Discriminating Lipid Biomarkers in Asiago PDO Cheese Production Systems

Alessandro Bianchi, Elena Russo

Department of Food Technology, University of Ferrara, 44121 Ferrara, Italy, and Department of Food and Nutrition Science, University of Bologna, 40126 Bologna, Italy

Citation: Alessandro Bianchi, Elena Russo (2026). Discriminating Lipid Biomarkers in Asiago PDO Cheese Production Systems. Psychiatria, 18(3), 26-34. https://doi.org/10.5281/zenodo.19096996

ABSTRACT: The lipid fraction of Asiago Protected Designation of Origin (PDO) cheese was analyzed to identify specific biomarkers of its main production systems through a canonical discriminant analysis. The three main production systems of the cheese were considered. Two were located in the upland (UL): pasture-based (P-UL) vs hay-based total mixed rations (H-UL). The third was located in the lowland (LL) and processed milk from cows fed maize silage-based rations (maize silage lowland: MS-LL). The discriminant analysis selected nine fatty acids and vitamin A as lipid biomarkers useful to separate the three production systems. High contents of conjugated linoleic acids, anteiso-C15:0, and vitamin A were discriminant factors for P-UL cheese. The separation between H-UL and MS-LL cheese was less marked with the former having the higher content of conjugated linoleic acids and some polyunsaturated n-6 fatty acids and with the latter being identified by cyclopropane fatty acid and C9:0.

KEYWORDS: Asiago cheese, production system, lipid fraction, canonical discriminant analysis

INTRODUCTION

Nowadays many types of cheese produced in the European Union are officially labeled according to specific production methods (i.e, organic or traditional specialties guaranteed) and/ or to the geographical location of the milk production system (i.e., mountain products). To avoid frauds, both consumers and producers would benefit from a set of identification biomarkers capable of distinguishing cheeses and dairy products from each other. In this regard, the lipid fraction of dairy products has a relevant role in the characterization of their nutritional[1] and organoleptic quality.[2,3] The outcomes of a recent meta-analysis on the existing scientific literature comparing conventional and organic bovine milk indicated that the supply of nutritionally desirable long-chain polyunsaturated n-3 fatty acids (PUFA) was improved in the lipid composition of organic milk.[4] The presence of conjugated linoleic acids (CLA), odd- and branched-chain fatty acids (OBCFA), and other secondary fatty acids have also shown to improve cheese nutritional properties, because these bioactive substances are positively associated with human health.[5] Fat-soluble vitamins are further bioactive constituents of the cheese lipid fraction due to their antioxidant activity that extends food shelf life and prevents rancidity phenomena.[6] Recent scientific evidence showed that the milk fatty acids (FA) profile and fat-soluble vitamins can be used to discriminate between different feeding systems of dairy cows.[3,7,8] Therefore, the identification of qualified biomarkers from the analysis of the lipid fraction could represent a promising approach to trace the different production systems for a given cheese.

Asiago is a semihard cheese produced from cow's milk in a restricted area of northeastern Italy. Despite being processed and ripened according to its Protected Designation of Origin (PDO),[9] Asiago cheese has a wide variability in quality due to its different milk production systems and cheese-making conditions.[10] This study aimed at analyzing lipid composition of Asiago PDO cheese to identify specific biomarkers that can be used to discriminate between the main production systems.

MATERIALS AND METHODS

Cheese Production Systems and Experimental Design. The study considered the three main cheese production systems (CPS) of Asiago PDO which differ for size and location of the cheese factories and for the cow feeding and management system (Table 1). Two production systems represent the historic center of Asiago cheese, and they are located in the upland (UL) of Asiago (around 1000 m above sea level) in the Veneto Region (Italy). The first is a seasonal pasture-

Table 1. Descriptive Traits of the Production Systems of Asiago PDO Cheese

	pasture-based-upland	hay-based-upland	maize silagebased-lowland
dairy farm location	mountain	mountain	lowland

rearing system	outdoor	indoor	indoor
feeding system	pasture grazing and concentrate	total mixed ration	total mixed ration
Dietary Compositio	(% dry matter)		
pasture	65−75		
permanent grass hay		50−55	5−10
lucerne hay		5−15	10−20
maize silage			30−40
energy-protein concentrate	25−35	35−45	40−50

