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The stability of fatty acids in yoghurts produced from bulk milk samples intentionally selected according to dairy production systems

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Abstract: The fatty acid (FA) composition of milk fat can be positively influenced by the modification of dairy farming and the possible selection of raw material for processing. The question arises as to whether the benefits of a better FA composition will be maintained even after processing dairy products. Thus, the aim was to obtain a more favourable FA composition by a targeted selection of cow's milk (grazing *vs* stabled herds) and then compare FAs of milk and fermented product (yoghurt). Bulk tank milk of grazing herds had significantly better FA composition than milk of stabled herds (for example, C16:0 28.07% *vs* 32.27%, $P < 0.001$; C18:3n-3 0.83% *vs* 0.41%, $P < 0.001$; C18:2 *cis*-9, *trans*-11 (conjugated linoleic acid; CLA) 1.02% *vs* 0.41%, $P < 0.01$). The differences between the FAs in milk and yoghurt samples were negligible (in relative values from 0.04% to 5.21%). The correlations between milk and yoghurt for nutritionally important FAs were high, from 0.925 0 (C18:2n-6) to 0.998 8 (CLA; both $P < 0.001$). The minimal effect of milk fermentation on the original FA composition of milk fat was found. In conclusion, systematic selection of raw cow's milk or modification of farming conditions can also provide a nutritionally desirable composition of final dairy products.

Keywords: dairy cow; fermentation; grazing herd; milk fat; stabled herd

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Milk, one of the most complete foods rich in energy and nutrients, is an important part of the human diet, especially in childhood. It provides the human metabolism with calcium, other minerals, biologically valuable proteins, vitamins, and fatty acids (FAs), in many cases essential. For these reasons, worldwide milk consumption has constantly been growing, despite some health disorders associated with milk consumption, such as lactose intolerance or milk protein allergy (Pratelli et al. 2024).

The previous adverse assessment of milk FAs composition has been reevaluated in the recent ten to twenty years, leading to the return of milk fat and dairy products to human nutrition. Thus, it is generally known that unsaturated FAs (UFAs), especially polyunsaturated FAs (PUFAs), in particular, conjugated linoleic acid (CLA; C18:2 *cis*-9, *trans*-11), in contrast to *trans* isomers of UFAs (TFAs) and some saturated FAs (SFAs), have a positive effect on reducing the incidence of chronic diseases, such as cardiovascular or cancer diseases (Parodi 1999; German et al. 2009; Gomez-Cortes et al. 2018).

The FA proportions in milk fat (FAPM) show considerable variability depending on several factors (Frelich et al. 2012; Samkova et al. 2012, 2018; Hanus et al. 2016, 2018; Sulejmani et al. 2023). These factors can be external such as season, type of dairy production system (DPS, e.g. housed *vs* pastured), feeding and nutrition of animals, or internal such as animal species and breed and the related genetic basis for the metabolism or biosynthesis of FAs, as well as the parity and stage of lactation.

For example, significant differences between goat's and cow's milk have been reported previously (Toral et al. 2015; Kala et al. 2016). Compared to cow, goat's milk has: a higher proportion of short-chain FAs; a lower proportion of palmitic acid; a higher proportion of essential FAs, i.e. linoleic acid and α-linolenic acid. Similarly, significant differences in the FAPM among cows, goats, sheep, buffalos, yaks, camels, and donkeys have been summarised (Ferrand-Calmels et al. 2014; Wang et al. 2022). Furthermore, the results with often significant influence of the dairy breed on the FAPM, which are based on genetic aspects and subsequent breeding of dairy cows, were presented (Soyeurt et al. 2011; Hanus et al. 2018). However, in some studies, the significance of these possible differences in FA composition between dairy breeds was moderated, e.g. compared to the observed effectiveness of nutritional effects (Hanus et al. 2016; Niero et al. 2021). Concerning the frequency of dairy cattle breeds globally, the most studied breeds have been (Soyeurt et al. 2011; Samkova et al. 2012; Hanus et al. 2018): Holstein, Jersey, Simmental (in the Czech Republic in the variant Czech Fleckvieh), Brown Swiss, Ayrshire, and Montbeliarde.

In terms of a DPS, a significant influence on the FAPM has been found in organic farming compared with conventional farming (Srednicka-Tober et al. 2016; Liu et al. 2020; Manuelian et al. 2022). For organic farming, a higher share of grazing and a lower share of feed concentrates are typical and usually result in a lower milk yield. This results in a higher proportion of PUFAs and MUFAs and a lower proportion of SFAs in organic milk than in conventional milk.

