February),267–279. https://doi.org/10.1016/j.tifs.2021.02.016

Elgersma, A., Tamminga, S., & Ellen, G. (2006). Modifying milk composition through forage. *Animal Feed Science and Technology*, *131*(3–4), 207–225. https://doi.org/10.1016/j.anifeedsci.2006.06.012

Emery, R. S. (1978). Feeding For Increased Milk Protein. *Journal of Dairy Science*, *61*(6), 825–828. https://doi.org/10.3168/JDS.S0022-0302(78)83656-X

European Food Safety Authority. (2010). Scientific opinion of the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies

(NDA) on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, tra. *EFSA Journal*, *8*(1461).

Falchero, L., Sala, G., Gorlier, A., Lombardi, G., Lonati, M., & Masoero, G. (2009). Electronic Nose analysis of milk from cows grazing on two different Alpine vegetation types. *Journal of Dairy Research*, *76*(3), 365–371. https://doi.org/10.1017/S0022029909004105

FAO. (2010). *Fats and fatty acids in human nutrition: Report of an expert consultation. FAO Food and Nutrition* (p. 91).

Fenaroli, G., Bellanca, N., & Furia, T. E. (1975). *Fenaroli's handbook of flavor ingredients* (Thomas E. Furia & N. Bellanca (eds.); 2nd ed.). CRC Press, Inc.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Furia, T.E. (1980). *Handbook of food additives* (2nd ed.). CRC Press, Inc.

Galetto, W. G., & Hoffman, P. G. (1978). Some benzyl ethers present in the extract of vanilla (Vanilla planifolia). *Journal of Agricultural and Food Chemistry*, *26*(1), 195–197. https://doi.org/10.1021/jf60215a065

García, J., & Cozzolino, D. (2006). Use of near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration models Uso de la espectroscopía de reflectancia en el infrarrojo cercano (NIR) para predecir la composición química de forrajes e. *Agricultura Tecnic (Chile)*, *66*(1), 41–47.

Genovese, A., Marrazzo, A., De Luca, L., Romano, R., Manzo, N., Masucci, F., Di Francia, A., & Sacchi, R. (2019). Volatile organic compound and fatty acid profile of milk from cows and buffaloes fed mycorrhizal or nonmycorrhizal ensiled forage. In *Molecules* (Vol. 24, Issue 8, p. 14). https://doi.org/10.3390/molecules24081616

Gerloff, B. J. (2000). Dry cow management for the prevention of ketosis and fatty liver in dairy cows. *Veterinary Clinics of North America: Food Animal Practice*, *16*(2), 283–292. https://doi.org/10.1016/S0749-0720(15)30106-7

Gomez-Cortes, P., Frutos, P., Mantecon, A. R., Juarez, M., Fuente de la, M. A., & Hervast, G. (2008). Addition of Olive Oil to Dairy Ewe Diets : Effect on Milk. *Journal of Dairy Science*, *91*, 3119–3127. https://doi.org/10.3168/jds.2007-0954

Grummer, R. R. (1991). Effect of Feed on the Composition of Milk Fat. *Journal of*

Dairy Science, *74*(9), 3244–3257. https://doi.org/10.3168/jds.S0022-0302(91)78510-X

Gulbas, S. Y., & Saldamli, I. (2005). The effect of selenium and zinc fortification on the quality of Turkish white cheese. *International Journal of Food Sciences and Nutrition*, *56*(2), 141–146. https://doi.org/10.1080/09637480500082579

Gumus, C. E., & Gharibzahedi, S. M. T. (2021). Yogurts supplemented with lipid emulsions rich in omega-3 fatty acids: New insights into the fortification, microencapsulation, quality properties, and health-promoting effects. *Trends in Food Science and Technology*, *110*(February 2020), 267–279. https://doi.org/10.1016/j.tifs.2021.02.016

Hashemi, H., Hadi, M., & Mesbahi, G. (2015). Scientific and technical aspects of yogurt fortification : A review. *Food Science and Human Wellness*, *4*(1), 1–8. https://doi.org/10.1016/j.fshw.2015.03.002

Hassen, A., Chavula, P., Mohammed, S. S., & Dawid, A. (2022). The Effect of Feed Supplementation on Cow Milk Productivity and Quality : A Brief Study. *International Journal of Agriculture and Veterinary Sciences*, *4*(1), 13–25.