Received: August 8, 2017
Revised: October 20, 2017
Accepted: October 24, 2017
Published: October 24, 2017

Table 2. Effect of the Production System on Chemical Composition of Asiago PDO Cheese

	production system[a]				orthogonal contrasts	
chemical composition	P-UL	H-UL	MS-LL	SEM[b]	P-HL vs (H-UL+MS-LL)/2	H-HL vs MS-LL
moisture (%)	33.6	34.3	36.4	0.36	<0.001	<0.001
total fat (%)	31.4	31.6	30.3	0.27	ns	0.001
total protein (%)	25.9	26.0	25.7	0.20	ns	ns
water-soluble N (% total N)	26.6	23.2	21.8	0.596	<0.001	ns
NaCl (%)	0.98	1.18	1.20	0.065	0.026	0.763

[a] [b]

P-UL, pasture-based-upland; H-UL, hay-based-upland; MS-LL, maize silage-based-lowland.SEM, standard error of the mean.

based system (P-UL) in which cows graze during the summer on natural pastures and receive a daily amount of concentrates [3.0−5.0 kg of dry matter (DM) per lactating cow] according to their milk yield. This is a very small scale production system that usually processes milk produced by a single or a few dairy herds. The other is a hay-based production system (H-UL) with cows fed total mixed rations (TMR) made of local permanent grass, cereal grains, and protein sources. It represents small/medium size alpine cheese factories that process milk produced from local dairy farms to produce cheese labeled as mountain product in compliance with the EU regulation no. 665/ 2014. A third production chain (MS-LL) features medium/large size cheese factories located in the lowland (LL) surrounding the highland of Asiago. These productive sites process milk collected from dairy farms in which high producing dairy cows are kept indoors and fed maize silage (MS)-based high energy TMR.

Three separate bulks of raw milk were collected within each CPS during the summer period (from July to September), and they were processed to produce Asiago PDO cheese in different sites. All wheels had a similar ripening time of about 6 months that was carried out in a storage bay at 10 ± 2 °C and 80−85% relative humidity. The sampling protocol considered 81 wheels of cheese (28 of P-UL, 26 of H-UL cheese, and 27 of MS-LL, respectively).

Chemical Analysis. Cheese samples were analyzed in duplicate for moisture,[11] total protein and water-soluble nitrogen,[12] and sodium chloride content.[13] Total lipids content was determined using an accelerated solvent extraction method by ASE 200 (Dionex Corporation, Sunnyvale, CA) with hexane/2-propanol (3:2, v/v) solution that was removed by means of a rotary evaporator. According to the method proposed by Christie et al.,[14] an aliquot of 40 mg of anhydrous fat was used to methylate the fatty acids (FAs). Sodium methoxide (CH_3ONa) was used as a catalyst and methyl 12tridecenoate as an internal standard (Nu Chek Prep Inc., Elysian, MN). The analyses of FA methyl esters were performed by twodimensional GC on a 7890A instrument (Agilent Technologies, Santa Clara, CA) connected to a chromatography data system (Agilent ChemStation, Agilent Technologies, Wilmington, DE) and fitted with two capillary columns: the first used was a 75 m × 180 μm i.d., 0.14 μm film thickness (Supelco, Bellefonte, PA); the second used was a 3.8 m × 250 μm i.d., 0.25 μm film thickness (J&W Scientific, Folsom, CA). The carrier gas was hydrogen, the injector temperature was 270 °C, and the flame ionization detector was operated at 250 °C. The oven temperature of the GC varied from 50 °C to 150 °C with an increase of 50 °C/min and then ramped up to 240 °C at 2 °C/min. The cheese FAs were identified by comparing their retention time and peak position to those of a mixture of 52 pure FAs [reference mixture (Nu Chek Prep Inc., Elysian, MN); reference mixture and bacterial acid methyl esters (Supelco); PUFA no.3 from menhaden oil (SigmaAldrich, St. Louis, MO); individual CLA isomers (Nu Chek Prep Inc.; Matreya LLC, State College, PA)]. A standard (dihydrosterculic acid methyl ester, purity >98%) of cyclopropane FA (CPFA) was commercially available (Abcam, Cambridge, UK) while ω-cyclohexyl tridecanoic acid (ω-C19) was synthesized as reported previously.[15] The two-dimensional chromatograms were analyzed with the comprehensive GC × GC image software (Zoex Corp., Houston, TX). On the basis of peak areas, single FAs were expressed as g/100 g of total FAs according to the correction factors of the AOAC 963.22 method.[16] The analysis of vitamin A (retinol) and vitamin E (αtocopherol) was performed by high performance liquid chromatography according to the methods proposed by Zahar and Smith[17] and Indyk,[18] respectively. A model 510 HPLC (Waters Corp., Milford, MA) was equipped with a 150 mm × 4.6 mm i.d., 5 μm, RP-C18 Discovery column (Supelco, St. Louis, MO) at 25 °C, mobile phase methanol/water (98:2, v/v), and model 2996 UV detector (Waters Corp.). The two vitamins were determined at 324 nm for retinol and 293 nm for α-tocopherol according to the retention times of the reference standard (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis. All the statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC). Cheese chemical data were submitted to ANOVA (PROC-GLM), adopting a linear model that considered the fixed effects of the production system and milk bulk. The two degrees of freedom of the CPS factor were used to perform the following set of orthogonal contrasts: P-UL vs (HUL + MS-LL)/2 and H-UL vs MS-LL. The data set of the cheese lipid fraction was subjected to a preliminary selection to avoid redundancy among variables. The procedure