Cow nutrition can, therefore, be considered dominant among many factors of the FAPM variability. In addition to DPS mentioned above, the following feed factors also have an influence: use of grazing (Niero et al. 2021; Loza et al. 2023), proportion and type of forage (unpreserved and preserved) (Kalac and Samkova 2010), and type of concentrate in feed rations (Toral et al. 2015; Ferlay and Chilliard 2020). Compared to cereal concentrates, oilseed supplementation can positively modify FAPM by increasing UFAs, i.e. MUFAs, PUFAs, especially CLA, at the expense of SFAs. Based on recent reviews and meta-analyses, including classifiable and quantifiable factors with the potential to influence the FAPM, predictive equations that allow estimation of some aspects of the FAPM variability according to a summary of technological influences of environment and other biological influences in dairy farming were calculated (Coppa et al. 2013). It is one of the methods that can positively contribute to the information of the processing industry for the possible production of functional foods with positive benefits to human health. At the same time, for the same reasons, analytical methods and their calibrations for routine rapid prediction of the FAPM were developed and validated in the field of infrared spectroscopy (Soyeurt et al. 2011; Ferrand-Calmels et al. 2014; Samkova et al. 2020), most often in the mid-region of infrared radiation spectrum modified with Fourier transformation (MIR-FT), according to the results of a reference gas chromatography. These

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procedures are also today, after achieving relatively successful (reliable) analytical results, an effective source of information for the possibility of dairy breeding (Tiplady et al. 2020) or selection of raw material with more desirable FAPM (Gomez-Cortes et al. 2018; Hanus et al. 2018).

Scientific knowledge about the FAPM variability in practice and the observed potential effects of the FAPM on the health of dairy product consumers, together with research on relevant analytical methods for the FAPM predicting, have led to ideas about improving the production of specific dairy products with beneficial effects on human health. The way might be a positive adjustment in DPS (Brodziak et al. 2021) and efficient dairy cow breeding or selection of the preferred composition of the FAPM of raw milk for processing (Gomez-Cortes et al. 2018; Hanus et al. 2018; Tiplady et al. 2020) according to routine information on the FAPM from dairy laboratories.

This topic still needs to be sufficiently investigated. The stability of FAPM during technological processing is also a question because the processes commonly applied to milk (heat treatment, high-pressure treatment, fermentation, etc.) may cause changes in FAPM (Bisig et al. 2007; Lauciene et al. 2019; Khan et al. 2020). Thus, some publications have focused on the FA stability in products made from the milk of small ruminants, rarely from dairy cows (Pecova et al. 2019; Bodnar et al. 2021; Buccioni et al. 2022).

This work aimed to assess the variability and stability of the FAPM during the fermentation of cow's bulk milk from different dairy production systems into yoghurt to expand the information to consider the possibilities of producing nutritionally desirable foods.

MATERIAL AND METHODS

The research was performed in suitable dairy herds selected according to DPS in two regions (north-eastern Bohemia and north-western Moravia and Silesia) of the Czech Republic (CR).

Dairy herds and bulk tank milk samples

Bulk tank milk samples in the experiment were taken at 16 commercial dairy farms (Table 1). Sampling took place at one time during the summer feeding period (July). On half of the farms (with a total number of cows, 1 344), the dairy

Table 1. General characteristics of herds involved in the experiment and characteristics of feed rations of dairy cows

Herd (No.)	DPS	MSL	Breed	AFC (day)	Type of forage (kg/cow/day)						Cereal concentrates (kg/cow/day)	Proportion of forage (%)
					grazing	silages	hay	straw	others	total		
1	GHe	432	H	856	30	10	3	–	9.5	52.5	8.5	86
2	GHe	633	CF	1 092	65	–	2	–	–	67	1	99
3	GHe	550	CF	873	70	–	–	1	–	71	2	97
4	GHe	567	CF	996	70	–	–	1	–	71	–	100
5	GHe	508	CF	1 024	60	–	3	–	–	63	2.5	96
6	GHe	645	H	827	35	9	–	–	–	44	5.5	89
7	GHe	545	CF	765	70	–	–	–	–	70	5	93
8	GHe	510	CF	798	40	8	–	–	–	48	3.2	94
9	SHe	364	CF	866	–	31	1	–	–	32	10.5	75
10	SHe	360	H	758	–	25.5	2.5	–	–	28	9.8	74
11	SHe	299	CF	726	–	28	2	–	12	42	8.7	83
12	SHe	259	H	717	–	28	0.7	0.7	8.2	37.6	8.3	82
13	SHe	267	H	755	–	31.1	–	–	6	37.1	11	77
14	SHe	286	H	761	–	35	3	–	15	53	10.2	84
15	SHe	268	H	826	–	37	0.2	–	–	37.2	8.4	82
16	SHe	555	H	968	–	27	0.5	–	–	27.5	10	73