Hopkins, D. W. (2001). What is a Norris Derivative? *NIR News*, *12*(3), 3–5. https://doi.org/10.1255/nirn.611

Jenkins, T. C., & McGuire, M. A. (2006a). Major advances in nutrition: Impact on milk composition. *Journal of Dairy Science*, *89*(4), 1302–1310. https://doi.org/10.3168/JDS.S0022-0302(06)72198-1

Jenkins, T. C., & McGuire, M. A. (2006b). Major advances in nutrition: Impact on milk composition. *Journal of Dairy Science*, *89*(4), 1302–1310. https://doi.org/10.3168/jds.S0022-0302(06)72198-1

Jenkins, T. C., & McGuire, M. A. (2006c). Major Advances in Nutrition: Impact on Milk Composition. *Journal of Dairy Science*, *89*, 1302–1310.

Kazmi, S. A., Vieth, R., & Rousseau, D. (2007). Vitamin D3 fortification and quantification in processed dairy products. *International Dairy Journal*, *17*(7), 753–759. https://doi.org/10.1016/j.idairyj.2006.09.009

Kennelly, J. J. (1996). The fatty acid composition of milk fat as influenced by feeding oilseeds. *Animal Feed Science and Technology*, *60*(3–4), 137–152. https://doi.org/10.1016/0377-8401(96)00973-X

Kertz, A. F. (2010). Urea Feeding to Dairy A Historical Perspective and Review. *The Professional Animal Scientist*,*26*(3),257–272. https://doi.org/10.15232/S1080-7446(15)30593-3

Knegsel, van A. T. M., Brand van den, H., Dijkstra, J., Strealen van, W. M., Heetkamp, M. J. W., Tamminga, S., & Kemp, B. (2007). Dietary Energy Source in Dairy Cows in Early Lactation : Energy Partitioning and Milk Composition. *Journal of Dairy Science*, *90*(3), 1467–1476. https://doi.org/10.3168/jds.S0022-0302(07)71632-6

Kruger, M. C., Ph, D., Booth, C. L., Sc, M., Coad, J., Ph, D., Schollum, L. M., Ph,

D., Kuhn-sherlock, B., Ph, D., Shearer, M. J., & Ph, D. (2006). Effect of calcium fortified milk supplementation with or without vitamin K on biochemical markers of bone turnover in premenopausal women. *Applied Nutritional Investigation*, *22*, 1120–1128. https://doi.org/10.1016/j.nut.2006.08.008

Kunkle, W. E., Johns, J. T., Poore, M. H., & Herd, D. B. (2000). Designing supplementation programs for beef cattle fed forage-based diets. *Journal of Animal Science*, *77*, 1–11. https://doi.org/10.2527/jas2000.00218812007700ES0012x

Lachman, J., Hejtmánková, A., Orsák, M., Popov, M., & Martinek, P. (2018). Tocotrienols and tocopherols in colored-grain wheat, tritordeum and barley. *Food Chemistry*, *240*, 725–735. https://doi.org/10.1016/J.FOODCHEM.2017.07.123

Leonardi, C., & Armentano, L. E. (2003). Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *Journal of Dairy Science*, *86*(2), 557–564. https://doi.org/10.3168/jds.S0022-0302(03)73634-0

Leskauskaite, D., & Jasutiene, I. N. A. (2016). Fortification of dairy products with vitamin D 3. *International Journal of Dairy Technology*, *69*(2), 177–183. https://doi.org/10.1111/1471-0307.12242

Marangoni, F., Pellegrino, L., Verduci, E., Ghiselli, A., Calvani, R., Cetin, I., Giampietro, M., Piretta, L., Giacco, R., Vecchia, C. La, Luisa, M., Ballardini, D., Banderali, G., Bellentani, S., Canzone, G., Cricelli, C., Faggiano, P., Ferrara, N., Flachi, E., Calvani, R. (2018). Cow 's Milk Consumption and Health : A Health Professional ' s Guide Cow ' s Milk Consumption and Health : A Health Professional ' s Guide. *Journal of the American College of Nutrition*, 1–12. https://doi.org/10.1080/07315724.2018.1491016