deleted variables strongly correlated with each other (r value >0.95 for the Pearson coefficient). A stepwise discriminant analysis (PROC STEPDISC) was then applied to the remaining FAs and fat-soluble vitamins to reduce the number of variables according to CPS. A canonical discriminant analysis (PROC CANDISC) was performed to achieve the most discriminant variables that maximize the distance among CPS groups. Two canonical functions named CAN1 and CAN2 were obtained, and the final pool of FAs and vitamins highly correlated to them (r value >0.60) was identified. The degree of dissimilarity among production systems was measured by squared Mahalanobis distances (D^2-Mahalanobis), and the reliability of the canonical discriminant model was finally assessed by a cross-validation.

RESULTS AND DISCUSSION

Cheese Chemical Composition, Fatty Acid Profile, and Fat-Soluble Vitamin Content. Chemical composition of the cheese samples belonging to the three CPS are reported in Table 2. Cheese from MS-LL had the highest moisture content and the lowest fat content. Limited differences in chemical composition were detected between P-UL and H-UL except for water-soluble nitrogen and salt content. The FA profiles of the cheese samples are reported in Table 3. Cheese from the pasture-based system had the highest content of the short-chain FA C4:0, whereas it had a lower concentration of mediumchain FAs (from C8:0 to C14:0) than that of H-UL and MS-LL cheese. Pasture-based cheese was also associated with a higher ($P < 0.001$) concentration of MUFAs and PUFAs and a reduction of SFAs. Moreover, consistent with previous studies on alpine pasture cheese and milk,[19,20] P-UL cheese had the richest CLAs content ($P < 0.001$). The concentration of these healthy constituents in milk and dairy foods is increased by the intake of fresh grasses that are rich in C18 precursors, such as linolenic and linoleic acid, as well as by the prevention of the reduction to stearic acid of the vaccenic acid produced in the rumen.[21,22] Under grazing conditions, the dietary C18:3 n-3 is mainly biohydrogenated and the derivatives are trans-11 C18:1

Table 3. Effect of the Production System on Fatty Acids (FAs) and Fat-Soluble Vitamin Concentrations of Asiago PDO Cheese