AFC = age at first calving; CF = Czech Fleckvieh; DPS = dairy production system; GHe = grazing herd; H = Holstein; MSL = mean sea level; SHe = stabled herd

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cows are permanently housed (stabled herds; SHe). In the second half of the farms (with a total number of cows 2 528), grazing is used (grazing herds; GHe). Nine farms are located in a mountainous area with an altitude of 432 m to 645 m, and seven in a lowland area with 267 m to 364 m. The Czech Fleckvieh (CF) breed is bred on half of the farms, and Holstein (H) breed on the other half. Milk samples were transported without chemical preservation to the laboratory under cold conditions of a thermobox (< 6 °C) within 24 hours. Each sample was divided into three parts (*i*) analysis of chemical composition and properties; (*ii*) FAPM analysis; (*iii*) processing into model yoghurt.

General characteristics of nutrition, and milk performance of selected dairy herds

Feed rations for dairy cows were compiled according to the required daily nutrient requirements for dairy cows (basic and production feed rations) with regard to current milk yield. Production feed mixtures were supplemented with vitamin and mineral supplements. The SHe used mainly feeds typical for the CR conditions (corn, alfalfa, clover or grass silage, hay, straw, malt, molasses, beet pulp). In GHe, the main component of the feed ration was grazing (grass and herb mixture as forage) used to varying degrees. The forage proportions were 73–84% (SHe) and 86–100% (GHe).

The milk yield, fat and protein content/yield (counted for whole lactation, first and all) were characterised according to official results of milk recording data of the Czech-Moravian Breeders' Corporation in Hradištko (ČMSCH), which is a representative of the CR in International Committee for Animal Recording (ICAR).

Milk composition and properties analyses

The composition and properties of bulk milk samples were analysed in the Dairy Research Institute the following day after sampling. The composition of milk [fat, crude protein, casein, lactose monohydrate, total dry matter (solids), solids non-fat, urea, acetone, citric acid, free FAs (FFAs)] was determined by indirect mid-infrared spectroscopy MIR-FT (with Michelson interferometer and Fourier transformations) using a Lactoscope FTIR (Delta Instruments, Drachten, The Netherlands).

Somatic cell count (SCC) was determined by fluoro-opto-electronic flow cytometry on a Somacount 300 (Bentley Instruments, Chaska, USA).

The milk freezing point depression (MFP) values were determined using the cryoscope Cryo-Star automatic (Funke-Gerber, Berlin, Germany). The selected measurement mode was Plateau Search (with parameters: interval = 23 s and delta t = 0.4 m°C).

The active acidity of the milk (pH) was measured by a potentiometric method using a pH-meter (pH 1100L; VWR, pHenomenal®, Darmstadt, Germany). The titratable acidity (TA) of milk was measured by titration of 100 ml of milk (Soxhlet-Henkel) using an alkaline solution of 0.25 N sodium hydroxide (NaOH; °SH = ml × 2.5 mmol/l) in an indicator medium (phenolphthalein) according to the relevant standard (Czech Normalization Institute: CSN 57 0530 (570530):1973 – Methods for testing of milk and milk products).

The rennetability (rennet coagulation time) was determined instrumentally (the nephelo-turbidimetric device as a reader of milk coagulation ML-2) as the time (s) of enzymatic coagulation of milk proteins (Pribyla et al. 2006).

Milk electrical conductivity was measured by OK 102/1 (Radelkis, Budapest, Hungary) conductometer at 20 °C (mS/cm) using a geometrical exactly defined bell glass electrode with platinum ring contacts. The instrument was calibrated with the appropriate salt (KCl) solution (10.2 mS/cm) to measure each milk sample set.

Milk alcohol stability was determined using a milk titration (5 ml) by 96% ethanol to create the first visible milk protein precipitated flakes (expressed in ml of alcohol) as a substitution for the classical thermostability test.

Model yoghurt processing and analyses

The examined milk sample (50 ml) was pipetted into a beaker, which was laboratory pasteurised at 85 °C for 5 minutes. Subsequently, it was cooled to 43 °C, and 2 ml of yoghurt culture was added, incubation for 4.5 h at 43 °C followed. The test was performed with a mixed thermophilic yoghurt culture YC-180 and Yo-Flex 50 U, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp.