Marina, R., Paola, D., & Mondello, L. (2021). *Hand Book of Dairy Food Analysis* (F. Toldrá & L. M. L. Nollet (eds.); 2nd edtion). CRC Press. https://doi.org/https://doi.org/10.1201/9780429342967

Marsili, R. T. (2000). Shelf-life prediction of processed milk by solid-phase microextraction, mass spectrometry, and multivariate analysis. *Journal of Agricultural and Food Chemistry*, *48*(8), 3470–3475. https://doi.org/10.1021/jf000177c

Mehanso, H. (2006). Symposium: Food Fortification in Developing Countries Biofortification of Staple Food Crops. *The Journal of Nutrition*, *136*, 1064–1067.

Mohd-Setapar, S. H., Abd-Talib, N., & Aziz, R. (2012). Review on Crucial Parameters of Silage Quality. *APCBEE Procedia*, *3*(May 2012), 99–103. https://doi.org/10.1016/j.apcbee.2012.06.053

Murray, I. (1986). The NIR spectra of homologous series of organic compounds. *NIR/NIT*, 13–28.

Næs, T., Isaksson, T., Fearn, T., & Davies, T. (2002). *A User-friendly guide to*

multivariate calibration and classification. NIR Publications.

Nagata, Y. (2003). Measurement of Odor Threshold by Triangle Odor Bag Method. *Measurement Review*, 118–127.

Ni, M., Dra, C., & P, G. (2001). Influence of the dietary protein level on the quality of cow milk. *Archiva Zootechnica*, *6*, 101–106.

Norris, K. H. (2001). Applying Norris Derivatives. understanding and correcting the factors which affect diffuse transmittance spectra. *NIR News*, *3*(12), 6–9.

Olson, R., Gavin-Smith, B., Ferraboschi, C., & Kraemer, K. (2021). Food fortification: The advantages, disadvantages and lessons from sight and life programs. *Nutrients*, *13*, 1–12. https://doi.org/10.3390/nu13041118

Palmquist, D. L. (2009). Milk fat: Origin of fatty acids and influence of nutritional factors thereon. *Advanced Dairy Chemistry*, *2*, 43–92. https://doi.org/10.1007/0-387-28813-9_2/COVER

Palmquist, D. L., Denise Beaulieu, A., & Barbano, D. M. (1993). Feed and Animal Factors Influencing Milk Fat Composition. *Journal of Dairy Science*, *76*(6), 1753–1771. https://doi.org/10.3168/jds.S0022-0302(93)77508-6

Pereira, P. C. (2014). Milk nutritional composition and its role in human health. *Nutrition*, *30*(6), 619–627. https://doi.org/10.1016/j.nut.2013.10.011

Pérez-Silva, A., Odoux, E., Brat, P., Ribeyre, F., Rodriguez-Jimenes, G., Robles-Olvera, V., García-Alvarado, M. A., & Günata, Z. (2006). GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (Vanilla planifolia G. Jackson) beans. *Food Chemistry*, *99*(4), 728–735. https://doi.org/10.1016/j.foodchem.2005.08.050

Pfrimer, K., Ferriolli, E., Takeuchi, L. P., Salles, S. M., Sara-Netto, A., Zanetti, A. M., Roma-Junior, C. L., Braga, M. B. C., Domenici, A. F., Valim, M. L. Y., Paschoalato, B. P. A., Marchi, F. L., Azzollini, E. C. S. A., Donadi, A. E., Martinez, E., & Vannucchi, H. (2018). Effects of the consumption of milk biofortified with selenium, vitamin E, and Different Fatty Acid Profile on Immune Response in Elderly. *Molecular Nutrition and Food Research*, 1–7.

Póti, P., Pajor, F., Bodnár, Á., Penksza, K., & Köles, P. (2015). Effect of micro-alga supplementation on goat and cow milk fatty acid composition. *Chilean Journal of Agricultural Research*, *75*(2), 259–263. https://doi.org/10.4067/S0718-58392015000200017

Randby, A. T., Selmer-Olsen, I., & Baevre, L. (1999). Effect of ethanol in feed on milk flavor and chemical composition. *Journal of Dairy Science*, *82*(2), 420–428. https://doi.org/10.3168/jds.S0022-0302(99)75248-3