lipid constituent	production system[a]			orthogonal contrasts		
	P-UL	H-UL	MS-LL	SEM[b]	P-UL vs (H-UL+MS-LL)/2	H-UL vs MS-LL
FAs (g/100 g of fatty acids)						
C4:0	2.72	2.58	2.60	0.033	<0.001	ns
C6:0	2.16	2.18	2.18	0.024	ns	ns
C8:0	1.33	1.40	1.37	0.011	<0.001	ns
C9:0	0.02	0.05	0.05	0.005	<0.001	ns
C10:0	2.89	3.23	3.17	0.030	<0.001	ns
C12:0	3.32	3.75	3.71	0.041	<0.001	ns
C13:0	0.17	0.18	0.19	0.003	<0.001	0.026
C14:0	11.89	12.41	12.50	0.072	<0.001	ns
C14:1	1.05	1.04	1.07	0.015	ns	ns
iso-C15:0	0.38	0.31	0.31	0.004	<0.001	ns
anteiso-C15:0	0.66	0.62	0.59	0.009	<0.001	0.037
C15:0	1.23	1.24	1.28	0.010	0.004	<0.001
iso-C16:0	0.46	0.34	0.34	0.005	<0.001	ns
C16:0	28.70	30.98	32.04	0.138	<0.001	<0.001
cis-7 C16:1	0.21	0.10	0.09	0.006	<0.001	0.040
cis-9 C16:1	1.29	1.39	1.52	0.035	<0.001	0.010
iso-C17:0	0.46	0.42	0.38	0.003	<0.001	<0.001
anteiso-C17:0	0.42	0.39	0.38	0.004	<0.001	ns
C17:0	0.62	0.57	0.61	0.007	<0.001	<0.001
C17:1	0.20	0.19	0.20	0.018	ns	ns
iso-C18:0	0.08	0.05	0.04	0.002	<0.001	ns
C18:0	8.18	7.98	7.93	0.092	0.034	ns
trans-11 C18:1	3.36	1.78	1.62	0.055	<0.001	0.042
cis-9 C18:1	19.26	18.30	17.91	0.145	<0.001	ns
cis-11 C18:1	0.43	0.39	0.41	0.010	0.009	ns
C18:2 n-6	1.85	2.35	1.94	0.044	<0.001	0.001
cis-9,trans-11 CLA	0.99	0.52	0.35	0.015	<0.001	<0.001
trans-11,cis-13 CLA	0.09	0.05	0.03	0.002	<0.001	ns
∑cis/trans + trans/cis CLA	0.11	0.08	0.05	0.002	<0.001	<0.001
∑trans/trans CLA	0.13	0.10	0.06	0.002	<0.001	<0.001
C18:3 n-3	0.58	0.42	0.39	0.011	<0.001	0.039
C18:3 n-6	0.06	0.06	0.06	0.002	ns	ns
ω-C19[c]	0.10	0.11	0.14	0.021	ns	ns
CPFA[d]	0.000	0.003	0.023	0.002	<0.001	<0.001
C20:0	0.19	0.15	0.14	0.002	0.004	ns
C20:1 n-9	0.13	0.08	0.07	0.002	0.003	ns
C20:2 n-6	0.03	0.05	0.02	0.003	ns	0.001
C20:3 n-6	0.07	0.10	0.09	0.002	<0.001	0.020
C20:4 n-6	0.08	0.11	0.11	0.002	<0.001	ns
C20:5 n-3	0.12	0.08	0.08	0.003	<0.001	ns
C22:6 n-3	0.06	0.02	0.02	0.001	<0.001	ns
∑SFA	65.90	68.73	69.86	0.191	<0.001	<0.001
∑MUFA	25.92	23.81	22.87	0.156	<0.001	0.020

and C18:0 that are strongly desaturated to CLA in the mammary gland. Odd- and branched chain FAs (OBCFA) are further healthy compounds of the lipid fraction in milk and dairy products, and in this study, their total content (P = 0.008) and in particular that of iso- and anteiso-C15:0, iso-C16:0, anteiso-C17:0, and iso-C18:0 (P < 0.001) increased in P-UL
cheese according to the higher forage content of the diets fed in this CPS.[23] Among cyclic FAs, ω-cyclohexyl tridecanoic (ωC19) was similar across CPS, whereas cyclopropane FA (CPFA) was detected only in MS-LL cheese. The statistical contrast between H-UL and MS-LL samples was not significant for most FAs (Table 3). However, H-UL cheese had a lower concentration of SFAs (P < 0.001) and higher concentration of MUFAs (P = 0.020), PUFAs (P < 0.001), and cis-9,trans-11 CLA (P < 0.001). Vitamin A (retinol) and vitamin E (αtocopherol) concentrations were highest (P < 0.001) in P-UL cheese, while there was no difference between H-UL and MSLL (Table 3). Fresh grass is rich in carotenoids and αtocopherol while sun exposure and oxidation during harvest, ensiling, and storage cause losses in preserved forages, explaining the lower content of their derivatives in food products from hay and/or silage-based dairy systems.[24]