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lactis and *Lactobacillus delbrueckii* subsp. *bulgaricus* whose inoculum was prepared according to the manufacturer's specifications (Chr. Hansen, Hørsholm, Denmark).

Analyses of yoghurts were performed by the same methods as described for milk.

FAPM analyses

Milk and yoghurt samples were analysed on their FAPM using reference gas chromatography (GC) after previous lyophilisation of the material, fat extraction, and derivatisation of FAs at the Department of Applied Chemistry (South Bohemia University in České Budějovice, Faculty of Agriculture and Technology).

Lyophilisation of materials. A sample of milk/yoghurt (30 ml) was placed in a 150 ml beaker and frozen at –18 °C. It was followed by lyophilisation for 48 h at –46 °C and a pressure of 0.07 mbar. Instrument Alpha 1-4 LD (Christ, Osterode am Harz, Germany) was used for lyophilisation. The lyophilised material was transferred to plastic sample vials and stored at –18 °C until the analysis.

Fat extraction. To a sample of 0.5 g of lyophilised milk/yoghurt in an 8 ml vial, 5 ml of petroleum ether was added. The vials were placed in a shaker at room temperature for 3 hours. After sedimentation, the petroleum ether extract was used directly for derivatisation.

Derivatisation of FAs. The FAs were converted to methyl esters by re-esterification of the petroleum ether extract of the fat with methanolic potassium hydroxide solution. To 1.5 ml of petroleum ether extract of milk was added 200 µl of a 2M solution of potassium hydroxide (KOH) in methanol, and the mixture was heated in a 60 °C water bath for 2 minutes. To the cooled mixture, 400 µl of 1M hydrogen chloride (HCl) in methanol and 1 ml of petroleum ether were added to neutralise KOH. 1 µl of petroleum ether solution was taken for GC analysis (Samkova et al. 2020).

Determination of FAs. Briefly, the determination of FAs was performed on a Varian 3800 instrument (Varian Techtron, Palo Alto, USA). The identification of FAs in milk fat was performed using Supelco standards (F.A.M.E. Mix C4-C24 and other; Supelco, Darmstadt, Germany), MS detection with chemical ionisation by acetonitrile (van Pelt et al. 1999), and MS detector with chemical ionisation of acetonitrile. A total of 56 FAs were detected in milk fat, and 48 FAs were identified. The proportion of individual FAs was determined from the ratio of the areas of their peaks to the total area of the peaks of all detected FAs. A flame-ionization detector (FID) was used (Christie et al. 1989), and the detector was calibrated using quantitative Supelco standards.

Details of the GC method and FAs are supplemented by parameters of the chromatographic analysis (Table S1), model chromatogram (Figure S1), and a tabular scheme of the distribution of analysed FAs into relevant groups according to their selected characteristics (Table S2).

Statistical analysis

Data of milk indicators for statistical evaluation in this work were used in the original, untransformed values. The somatic cell count, which usually requires a logarithmic transformation in the values of individual milk samples, has also not been transformed, as the values of bulk tank milk samples in the current conditions are already approaching the normal frequency distribution.

Data were evaluated using the program Statistica Cz v12 (StatSoft CR, Prague, Czech Republic). The assumptions for parametric methods (normality of data, homogeneity of variances) were evaluated for the set.

Student's *t*-test (unpaired) was used for the comparison of two data sets in the independent variable, i.e. DPS: GHe *vs* SHe. The differences between FAPM of milk and yoghurt were assessed using: *i*) Student's *t*-test (paired); *ii*) correlation and regression analysis [r = Pearson correlation coefficient; R^2 = coefficient of determination = $r^2 \times 100$ (%)]; regression equation as:

$$Y = A + B \times X_1 \quad (1)$$

where:

A – intercept;
B – slope coefficient;
X_1 – individual FAs or FA groups in milk;

In all cases, the statistical significance of differences was expressed as a *P*-value.

Subsequently, the individual samples (32 vectors with information about particular FA proportions) were visualised concerning DPS (SHe, GHe)

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by projection into the first two latent dimensions. Due to the dimension of the data matrix ($n < p$), the sparsePLS Discriminant Analysis (Le Cao et al. 2011) method was used for this purpose, enabling the automatic selection of variables contributing to the discrimination between groups. In this case, the necessary numerical calculations were performed using the R programming environment v4.0.2 (R Core Team 2020) with a connected mixOmix library (Rohart et al. 2017).

