Authentication and Nutritional Benefits of Cheeses Based on Vegetable Oils

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The fatty acid profile of a number of commercial fermented cheeses, based on vegetable oils or milk lipids have been evaluated by GC-FID (gas chromatography with flame ionization detector), disclosing the quantity of SFAs (saturated fatty acids), MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids), as well as the presence of small quantities of TUFAs (trans unsaturated fatty acids) in the dairy products. The authentication of the cheeses based on vegetable oil or milk was performed by GC or IR fingerprinting.

Keywords: vegetable oil based cheese, GC fingerprinting, IR fingerprinting, Principal Component Analysis

Dairy products registered recently a reduced consume due to milk association with a number of diseases like: lactose intolerence [1], obesity [2], diabetes [3], high cholesterol and cardiovascular diseases [4] and even cancer [5]. The market of plant based milk substitutes is rising based on objections to cow's milk consume, but also due to the vegetarian or vegan diet choice [4]. On the contrary, there are studies that claimed a positive effect of dairy products on cardiovascular diseases [6], diabet [7] or cancer [8]. Nevertheless, the SFAs from milk are generally considered as a cause for health damages [9-11].

This paper presents a comparative analysis of six commercial products: three fermented cheeses manufactured with vegetable oils and three similar chesees based on milk.

The content in SFAs, MUFAs, PUFAs and TUFAs has been determined by extraction followed by esterification and GC analyses of the mixtures of the corresponding methyl esters. By using the PCA (Pricipal Component Analysis) method [12], the fatty acid profile proved to be a tool for the cheese content discrimination (based on vegetable oil or on milk). Another procedure for cheese discrimination, based on the PCA, was established using the FTIR (Fourier Transform Infrared) spectroscopy.

Experimental part

Materials and methods

The petroleum ether (fraction 40-60°C) was purchased from Merck (Darmstadt, Germany); analytical grade CH_3OH and GC grade CH_2Cl_2 were from Sigma-Aldrich (Steinheim, Germany). All the other chemicals of analytical grade were purchased from the same supplier.

The analyzed cheese samples are packed commercial products, purchased at local markets, and belong to two classes, based on the origin of the lipid source, namely: vegetable oil or milk. The cheeses have different composition, taste and texture. The commercial vegetable based cheeses (class 1), are Toast (sort 1 - Edam like flavour and texture), Pizza (sort 2 - Gouda taste), Viofast (sort 3 - vegan product). The milk based products (class 2) are Rucar (sort 4), Dalia (sort 5) and Penteleu (sort 6). The macronutrient (proteins, carbohydrates and lipids) contents indicated by the producers are presented in Table 1. The cheeses were stored at 4 °C and worked up right after the package opening.

The fats from all the samples have been extracted with light petroleum ether in a Soxhlet device, according to the known standard procedure [13]. The fatty acid extract was evaporated and transformed in methyl esters by treatment with methanol and BF_3 as catalyst, according to the standard method [14]. The mixture of fatty acid methyl esters (FAMEs) was analyzed by GC the components being identified with the help of the Supelco™ certified reference material, containing 37 FAME species. The analyses were performed with an Agilent Technologies 7890 A instrument, with FID detector, on a poly(biscyanopropyl)siloxane capillary column, having the following characteristics: 100 m length, 0.25 mm inner diameter, 0.2μm film thickness (Supelco SPTM 2560). The working temperature interval was 140°C-240°C. The analyses were performed in triplicate. The average values are presented in Table 2.

FTIR spectra were recorded on a Bruker Equinox 55 Spectrometer, with ATR (Attenuated Total Reflectance) ZnSe crystal, with a spectral window 600 to 4000 cm^{-1}, and a resolution of 2 cm^{-1}, at room temperature (~25°C). The background was the empty, dry ATR crystal. Before each measurement the baseline was established and the contamination between samples was avoided by the proper cleaning of the ATR crystal window.

The experimental data used for statistical analyses were the fatty acid compositions (determined by the GC of FAMEs) and the IR *fingerprint* spectral region (900-1500 cm^{-1}). The PCA was performed using the XLStat 2015 software (Addinsoft).