Canonical Discriminant Analysis. A canonical discriminant analysis (CDA) was carried out by using the data set relative to the lipid fraction of the 81 cheese samples to categorize the three CPS. Two significant functions (CAN1 and CAN2) (Wilks′s λ = 0.0023, approximately F value = 60.5, df1 = 38, df2 = 116, P < 1×10^{-4}) accounted for 91.4% and 8.6% of the total variability, respectively. The stepwise procedure used by the discrimination model selected 15 variables among all lipid compounds: 14 FAs and vitamin A (Table 4).

Table 4. Significant Predictors of the Production Systems of Asiago PDO Cheese Retained by the Stepwise Procedure

		statistics			
step	lipid constituent	Wilks′s λ	F-value	p-level	$R2_{partial}$
1	cis-9,trans-11 CLA	0.078	452.1	<0.001	0.92
2	∑trans/trans CLA	0.032	54.3	<0.001	0.59
3	cyclopropane FA (CPFA)	0.022	17.1	0.001	0.32
4	trans-11,cis-13 CLA	0.014	7.2	0.002	0.17
5	ω-cyclohexyl tridecanoic (ω-C19)	0.012	5.7	0.005	0.14
6	C20:3 n-6	0.009	8.1	0.001	0.19
7	C18:2 n-6	0.008	5.6	0.006	0.14
8	cis-9 C18:1	0.006	9.4	0.002	0.22
9	C20:4 n-6	0.005	5.9	0.004	0.15
10	C18:0	0.004	3.0	0.046	0.09
11	vitamin A (retinol)	0.003	4.0	0.023	0.11
12	C22:0	0.003	4.5	0.015	0.12
13	C20:1 n-9	0.003	3.4	0.039	0.10
14	anteiso-C15:0	0.002	2.9	0.045	0.09
15	C9:0	0.002	2.4	0.048	0.06

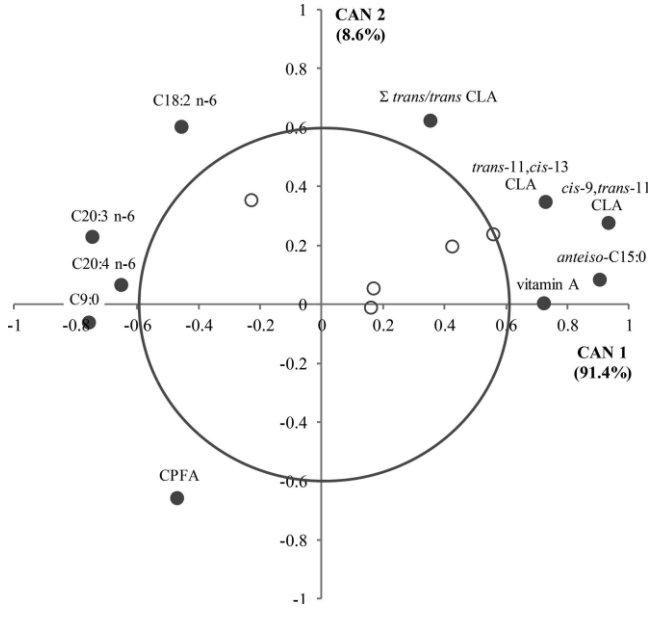

Figure 1. Pool of fatty acids (FA) and the fat-soluble vitamins selected by the stepwise regression procedure. The gray circles represent lipid constituents that had correlation coefficient values with the total canonical structure higher than 0.60. The axes (CAN1 and CAN2) account for the total variability of the measured variables for 91.4% and 8.6%, respectivley.

However, only 9 FAs and vitamin A had correlation coefficient values with the total canonical structure higher than 0.60 (Figure 1). A scattergram based on the standardized canonical coefficients was created to plot the

separation of the cheese samples according to the three CPS (Figure 2). The squared Mahalanobis distances among them indicated that P-UL samples were extremely different from both H-UL (D^2Mahalanobis = 272; $P < 0.001$) and MS-LL (D^2-Mahalanobis = 260; $P < 0.001$) samples. The degree of dissimilarity between H-UL and MS-LL was lower but still significant (D^2Mahalanobis = 36; $P < 0.001$). The cross-validation used to assess the reliability of the canonical discriminant model showed a correct classification for all the samples except for one H-UL sample that was categorized as MS-LL (Figure 2).