Institutional Review Board Statement

Our research was done within a project of the National Agency for Agricultural Research (Národní Agentura pro Zemědělský Výzkum, NAZV) under the Ministry of Agriculture of the Czech Republic, applying methodological demands for animal health protection. Thus, all experiments were performed in accordance with relevant guidelines and regulations recommended by the Ministry of Agriculture of the Czech Republic. In the case of our work, milk samples were taken from commercial herds and only during the regular testing of milk performance and milk quality, where approval by a properly constituted research ethics committee is not required.

RESULTS AND DISCUSSION

Evaluation of systematic selection of bulk tank milk samples

Bulk tank milk samples naturally show lower variability in milk composition and FAPM than individual samples. On the other hand, they represent a better (more representative) experimental material for model tests of technological properties, which are thus closer to the real situation in dairy practice. At the same time, they also mean a higher number of animals included in the experiment (in our experiment, n = 3 872). Therefore, to ensure the necessary variability (sufficient variation range) for efficient experiment performance in the case of bulk tank milk sampling, the samples were taken at significantly differentiated localities in the applied DPS. This selection of dairy herds has been carried out systematically following the relevant scientific works (Soyeurt et al. 2011; Coppa et al. 2013; Hanus et al. 2016). The main selection factor was the type of DPS, which is related to nutrition as the dominant factor (Coppa et al. 2013; Hanus et al. 2016).

The results showed (Tables 2–6) that the necessary variability for the experiment in milk performance, composition, and properties of bulk tank milk and model yoghurts, but especially the FAPM of raw milk and yoghurts, was achieved. In addition, it can be stated that the sample selection methodology reflected the expected specific trends and tendencies in FAPM differences between GHe and SHe.

Milk performance of selected dairy herds

According to the results of milk recording data (Table 2), some significant differences in the characteristics of dairy herds were found. In comparison to GHe, milk yield was significantly higher ($P < 0.01$) in SHe, while fat content was lower ($P < 0.01$). Substantial differences in milk yield were then reflected in fat ($P < 0.05$) and protein yield ($P < 0.01$). The differences could

Table 2. Milk yield, fat and protein content/yield in selected dairy herds according to the results of the milk recording data depending on the dairy production system

Item	First lactations (n = 8)			All lactations (n = 8)		
	GHe (mean ± SD)	SHe (mean ± SD)	P	GHe (mean ± SD)	SHe (mean ± SD)	P
Number of cows	57 ± 44	123 ± 62	0.036 4	157 ± 119	316 ± 170	0.059 6
Days	294 ± 9	297 ± 5	0.494 4	291 ± 9	296 ± 6	0.253 5
Milk (kg)	5 797 ± 1 859	8 373 ± 1 402	0.009 2	6 205 ± 1 794	9 117 ± 1 593	0.005 4
Fat (g/100 g)	4.01 ± 0.24	3.69 ± 0.10	0.004 4	4.05 ± 0.20	3.72 ± 0.14	0.002 3
Protein (g/100 g)	3.24 ± 0.10	3.34 ± 0.14	0.131 4	3.30 ± 0.11	3.31 ± 0.11	0.786 8
Fat (kg)	229 ± 61	308 ± 48	0.014 6	249 ± 62	339 ± 57	0.012 2
Protein (kg)	188 ± 62	279 ± 39	0.004 7	204 ± 58	301 ± 45	0.003 2

GHe = grazing herd; n = number of cases; P = probability value; SHe = stabled herd; SD = standard deviation

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be influenced by nutrition and the share of breeds in the GHe and SHe systems. In general, these results correspond to previous findings in similar conditions (Janu et al. 2007; Frelich et al. 2012; Hanus et al. 2016). The results also show what differences in the characteristics of dairy herds concerning milk yield can be expected in the implementation of the proposed type of raw milk selection according to the type of DPS.

Composition and properties of bulk tank milk and model yoghurt samples

The composition and properties of selected bulk tank milk samples used to prepare model yoghurts are expressed as the basic statistical characteristics in Table 3. The mean values and variability, including the range of variation, correspond to previous results for similar sample types and the sampling season (Janu et al. 2007; Frelich et al. 2012). As expected, the lowest variability was found for lactose, citric acid, pH, and MFP (from 0.6% to 1.7%), and the highest for urea, acetone, FFAs, and SCC (from 21.1% to 45.1%), i.e. indicators that are closely related to nutrition and mammary gland health. High variability was also found for rennetability (38.8%). Overall, the values correspond to the standard quality of raw cow's milk in the EU.