Salinas-Martínez, J. A., Posadas-Domínguez, R. R., Morales-Díaz, L. D., Rebollar-Rebollar, S., & Rojo-Rubio, R. (2020). Cost analysis and economic optimization of small-scale dairy production systems in Mexico. *Livestock Science*, *237*(March), 104028. https://doi.org/10.1016/j.livsci.2020.104028

Sampelayo, M. R. S., Chilliard, Y., Schmidely, P., & Boza, J. (2007). *Influence of*

type of diet on the fat constituents of goat and sheep milk &. 68, 42–63. https://doi.org/10.1016/j.smallrumres.2006.09.017

Schroeder, G. F., Gagliostro, G. A., Bargo, F., Delahoy, J. E., & Muller, L. D. (2004). Effects of fat supplementation on milk production and composition by dairy cows on pasture : a review. *Livestock Production Science*, *86*, 1–18. https://doi.org/10.1016/S0301-6226(03)00118-0

Secchiari, P., Antongiovanni, M., Mele, M., Serra, A., Buccioni, A., Ferruzzi, G., Paoletti, F., & Petacchi, F. (2003). *E ffect of kind of dietary fat on the quality of milk fat from Italian Friesian cows*. *83*, 43–52.

Seguel, G., Keim, J. P., Vargas-Bello-Pérez, E., Geldsetzer-Mendoza, C., Ibáñez, R. A., & Alvarado-Gilis, C. (2020). Effect of forage brassicas in dairy cow diets on the fatty acid profile and sensory characteristics of Chanco and Ricotta cheeses. *Journal of Dairy Science*, *103*(1), 228–241. https://doi.org/10.3168/jds.2019-17167

Shipe, W. F., Ledford, R. A., Peterson, R. D., Scanlan, R. A., Geerken, H. F., Dougherty, R. W., & Morgan, M. E. (1962). Physiological Mechanisms Involved in Transmitting Flavors and Odors to Milk. II. Transmission of Some Flavor Components of Silage. *Journal of Dairy Science*, *45*(4), 477–480. https://doi.org/10.3168/jds.S0022-0302(62)89430-2

Simopoulos, A. (1989). Summary of the NATO Advanced Research Workshop on Dietary w3 and w6 Fatty Acids: Biological Effects and Nutritional Essentiality. *Journal of Nutrition*, *119*, 521-528.

Simopoulos, A. P., Leaf, A., & Salem, N. (2000). Workshop Statement on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids. *Prostaglandins Leukot. Essent. Fatty Acids*, *63*, 119–121.

Sutton, J. D. (1989). Altering Milk Composition by Feeding. *Journal of Dairy Science*, *72*(10), 2801–2814. https://doi.org/10.3168/jds.S0022-0302(89)79426-1

Sympoura, F., Cornu, A., Tournayre, P., Massouras, T., Berdagué, J. L., & Martin, B. (2009). Odor compounds in cheese made from the milk of cows supplemented with extruded linseed and α-tocopherol. *Journal of Dairy Science*, *92*(7), 3040–3048. https://doi.org/10.3168/jds.2008-1802

Torrejo, C. S., Castillo-dura, C., Hertrampf, E. D., & Ruz, M. (2004). Zinc and Iron Nutrition in Chilean Children Fed Fortified Milk Provided by the Complementary National Food Program. *AApplied Nutrition Investigation*, *20*, 177–180. https://doi.org/10.1016/j.nut.2003.10.013

Tufarelli, V., Naz, S., Khan, R. U., & Mazzei, D. (2012). *Milk quality , manufacturing properties and blood biochemical profile from dairy cows fed peas (Pisum sativum L .) as dietary protein supplement*. *55*, 132–139.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597.

https://doi.org/10.3168/jds. S0022-0302 (91)78551-2

Weiss, W. P., & Wyatt, D. J. (2003). Effect of Dietary Fat and Vitamin E on α - Tocopherol in Milk from Dairy Cows 1. *Journal of Dairy Science*, *86*(11), 3582–3591. https://doi.org/10.3168/jds.S0022-0302(03)73964-2

Workman Jr., J., & Weyer, L. (2012). *Practical Guide and Spectral Atlas for Interpretive Near-Infrared Spectroscopy*. CRC Press. https://doi.org/http://dx.doi.org/10.1201/b11894