The combined analysis of Figures 1 and 2 allowed identification of the lipid compounds that are useful to distinguish the different production chains of Asiago PDO cheese. Cheese samples from P-UL migrated toward the right side of the plot that was characterized by higher contents of CLAs, anteiso-C15:0, and vitamin A, and these lipid constituents were considered specific biomarkers of uplandpasture-based Asiago PDO. Most of the long-chain n-3 PUFA were increased in P-UL samples, but none of them resulted in a relevant biomarker for this production system likely because for pasture only a small quantity of the main precursor (C18:3 n-3) escapes the rumen in its native form, lowering their endogenous synthesis.[25] The separation between cheese from H-UL and MS-LL was less marked. The samples of H-UL tended to aggregate in the upper-left side of the plot where canonical axes are mainly correlated with n-6 PUFA fraction (C18:2 n-6, C20:3 n-6, and C20:4 n-6). The MS-LL samples aggregated in the lower-left side of the plot, and they had CPFA and C9:0 as main lipid markers. These findings are supported by the literature,[2,26] that also reports an increased de novo synthesis of SFAs as well as a lower content of cis-9 C18:1 for milk and cheese from maize silage and high concentrate dairy diets in comparison with hay- or grass-silage-based ones. Furthermore, the larger botanical biodiversity of mountain hay compared to lowland forage may also explain the different content of individual FAs between H-UL and MS-LL cheese. Many positive correlations between the synthesis of specific long-chain FA and some botanical families of upland pastures have been reported,[27] such as those observed for the consumption of Fabaceae and Cyperaceae by grazing cows and MUFA and PUFA content in milk.[28]

Recently, the presence of cyclic FAs in dairy products has been investigated as potential markers of the inclusion of specific feedstuffs in dairy cattle diets. Cyclopropane FAs (dihydrosterculic and lactobacillic acids) and ω-cyclohexyl FAs (ω-cyclohexyltridecanoic and ω-cyclohexylundecanoic acids) were respectively associated with the administration of maize silage and cereal grains.[15,29] Results of this study confirm the role of CPFA as a tool to trace the inclusion of maize silage in dairy cow diets, while ω-C19 (ω-cyclohexyltridecanoic acid) was not a reliable marker for P-UL cheese, likely because alpine

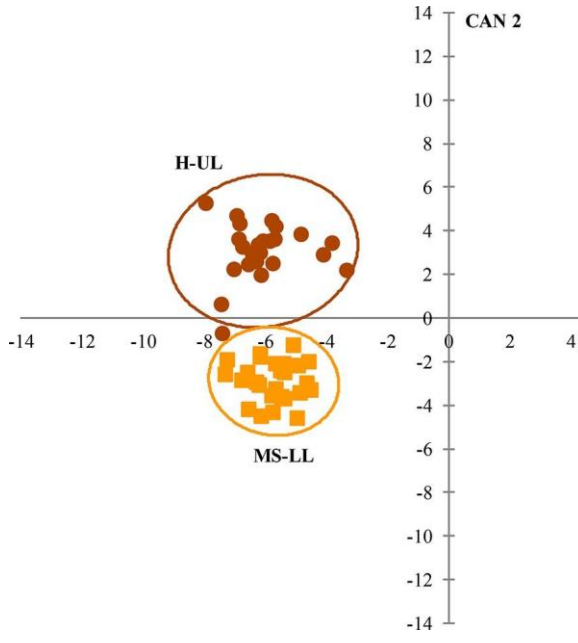

Figure 2. Canonical discriminant analysis scattergram of the three production systen ellipses are drawn around each centroid of groupings (misclassified cheese samp pasture-based-upland; brown circles, H-UL, hay-based-upland; orange squares, MS

grazing cows are often supplemented with cereal-based concentrates.

The outcomes of this study indicated that selected lipid biomarkers can discriminate between Asiago PDO cheese produced according to diverse dairy production systems. The content of the principal beneficial lipid compounds such as PUFAs, CLAs, and fat-soluble vitamins has shown to increase in upland cheeses and particularly in those processing pasture milk. Cyclopropane FA and C9:0 were specific biomarkers for maize silage-based dairy diets fed in the lowland.