The results of the composition of yoghurts can be considered rather as estimates because the indirect measurement (MIR-FT) of the composition of dairy products is burdened by the great variability of these materials during technological processing. The validity of the calibrations is only approximate. Nevertheless, the correlation coefficients between the same components of milk and yoghurt were significant ($P < 0.01$), for crude protein 0.60, casein 0.64, fat 0.89, lactose 0.62, and urea 0.81 (data not given in the Table). From these values, a greater fermentation intervention in lactose than in fat, for example, is logically evident. The results of active and titratable acidity showed a logically marked technological change in yoghurt compared to raw milk in terms of the expected increase in acidity: *i*) pH from 6.67 to 4.42 (by –34%); *ii*) titratable acidity from 7.70 °SH to 30.79 °SH (by 300%).

Table 3. Basic statistical characteristics of monitored indicators of bulk tank milk and model yoghurt samples

Composition	Milk (n = 16)					Yoghurt (n = 16)				
	mean	SD	min	max	RSD (%)	mean	SD	min	max	RSD (%)
Fat (g/100 g)	3.71	0.32	3.02	4.25	8.6	3.66	0.25	2.98	4.14	6.8
CP (g/100 g)	3.27	0.14	3.09	3.62	4.2	3.45	0.22	3.16	4.02	6.5
Casein (g/100 g)	2.59	0.14	2.42	2.92	5.3	2.39	0.21	2.12	2.96	8.9
Lactose (g/100 g)	4.86	0.08	4.67	4.98	1.7	4.40	0.12	4.12	4.54	2.6
SNF (g/100 g)	8.65	0.22	8.04	9.01	2.5	9.27	0.32	8.64	9.94	3.5
Urea (mg/100 ml)	38.1	8.0	27.6	52.5	21.1	67.7	7.4	53.8	80.8	11.0
Acetone (mg/l)	4.93	2.22	2.43	10.21	45.1	–	–	–	–	–
Citric acid (mmol/l)	6.10	0.08	5.99	6.24	1.3	–	–	–	–	–
FFA (mmol/100 g of fat)	1.01	0.36	0.62	1.81	36.0	–	–	–	–	–
Other indicators										
MFP (°C)	–0.524	0.007	–0.532	–0.507	1.3	–	–	–	–	–
SCC (10^3/ml)	339	126	139	525	37.2	–	–	–	–	–
pH	6.67	0.04	6.55	6.73	0.6	4.42	0.04	4.35	4.48	0.9
TA (°SH)	7.70	0.44	6.88	8.44	5.7	30.79	2.61	24.40	34.32	8.5
EC (mS/cm)	3.83	0.12	3.60	4.00	3.1	–	–	–	–	–
AS (ml)	0.67	0.10	0.49	0.89	14.7	–	–	–	–	–
Rennetability (s)	58	22	11	99	38.8	–	–	–	–	–

AS = alcohol stability; CP = crude protein; EC = electrical conductivity; FFA = content of free fatty acids in milk fat; MFP = milk freezing point; n = number of cases; pH = active acidity; RSD = relative standard deviation (variation coefficient in %) = (SD/mean) × 100; SD = standard deviation; SCC = somatic cell count; SNF = solids-non-fat; TA = titratable acidity

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When milk samples were divided according to DPS, the significant differences in the milk indicators between both variants (GHe and SHe) were observed only in the citric acid ($P < 0.05$), alcohol stability ($P < 0.001$), and rennetability ($P < 0.05$) (Table 4). It may be due to the lower number of samples in the groups. Considerable differences are known from the literature (Frelich et al. 2012; Srednicka-Tober et al. 2016; Manuelian et al. 2022), although there is enormous variability within the DPS (type of forage, proportion and quality of pasture, etc.). Statistically significant differences were found mainly in milk yield and fat content (Hanus et al. 2016; Fretin et al. 2017). However, rarely found differences do not mean a substantial practical effect on the production of model yoghurts in this experiment.

The FAPM of bulk tank milk and model yoghurt samples

Basic statistical characteristics of the FAPM for raw milk and model yoghurt are given in Table 5. The average values for raw milk were approximately in agreement with other scientific papers (Soyeurt et al. 2011; Frelich et al. 2012; Coppa et al. 2013; Niero et al. 2021). For individual FAs, the lowest and highest variability was found for C6:0 (7.4%) and CLA (61.4%). For FA groups, the lowest and highest variability was found for SFAs and TFAs (4.6% and 39.4%), where, at the same time, these groups have the highest and lowest proportions of 65.12% and 2.52% in cow's milk fat. Comparable results were found in model yoghurt samples, both for individual FAs and FA groups.