Results and discussion

The diet based largely on meat and dairy products has a negative impact on both human health, and also environment due to the pollution generated by the livestock production. Dietary changes to plant based food may lead to lower levels of nitrogen and greenhouse emissions [15] diminishing the environmental pollution. In order to choose the best diet it is of interest to compare the nutritional and health benefits of a number of vegetarian or vegan cheeses based on palm oil (sort 1-3) , with milk based cheeses (sort 4-6). The total content of macronutrients (table 1) is similar for all the six sorts of cheese (42-47%).

Differences concerning the content are obvious for proteins and carbohydrates, the lipids being around 20% in all the analyzed cheeses. The protein from the sort 1 and 2 cheeses is casein in a proportion of 2-8%, while in the milk

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Table 1
MACRONUTRIENT CONTENT OF THE ANALYZED CHEESES

Component [%]	Vegetarian and vegan cheeses (class 1)			Milk cheeses (class 2)		
	sort 1	*sort 2*	*sort 3*	*sort 4*	*sort 5*	*sort 6*
Proteins	8	2	0	22	22	23
Carbohydrates	10	19	20	0.1	0.1	0.1
Lipids	28	21	23	21	25	19

products (sort 4-6) casein is accompanied by whey [16], with a total amount of proteins of 22-23%. According to recent data, food with a reduced content in proteins is recommended as healthier in some cases, as for adults below 65 years. A high protein diet may lead to different health problems like: high body mass index and obesity risk for children [17]; increased incidence of cardiac diseases [18]; risk for cancer and mortality for people below 65 [19]. The carbohydrate amount is also different in the vegetarian and dairy cheeses. According to literature a high protein and low carbohydrate diet, as in the analyzed dairy cheeses, affects negatively the health [20].

The fatty acid profile of the food lipids is also important. A diet based on less SFAs proved to be benefic for health [21]. In the cheese case, there are a number of solutions for solving the problem of SFA excess. One way is the addition, at specific stages of cheese fabrication, of omega-3 (Δ^9) fatty acids (PUFAs) extracted from flaxseed or fish oils [22]. A vegetable oil known for its positive effects on human health, due to the low amount of hyper-cholesterolemic acids and high amounts of vitamin E and carotenoids, is palm oil [23]. These assumptions recommend the palm oil based cheese as healthier food than the dairy products. The fatty acid profile is an asset for sustaining this assertion. For the determination of fatty acid profile a traditional technique is the GC analysis of the mixture of the corresponding methyl esters. Thus, a polar stationary phase, enabling the FAME separation according to the number of carbon atoms, number and position of double bonds and also the *cis* or *trans* geometry [24], was employed. The fatty acid profile for all the analyzed cheeses is presented in table 2. Based on the experimental results the products with vegetable oil are rich in unsaturated fatty acids (UFAs). The ratio MUFAs+PUFAs/ SFAs is around 0.90 in the vegetarian cheeses and 0.93 in the vegan product, being only 0.38-0.39 for the dairy products.

Taking into account the damages caused by a diet rich in SFAs [25], the cheeses based on vegetable oil with higher