ABBREVIATIONS

CDA, canonical discriminant analysis; CLA, conjugated linoleic acids; CPS, cheese production system; CPFA, cyclopropane fatty acid; DM, dry matter; FA, fatty acid; H-UL, hay-upland; MS-LL, maize silage-lowland; MUFA, monounsaturated fatty acid; OBCFA, odd- and branched-chain fatty acids; PDO, Protected Designation of Origin; PUFA, polyunsaturated fatty acid; P-UL, pasture-upland; SFA, saturated fatty acid; TMR, total mixed ration; ω-C19, ω-cyclohexyl tridecanoic acid

REFERENCES

(1) Parodi, P. W. Milk fat in human nutrition. Aust. J. Dairy Technol. 2004, 59, 3−59.

(2) Giaccone, D.; Revello-Chion, A.; Galassi, L.; Bianchi, P.; Battelli, G.; Coppa, M.; Tabacco, E.; Borreani, G. Effect of milk

thermisation and farming system on cheese sensory profile and fatty acid composition. Int. Dairy J. 2016, 59, 10−19.

(3) Romanzin, A.; Corazzin, M.; Favotto, S.; Piasentier, E.; Bovolenta, S. Montasio cheese liking as affected by information about cows breed and rearing system. J. Dairy Res. 2016, 64, 15−21.

(4) Srednicka-Tober, D.; Barań ski, M.; Seal, C. J.; Sanderson, R.; Benbrook, C.; Steinshamn, H.; Gromadzka-Ostrowska, J.; Rembiałkowska, E.; Skwarło-Sońta, K.; Eyre, M.; Cozzi, G.; Larsen, M. K.; Jordon, T.; Niggli, U.; Sakowski, T.; Calder, P. C.; Burdge, G. C.; Sotiraki, S.; Stefanakis, A.; Stergiadis, S.; Yolcu, H.; Chatzidimitriou, E.; Butler, G.; Stewart, G.; Leifert, C. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta- and redundancy analyses. Br. J. Nutr. 2016, 115, 1043−1060.

(5) Astrup, A.; Bradley, B. H. R.; Brenna, J. T.; Delplanque, B.; Ferry, M.; Torres-Gonzalez, M. Regular-fat dairy and human health: A synopsis of symposia presented in Europe and North America (2014− 2015). Nutrients 2016, 8, 463.

(6) Dhankhar, J.; Sharma, R.; Indumathi, K. P. Bioactive lipids in milk. Int. Food Res. J. 2016, 23, 2326−2334.

(7) Vicente, F.; Santiago, C.; Jimenez-Calderó n, J. D.; Martínez- Fernandez, A. Capacity of milk composition to identify the feeding system used to feed dairy cows. J. Dairy Res. 2017, 84, 254−263.

(8) Adler, S. A.; Jensen, S. K.; Govasmark, E.; Steinshamn, H. Effect of short-term versus long-term grassland management and seasonal variation in organic and conventional dairy farming on the composition of bulk tank milk. J. Dairy Sci. 2013, 96, 5793−5810.

(9) Commission Regulatioan (EC) No. 1107/96 of 12 June 1996 on the registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No. 2081/92. Off. J. Eur. Commun. 1996, L148, 1−15.

(10) Cozzi, G.; Ferlito, J.; Pasini, G.; Contiero, B.; Gottardo, F. Application of near-infrared spectroscopy as an alternative to chemical and color analysis to discriminate the production chains of Asiago d′Allevo cheese. J. Agric. Food Chem. 2009, 57, 11449−11454.

(11) IDF. Cheese and processed cheese. Determination of the total solids content. IDF Standard 4A; International Dairy Federation: Brussels, Belgium, 1982.

(12) IDF. Milk. Determination of the nitrogen (Kjeldahl method) and calculation of the crude protein content. IDF Standard 20B; International Dairy Federation: Brussels, Belgium, 1993.

(13) International Dairy Federation. Cheese and processed cheese products. Determination of chloride content. Potentiometric titration method. IDF Standard 88A; International Dairy Federation: Brussels, Belgium, 1988.