The distribution of these results for raw milk according to DPS is given in Table 6. A higher incidence of significant differences can be seen in the FAPM than in the observed milk indicators. It means that the methodology of milk source selection is much more effective in influencing the FAPM than in components and properties of milk, where, on the contrary, it does not bring significant differences (Table 4).

Depending on the DPS (Table 6), statistical significances in proportions of C16:0, C18:0, C18:1 *cis*-9, C18:3n-3, CLA, hypercholesterolemic FAs (HFAs), TFAs, SFAs, PUFAs, medium-chain FAs (MCFAs), and long-chain FAs (LCFAs) were found. These individual FAs and FA groups are mainly nutritionally interesting items (Parodi 1999; German

Table 4. Chemical composition, and other indicators of bulk tank milk samples for yoghurt processing depending on the dairy production system

Item	GHe (n = 8) mean ± SD)	SHe (n = 8) (mean ± SD)	P
Fat (g/100 g)	3.72 ± 0.37	3.69 ± 0.28	0.887 3
CP (g/100 g)	3.29 ± 0.14	3.26 ± 0.14	0.745 2
Casein (g/100 g)	2.59 ± 0.14	2.58 ± 0.14	0.917 3
Lactose (g/100 g)	4.85 ± 0.09	4.88 ± 0.08	0.509 4
SNF (g/100 g)	8.58 ± 0.24	8.71 ± 0.19	0.254 5
Urea (mg/100 ml)	36.7 ± 9.0	39.5 ± 7.2	0.506 7
Acetone (mg/l)	5.19 ± 2.70	4.68 ± 1.77	0.658 9
Citric acid (mmol/l)	6.13 ± 0.07	6.06 ± 0.07	0.040 2
FFA (mmol/100 g of fat)	1.03 ± 0.46	0.98 ± 0.26	0.803 1
MFP (°C)	−0.523 ± 0.006	−0.524 ± 0.008	0.937 4
SCC (10^3/ml)	329 ± 140	349 ± 119	0.759 9
pH	6.66 ± 0.05	6.68 ± 0.03	0.161 4
TA (°SH)	7.69 ± 0.45	7.71 ± 0.46	0.913 7
EC (mS/cm)	3.79 ± 0.14	3.88 ± 0.09	0.148 9
AS (ml)	0.74 ± 0.07	0.60 ± 0.06	0.000 6
Rennetability (s)	47 ± 19	68 ± 21	0.048 7

AS = alcohol stability; CP = crude protein; EC = electrical conductivity; FFA = content of free fatty acids in milk fat; GHe = grazing herd; MFP = milk freezing point; n = number of cases; P = probability value; pH = active acidity; SCC = somatic cell count; SD = standard deviation; SHe = stabled herd; SNF = solids-non-fat; TA = titratable acidity

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Table 5. Basic statistical characteristics of individual fatty acids (FAs) and FA groups (g/100 g of FAs) in bulk tank milk and model yoghurt samples

FAs	Milk (n = 16)					Yoghurt (n = 16)				
	mean	SD	min	max	RSD (%)	mean	SD	min	max	RSD (%)
C4:0	1.70	0.22	1.36	2.27	12.8	1.61	0.22	1.30	2.12	13.9
C6:0	1.51	0.11	1.34	1.75	7.4	1.48	0.11	1.30	1.71	7.6
C8:0	1.14	0.13	0.88	1.35	11.0	1.14	0.12	0.84	1.28	10.1
C10:0	2.69	0.40	1.90	3.42	14.9	2.74	0.40	1.92	3.33	14.5
C12:0	3.21	0.56	2.16	4.19	17.4	3.26	0.57	2.14	4.15	17.4
C14:0	10.69	1.19	8.33	12.66	11.1	10.81	1.16	8.46	12.53	10.7
C16:0	30.17	2.85	24.54	34.76	9.5	29.99	2.74	24.59	34.29	9.1
C18:0	11.08	1.47	9.01	14.39	13.2	10.80	1.35	8.90	13.54	12.5
C18:1 *cis*-9	20.64	1.83	17.21	23.90	8.9	20.60	1.79	17.30	23.23	8.9
C18:2n-6	2.29	0.33	1.62	2.92	14.5	2.30	0.29	1.95	2.97	12.5
C18:3n-3	0.62	0.26	0.31	1.13	41.8	0.61	0.24	0.32	1.09	39.8
CLA	0.71	0.44	0.29	1.71	61.4	0.71	0.41	0.29	1.62	57.4
FA groups										
VFA	7.04	0.60	6.01	8.23	8.5	6.97	0.57	5.67	7.67	8.1
HFA	44.08	4.24	35.10	49.93	9.6	44.06	4.11	35.33	49.60	9.3
TFA	2.52	0.99	1.48	5.48	39.4	2.45	1.08	1.47	5.17	43.9
SFA	65.12	3.01	58.77	69.98	4.6	64.83	3.01	58.34	68.61	4.6
MUFA	25.98	1.89	21.32	28.43	7.3	26.17	1.65	22.67	28.41	6.3
PUFA	4.29	0.91	3.10	6.78	21.2	4.37	0.83	3.34	6.71	19.0
UFA	30.50	2.46	25.74	34.51	8.1	30.81	2.33	27.21	35.62	7.6
SCFA	10.85	1.20	8.66	13.23	11.0	10.84	1.17	8.38	12.48	10.8
MCFA	47.75	3.76	40.62	54.23	7.9	47.98	3.66	40.79	53.95	7.6
LCFA	41.40	4.69	34.76	50.71	11.3	41.18	4.67	34.81	50.80	11.3