Fatty acid Type	Cheese sample fatty acid content [%]					
	sort 1	*sort 2*	*sort 3*	*sort 4*	*sort 5*	*sort 6*
Caproic ($C_6H_{12}O_2$)	nd	nd	nd	2.80±1.34	1.93±0.48	3.59±1.77
Caprylic ($C_8H_{16}O_2$)	0.35±0.00	nd	0.06±0.01	1.87±0.21	2.24±0.57	1.93±0.25
Capric ($C_{10}H_{20}O_2$)	0.20±0.00	nd	0.34±0.01	3.79±0.21	3.70±0.16	3.65±0.37
Lauric ($C_{12}H_{24}O_2$)	1.70±0.01	0.36±0.05	2.52±	3.79±0.15	3.76±0.28	3.60±0.34
Myristic ($C_{14}H_{28}O_2$)	1.47±0.01	1.03±0.02	1.69±0.01	12.87±0.07	12.98±0.26	12.59±0.41
Myristoleic ($C_{14}H_{26}O_2$; Δ^9 *cis*)	nd	nd	nd	0.86±0.02	0.93±0.03	0.83±0.10
Pentadecanoic ($C_{15}H_{30}O_2$)	nd	nd	nd	1.47±0.18	1.57±0.05	1.46±0.09
Palmitic ($C_{16}H_{32}O_2$)	45.24±0.04	47.65±0.03	42.87±0.07	35.14±1.51	35.66±1.06	34.39±1.1
Palmitoleic ($C_{16}H_{30}O_2$; Δ^9*cis*)	nd	nd	0.13±0.01	2.33±0.06	2.69±0.43	2.29±0.33
Heptadecanoic ($C_{17}H_{34}O_2$)	nd	nd	nd	0.91±0.05	0.98±0.06	0.91±0.13
Stearic ($C_{18}H_{36}O_2$)	3.53±0.03	3.54±0.03	3.49±0.01	9.67±0.22	9.14±0.30	9.35±1.51
Elaidic ($C_{18}H_{34}O_2$; Δ^9 *trans*)	nd	nd	nd	1.77±0.25	1.67±0.13	1.64±0.68
Oleic ($C_{18}H_{34}O_2$; Δ^9 *cis*)	37.35±0.03	37.95±0.00	38.19±0.03	19.67±0.35	20.83±1.35	21.10±2.10
Linolelaidic ($C_{18}H_{32}O_2$; Δ^9 Δ^{12} all *trans*)	nd	nd	nd	0.08±0.01	nd	nd
Linoleic ($C_{18}H_{32}O_2$; Δ^9 Δ^{12}all *cis*)	9.95±0.00	9.24±0.01	9.95±0.02	2.18±0.14	1.80±0.20	1.84±0.31
Linolenic ($C_{18}H_{30}O_2$; Δ^9 Δ^{12} Δ^{15}all *cis*)	nd	nd	nd	0.75±0.18	0.62±0.23	0.48±0.10
Arachidic ($C_{20}H_{40}O_2$)	0.20±0.01	0.22±0.02	0.22±0.00	nd	nd	nd
Arachidonic ($C_{20}H_{32}O_2$; Δ^5 Δ^8 Δ^{11} Δ^{14} all *cis*)	nd	nd	nd	nd	0.14±0.25	0.36±0.35
Heneicosanoic ($C_{21}H_{42}O_2$)	nd	nd	nd	0.03±0.05	nd	nd
SFAs	52.69	52.80	51.73	72.30	72.10	71.82
MUFAs	37.35	37.80	38.32	24.63	26.12	25.86
PUFAs	9.95	9.24	9.95	3.04	2.42	2.32
MUFAs+PUFAs/SFAs	0.90	0.89	0.93	0.38	0.39	0.39

*nd - not detected

Table 2
FAME COMPOSITION OF FATS EXTRACTED FROM THE STUDIED CHEESES

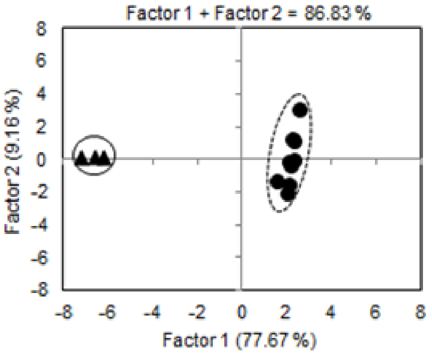

Fig. 1. Differentiation by PCA based on GC-FID analysis of FAMEs of vegetable (▲) and dairy (•) cheeses