Worku, A., Tóth, T., Orosz, S., Fébel, H., Kacsala, L., Húth, B., Hoffmann, R., Yakubu, H. G., Bazar, G., & Tóthi, R. (2021). Aroma profile, microbial and chemical quality of ensiled green forages mixtures of winter cereals and italian ryegrass. *Agriculture (Switzerland)*, *11*(6). https://doi.org/10.3390/agriculture11060512

Xia, S., & Xu, S. (2005). Ferrous sulfate liposomes: Preparation, stability and application in fluid milk. *Food Research International*, *38*(3), 289–296. https://doi.org/10.1016/j.foodres.2004.04.010

Yang, Z., Nie, G., Pan, L., Zhang, Y., Huang, L., Ma, X., & Zhang, X. (2017). Development and validation of near-infrared spectroscopy for the prediction of forage quality parameters in Lolium multiflorum. *PeerJ*, *2017*(10). https://doi.org/10.7717/peerj.3867

Zahedirad, M., Asadzadeh, S., Nikooyeh, B., Neyestani, T. R., Khorshidian, N., Youse, M., & Mohammad, A. (2019). Fortification aspects of vitamin D in dairy products : A review study. *International Dairy Journal*, *94*, 53–64. https://doi.org/10.1016/j.idairyj.2019.01.013

13. PUBLICATIONS AND PRESENTATIONS

13.1. SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE THESIS

13.1.1. Peer-reviewed papers published in foreign scientific journals

Yakubu, H. G., Kovacs, Z., Toth, T., and Bazar, G. (2022). The recent advances of near-infrared spectroscopy in dairy production– a review. *Critical Reviews in Food Science and Nutrition*, 62(3), 810-831. doi: 10.1080/10408398.2020.1829540, **D1, IF: 11.208**.

Yakubu, H.G., Kovacs, Z., Toth, T., and Bazar, G. (2022). Trends in artificial aroma sensing by means of electronic nose technologies to advance dairy production – a review. *Critical Reviews in Food Science and Nutrition*, 63(2), 234-248. doi: 10.1080/10408398.2021.1945533, **D1, IF: 11.208**.

Yakubu, H.G., Worku, A., Tóthi, R., Tóth, T., Orosz, S., Fébel, H., Kacsala, L., Húth, B., Hoffmann, R., and Bazar, G. (2023). Near-infrared spectroscopy for rapid evaluation of winter cereals and Italian ryegrass forage mixtures. *Animal Science Journal*, 94(1), 1-13.doi: 10.1111/asj.13823, **Q2, IF: 2.000**

Yakubu, H.G., Ali, O., Szabó, A., Tóth, T., and Bazar, G. (2023). Feeding mixture silages of winter cereals and Italian ryegrass can modify the fatty acid and odor profile of bovine milk. *Agriculture,* 13, 381, 1-15.doi: 10.3390/agriculture13020381, **Q1, IF 3.600** (2022).

Yakubu, H.G., Ali, O., Ilyés, I., Vigyázó, D., Bóta, B.; Bazar, G., Tóth, T., and Szabó, A. (2022). Micro-encapsulated microalgae oil supplementation has no systematic effect on the odor of vanilla shake– test of an electronic nose. *Foods*, 11, 3452. 1-19, doi: 10.3390/foods11213452, **Q1, IF: 5.200**.

13.1.2. Peer-reviewed paper published in Hungarian scientific journal

Yakubu H.G., Bázár, G., Radó-Nyiczky, É., Orosz S., and Tóth T. (2022). Description of the odor profile of fermented alfalfa and ryegrass silages using an electronic nose. Állattenyésztés és Takarmányozás (Hungarian Journal of Animal Production), 71(1), 1-10.

13.1.3. Scientific papers on the subject of the dissertation but not incorporated for some reasons

Yakubu, H.G., Kovács, Z., Vitális, F., and Bázár, G. (2021). Near-infrared spectroscopy: rapid and effective tool for measuring fructose content. Élelmiszervizsgálati Közlemények (Journal of Food Investigation) (67) 1, 3259-3268. doi: 10.52091/JFI-2021/1- 1-ENG, **Q4**.