(14) Christie, W. W.; Sebé dio, J. L.; Juané da, P. A practical guide tó the analysis of conjugated linoleic acid (CLA). Inform 2001, 12, 147− 152.

(15) Marseglia, A.; Caligiani, A.; Comino, L.; Righi, F.; Quarantelli, A.; Palla, G. Cyclopropyl and ω-cyclohexyl fatty acids as quality markers of cow milk and cheese. Food Chem. 2013, 140, 711−716.

(16) AOAC. Official methods of analysis, 17th ed.; AOAC International: Gaithersburg, MD, 2000.

(17) Zahar, M.; Smith, D. E. Vitamin A quantification in fluid dairy products: rapid method for vitamin A extraction for high performance liquid chromatography. J. Dairy Sci. 1990, 73, 3402−3407.

(18) Indyk, H. E. Simultaneous liquid chromatographic determination of cholesterol, phytosterols and tocopherols in food. Analyst 1990, 115, 1525−1530.

(19) Schievano, E.; Pasini, G.; Cozzi, G.; Mammi, S. Identification of the production chain of Asiago d′Allevo cheese by nuclear magnetic resonance spectroscopy and principal component analysis. J. Agric. Food Chem. 2008, 56, 7208−7214.

(20) Stergiadis, S.; Bieber, A.; Franceschin, E.; Isensee, A.; Eyre, M. D.; Maurer, V.; Chatzidimitriou, E.; Cozzi, G.; Bapst, B.; Stewart, G.; Gordon, A.; Butler, G. Impact of US Brown Swiss genetics on milk quality from low-input herds in Switzerland: interactions with grazing intake and pasture type. Food Chem. 2015, 175, 609−618.

(21) Kay, J. K.; Mackle, T. R.; Auldist, M. J.; Thomson, N. A.; Bauman, D. E. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 2004, 87, 369−378.

(22) Egger, P.; Holzer, G.; Segato, S.; Werth, E.; Schwienbacher, F.; Peratoner, G.; Andrighetto, I.; Kasal, A. Effect of oilseed supplements on milk production and quality in dairy cows fed a hay-based diet. Ital. J. Anim. Sci. 2007, 6, 395−405.

(23) Vlaeminck, B.; Fievez, V.; Cabrita, A. R. J.; Fonseca, A. J. M.; Dewhurst, R. J. Factors affecting odd-and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 2006, 131, 389−417.

(24) Noziere, P.; Graulet, B.; Lucas, A.; Martin, B.; Grolier, P.; Doreau, M. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Technol. 2006, 131, 418−450.

(25) Leiber, F.; Kreuzer, M.; Nigg, D.; Wettstein, H. R.; Scheeder, M. R. L. A study on the causes for the elevated n-3 fatty acids in cows′ milk of Alpine origin. Lipids 2005, 40, 191−202.

(26) Coppa, M.; Chassaing, C.; Ferlay, A.; Agabriel, C.; Laurent, C.; Borreani, G.; Barcarolo, R.; Baars, T.; Kusche, D.; Harstad, O. M.; Verbic, J.; Golecký , J.; Delavaud, C.; Chilliard, Y.; Martin, B. Potential of milk fatty acid composition to predict diet composition and authenticate feeding systems and altitude origin of European bulk milk. J. Dairy Sci. 2015, 98, 1539−1551.

(27) Coppa, M.; Ferlay, A.; Monsallier, F.; Verdier-Metz, I.; Pradel, P.; Didienne, R.; Farruggia, A.; Montel, M. C.; Martin, B. Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. J. Dairy Sci. 2011, 94, 1132−1145.

(28) Gorlier, A.; Lonati, M.; Renna, M.; Lussiana, C.; Lombardi, G.; Battaglini, L. M. Changes in pasture and cow milk composition during a summer transhumance in the western Italian Alps. J. Appl. Bot. Food Qual. 2012, 85, 216−223.

(29) Caligiani, A.; Nocetti, M.; Lolli, V.; Marseglia, A.; Palla, G. Development of a quantitative GC-MS method for the detection of cyclopropane fatty acids in cheese as new molecular markers for Parmigiano Reggiano authentication. J. Agric. Food Chem. 2016, 64, 4158−4164.