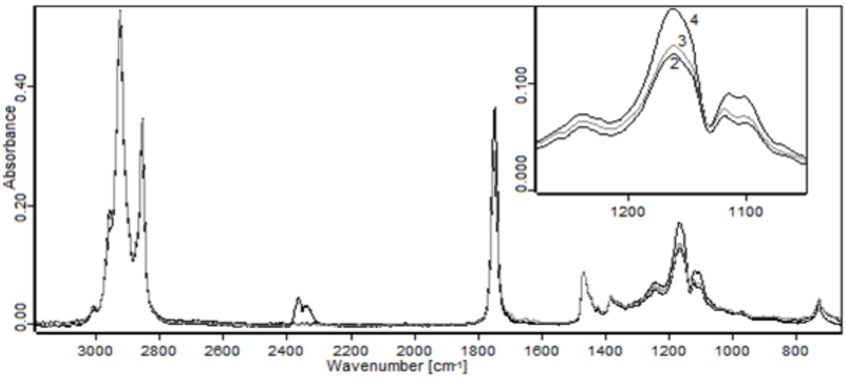

Fig. 2. FTIR spectra of the FAMEs from the studied cheeses of sorts **2, 3** and **4**

content of UFAs, especially PUFAs [26] are healthier products. The milk based products contain also small amounts of *trans* unsaturated acids (TUFAs), like: elaidic and linoelaidic acids. Their presence is unwanted, due to the consequences of their consumption on human health, such as high risk for coronary diseases [27, 28]. Thus, the vegetable oil based cheeses are recommended due to UFA content and lack of TUFAs.

The fatty acid profile is also a tool for discriminating the products. The chromatographic fingerprinting was performed based on the GC fatty acid compositions of a number of samples from the two classes of cheeses. The results are presented in figure 1. Two well separated groups appeared, corresponding to the two classes of products, based on the lipid sources (palm oil or milk).

The vegetable oil based cheeses are grouped (overlapped samples) due to the similar fatty acid profile of palm oil raw material. For the dairy products a separation along Factor 2 axis is observed, due mainly to the presence of linolelaidic or arachidonic acid in some samples. It is based on the different geographical sources of milk used by the producers for obtaining different cheese batches.

Infrared spectroscopy is another analytical method which coupled with chemometric tools may designate the quality and the identity of food products [29, 30]. The Fourier transform technique provides FTIR spectra with fast and high throughput. The measurements were characterized by three groups assigned to specific bonds: 3010-3100 (C=C), 2800-3000 (C-C), 1735-1770 cm^{-1} (C=O from esters) and the *fingerprint region* (900-1500 cm^{-1}) containing C-O and C-C streaching vibrations (950-1150 cm^{-1}) together with O–C–H, C–C–H and C–O–H bendings (1200-1474 cm^{-1}), in agreement with literature data [31, 32]. The experimental spectra are presented in figure 2.

The IR *fingerprint region* 900-1500 cm^{-1}, rich in information and displaying a unique pattern for each organic components, was choosen for performing the PCA. Due to the similar contents all the cheeses have comparable spectral features, the differences emerging from the intensity values. One may notice, that at almost identical absorbance values for the C=C (double bond) vibrations (λ=1490 cm^{-1}) the absorbance for C-C (saturated bond) vibrations (λ =1170) is higher for the dairy products (fig. 2). It confirms the results of GC analyses which evidenced higher quantity of SFAs in milk based cheeses. From each cheese sample a number of 13 vectors have been obtained from the experimental spectrum by measuring the absorbance values at each 50 cm^{-1}, in the analyzed spectral interval. The graphical representation of PC scores is presented in figure 3. The two selected factors explained 92.50% of the variance in the original data.

From the graphical representation the clear separation of the two classes of products (based on vegetable oil and based on milk) is observed (fig. 3). Thus, the method based on IR fingerprinting may be successfully used for authentication of the cheeses. The advantage of the IR method, compared to the GC fingerprinting, is the rapid feedback. Lately the consumer behaviour has been largely influenced by the origin and composition of food [33]. Thus, finding a method for discrimination and authentication of looking alike products is of great interest.