Worku, A., Tóth, T., Orosz, S., Fébel, H.; Kacsala, L., Húth, B., Hoffmann, R., **Yakubu, H.G**., Bazar, G., and Tóthi, R. (2021). Aroma Profile, Microbial and Chemical Quality of Ensiled Green Forages Mixtures of Winter Cereals and Italian Ryegrass. *Agriculture,* 11,512. doi: 10.3390/agriculture11060512, **Q2, IF: 2.925** (2021).

Bazar, G., Hajnalka, H., Éva,C., Csaba, P., **Yakubu, H.G**., Carlos, P.V., Didier, D., Francesco, C., Alessandra, B., and Toth, T. (2022). Machine Olfaction to Evaluate the Stability of the Odor Profile of Pancakes Enriched with Docosahexaenoic Acid and Anthocyanins. Food Analytical Methods, 15(7), 1961-1967. doi: 10.1007/s12161-022-02232-3, **Q2, IF: 3.498**.

13.1.4. Abstracts

13.1.5. Poster presentation

Yakubu, H.G., Tóth, T., and Bazar, G. Rapid method to monitor the effect of feeding on the odor profile of bovine milk, 14th Conference on Rapid Methods Europe, Amsterdam, The Netherlands, 3-5 October, 2022, poster presentation.

Yakubu, H.G., Aguinaga-Bósquez, J.P., Kovács, Z., Roszkos, R., Tóth, T., and Bazar, G. Establishing the impact of improved feeding on the quality of dairy products using correlative analytical technology (E-nose), 4th International Conference on Food Science and Technology, MATE, Budapest, Hungary, 10-11 June, 2022, poster presentation.

13.1.6. Oral Presentations

Yakubu, H. G. Feed related quality differences of cheese described with near-infrared (NIR) spectroscopy. Innovative scientific workshops in the Hungarian agricultural higher education (Innovatív tudományos műhelyek a hazai agrár felsőoktatásban), EFOP-3.6.3-VEKOP-16-2017-00008 project, MATE, Kaposvar, Hungary, 4 May, 2021, oral presentation.

Yakubu, H. G., Tóth T., Tóthi R., Worku, A., and Bázár G. Feed-related quality differences of dairy products described with near-infrared spectroscopy (NIRS) and electronic nose (E-nose). 4th International Conference on Biosystems and Food Engineering, Lurdy Conference and Event Centre, Budapest, Hungary, 4 June, 2021, oral presentation.

14. CURRICULUM VITAE

Mr. Haruna Gado Yakubu was born in Tamale, Ghana, on the **31st of March, 1992**, into the Mabunwura Yakubu family of Kpembe. Mr. Yakubu had his basic education between the years **1997 to 2007,** at Kpembe Local Government Basic Schools, and passed the Basic Education Certificate Examination (BECE) in **2007,** for enrolment into Senior High School.

Between the years **2007 to 2011**, Mr. Yakubu attended Salaga Senior High, where he studied General Agriculture, and passed the West Africa Senior High Certificate Examination in **June 2011**.

In **August 2012**, he was admitted into the School of Agriculture, University of Cape Coast, Cape Coast, Ghana, to read BSc Agriculture.

Mr. Yakubu, in **May 2016**, successfully completed the Bachelor of Agriculture degree, having passed all required examinations and dissertation, and graduated with **First Class Honours.**

Between the years **2016 to 2017**, he worked as a Teaching Assistant at the School of Agriculture, University of Cape Coast, Cape Coast, Ghana.

In **September 2017**, he was awarded the Stipendium Hungaricum Scholarship, tenable in Hungary, where he studied and acquired a Master of Animal Nutrition and Feed Safety Engineering (top of his class) in **June 2019,** from Kaposvár University, now Hungarian University of Agriculture and Life Science, MATE. During the **2019** Students' Scientific Cycle Competition, Mr. Yakubu was adjudged the 2nd Best Student Researcher, in the Life Science Session, amongst 12 competitors.

In **September 2019**, Mr. Yakubu was admitted into the Doctoral School of Animal Science, for his Ph.D studies under the supervision of Dr. György Bázár. His Ph.D theme was "the development of rapid analytical methods based on electronic nose technology and near infrared spectroscopy to advance dairy production."

Mr. Yakubu also worked as a Research Assistant at the Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, from **January 2020 to November 2022**. Mr. Yakubu joined Agrofeed Kft, **in April 2023**, as Export Manager in charge of the Ghanaian Market.