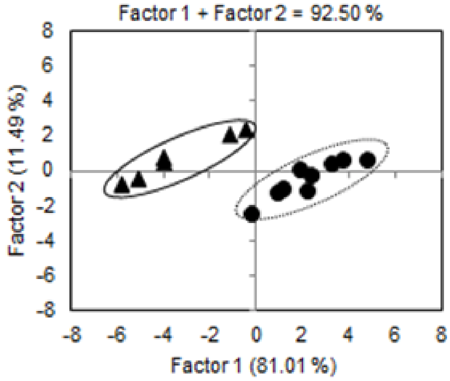

Fig. 3. Differentiation by PCA of vegetable (▲) and dairy (•) cheeses based on FTIR spectral data of FAMEs

Conclusions

The comparative analysis of vegetable oil and dairy cheeses recommends the first class as healthy products for some consumers based on the following criteria:

- low content in proteins;
- the fatty acid profile characterized by high PUFA + MUFA and low SFA content, as well as lack of TUFAs.

Besides the health aspects the vegetable oil based cheeses contribute to a diet diversification for a variety of consumers (vegetarian, vegan or patients with chronic diseases). The application of the PCA statistical method leads to a definite authentication of the two classes of cheeses, and so fulfils consumer demands. The IR fingerprinting seems a better solution considering that it is less time consuming and has a quick feedback.

References

1.MISSELWITZ, B., POHL, D., FRUHAUF, H., FRIED, M., VAVRICKA, S.R., FOX, M., United European Gastroenterol. J., **1**(3), 2013, p. 151.
2.O'SULLIVAN, T.A., BREMNER, A.P., BREMER, H.K., SEARES, M.E., BEILIN, L.J., MORI, T.A., LYONS-WALL, P., DEVINE, A., ODDY, W.H., J. Hum. Nutr. Diet., **28**, 2015, p. 452.
3.ZONG, G., SUN, Q., YU, D., ZHU, J., SUN, L., YE, X., LI, H., JIN, Q., ZHENG, H., HU, F.B., LIN, X., Diabetes Care, **37**, 2014, p. 56.
4.JESKE, S., ZANNINI, E., ARENDT, E.K., Plant Foods Hum. Nutr., **72**(1), 2017, p. 26.
5.STROM, S.S., YAMAMURA, Y., FORMAN, M.R., PETTAWAY, C.A., BARRERA, S.L., DIGIOVANNI J., Int. J. Cancer, **122**, 2008, p. 2581.

6.SONESTEDT, E., WIRFALT, E., WALLSTROM, P., GULLBERG, B., ORHO-MELANDER, M., HEDBLAD, B., Eur. J. Epidemiol., **26**, 2011, p. 609.
7.AUNE, D., NORAT, T., ROMUNDSTAD, P.,VATTEN, L.J., Am. J.Clin. Nutr., **98**(4), 2013, p. 1066.
8.YANG, Y., WANG, X., YAO, Q., QIN, L., XU, C., Sci. Rep., **6**, 2016, 20624.
9.SIRI-TARINO, P.W., CHIU, S., BERGERON, N., KRAUSS, R.M., Annu. Rev. Nutr., **35**, 2015, p. 517.
10.MORIO, B., FARDET, A., LEGRAND, P., LECERF, J.M., Nutr. Rev., **74**, 2015, p. 33.
11.PETRUS, P., ROSQVIST, F., EDHOLM, D., MEJHERT, N., ARNER, P., DAHLMAN, I., RYDEN, M., SUNDBOM, M., RISERUS, U., Lipids Healt. Dis., **14**, 2015, p. 42.
12.CAPUANO, E. - van RUTH, S. M.: QA: Fraud Control for Foods and Other Biomaterials by Product Fingerprinting [on line] In Akyar I (ed) Latest Research into Quality Control, INTECH, 2012, ISBN 978-953-51-0868-9.
13.*** ISO 659:2009. Oilseeds - Determination of oil content (Reference method), 2009.
14.*** ISO 12966-4:2015. Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 4: Determination by capillary gas chromatography, 2015.
15.WESTHOEK, H., LESSCHEN, J.P., ROOD, T., WAGNER, S., DE MARCO, A., MURPHY-BOKERN, D., LEIP, A., VAN GRINSVEN, H., SUTTON, M. A., OENEMA, O., Glob. Environ. Change, **26**, 2014, p. 196.
16.NICASTRO, H., BARQUILHA, G., TOMAIOLO, C., MARQUES, N., BRAGANÇA, E., J. Nutrit. Health Food Sci., **4**(1), 2016, p. 1.
17.WEBER, M., GROTE, V., CLOSA-MONASTEROLO, R., ESCRIBANO, J., LANGHENDRIES, J.P., DAIN, E., GIOVANNINI, M., VERDUCI, E., GRUSZFELD, D., SOCHA, P., KOLETZKO, B., Am. J. Clin. Nutr., **99**, 2014, p. 1041.
18.LAGIOU, P., SANDIN, S., LOF, M., TRICHOPOULOS, D., ADAMI, H.O., WEIDERPASS, E., Br. Med. J., **344**, 2012, e4026.
19.LEVINE, M.E., SUAREZ, J.A., BRANDHORST, S., BALASUBRAMANIAN, P., CHENG, C.W., MADIA, F., FONTANA, L., MIRISOLA, M.G., GUEVARA-AGUIRRE, J., WAN, J., PASSARINO, G., KENNEDY, B.K., WEI, M., COHEN, P., CRIMMINS, E.M., LONGO, V.D., Cell Metab., **19**, 2014, p. 407.
20.RUSSELL, W.R., GRATZ, S.W., DUNCAN, S.H., HOLTROP, G., INCE, J., SCOBBIE, L., DUNCAN, G., JOHNSTONE, A.M., LOBLEY, G.E., WALLACE, R.J., DUTHIE, G.G., FLINT, H.J., Am. J.Clin. Nutr., **93**, 2011, p. 1062.
21.KURAHASHI, N., INOUE, M., IWASAKI, M., SASAZUKI, S., TSUGANE, A.S., Cancer Epidemiol. Biomarkers Prev., **17**, 2008, p. 930.
22.BERMUDEZ-AGUIRRE, D., BARBOSA-CANOVAS, G.V., Food Chem., **133**, 2012, p. 787.
23.MANCINI, A., IMPERLINI, E., NIGRO, E., MONTAGNESE, C., DANIELE, A., ORRU, S., BUONO, P., Molecules, **20**, 2015 p. 17339.
24.RUIZ-RODRIGUEZ, A., REGLERO, G., IBANEZ, E., J. Pharm. Biomed. Anal., **51**(2), 2010, p. 305.
25.DE SOUZA, R.J., MENTE, A., MAROLEANU, A., COZMA, A.I., HA, V., KISHIBE, T., ULERYK, E., BUDYLOWSKI, P., SCHÜNEMANN, H., BEYENE, J., ANAND, S.S., Br. Med. J., **351**, 2015, p. 3978.
26.HAAST, R.A.M., KILIAAN, A.J., Prostag, Leukotr. ESS., **92**, 2015, p. 3.
27.LICHTENSTEIN, A.H., Curr. Atheroscler. Rep., **16**, 2014, p. 433.
28.BERMUDEZ-AGUIRRE, D., BARBOSA-CANOVAS, G.V., Food Sci. Technol., **44**, 2011, p. 1577.
29.KAROUI, R., DE BAERDEMAEKER, J., Food Chem., **102**, 2007, p. 621.
30.GORI, A., MAGGIO, R.M., CERRETANI, L., NOCETTI, M., CABONI, M.F., Int. Dairy J., **23**, 2012, p. 115.
31.SILVERSTEIN, R.M. WEBSTER, F.X., KIEMLE, D.J., BRYCE, D.L., Spectrometric Identification of Organic Compounds, 8th Edition, Hoboken: John Wiley & Sons, 2015, p. 81-108.
32.KAROUI, R., DUFOUR, E., PILLONEL, L., SCHALLER, E., PICQUE, D., CATTENOZ, T., BOSSET J.O., Int. Dairy J., **15**, 2005, p. 287.
33.RODRIGUES ARRUDA PINTO, V., FERNANDES MELO, L., BALBINO, D.F., FARIAS DE NOVAES, J., NEGRETE, M.C., DE SOUSA, T.D., J. Food Nutr. Res., **4**(1), 2016, p. 51.

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