CLA = conjugated linoleic acid, isomer C18:2 *cis*-9, *trans*-11; FA = fatty acid; n = number of cases; HFA = hypercholesterolemic FAs; LCFA = long-chain FAs; MCFA = medium-chain FAs; MUFA = monounsaturated FAs in *cis* configuration; PUFA = polyunsaturated FAs; RSD = relative standard deviation (variation coefficient in %) = (SD/mean) × 100; SD = standard deviation; SCFA = short-chain FAs; SFA = saturated FAs; TFA = *trans* isomers of unsaturated FAs; UFA = unsaturated FAs; VFA = volatile FAs

et al. 2009; Gomez-Cortes et al. 2018; Hanus et al. 2018). In comparison to non-grazing or conventional milk, higher values of nutritionally beneficial FAs (C18:0, C18:1 *cis*-9, C18:3n-3, CLA, PUFAs, and LCFAs) in the milk of grazing cows were also reported by other authors (Frelich et al. 2012; Hanus et al. 2016; Srednicka-Tober et al. 2016; Niero et al. 2021; Manuelian et al. 2022). An apparent separation of milk and yoghurt samples according to the DPS is also shown in Figure 1. A sample plot also confirmed a higher variability in GHe than in SHe.

It follows from the above that the methodology used to select the milk source [e.g. also according to a possible prediction (Coppa et al. 2013; Srednicka-Tober et al. 2016; Liu et al. 2020; Bodnar et al. 2021; Manuelian et al. 2022)] is a persuasive factor for obtaining a raw material with significantly different FAPM and, in the case of GHe also in terms of nutritionally and health-advantageous combination in the FAPM, of course at the cost of lower milk yield (Table 2). This trend also broadly corresponds to the findings of the above papers. The GHe dairy cow milk also has a more favourable ratio for consumers between the nutritionally and health-friendly oleic acid and the health-risk (Hanus et al. 2018) group of HFAs (C12:0, C14:0, and C16:0). This ratio is 1 : 1.9 for GHe *vs* 1 : 2.4 for SHe. Thus, DPS appears to be another practical aspect for the targeted selection of raw milk and subsequently modifying FAPM in final products.

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Table 6. Proportion of individual fatty acids (FAs) and groups of FAs (g/100 g of FAs) in bulk tank milk samples for yoghurt processing depending on the dairy production system

Item	GHe (n = 8) mean ± SD	SHe (n = 8) mean ± SD	P
C4:0	1.75 ± 0.15	1.65 ± 0.27	0.367 5
C6:0	1.50 ± 0.12	1.51 ± 0.12	0.981 0
C8:0	1.11 ± 0.17	1.17 ± 0.06	0.391 3
C10:0	2.55 ± 0.50	2.83 ± 0.22	0.171 7
C12:0	3.00 ± 0.66	3.43 ± 0.35	0.123 5
C14:0	10.24 ± 1.43	11.14 ± 0.70	0.132 7
C16:0	28.07 ± 2.23	32.27 ± 1.54	0.000 6
C18:0	11.87 ± 1.54	10.29 ± 0.89	0.024 9
C18:1 *cis*-9	21.56 ± 2.08	19.71 ± 0.95	0.037 5
C18:2n-6	2.24 ± 0.39	2.34 ± 0.27	0.538 3
C18:3n-3	0.83 ± 0.19	0.41 ± 0.08	0.000 1
CLA	1.02 ± 0.44	0.41 ± 0.10	0.002 2
VFA	6.92 ± 0.73	7.15 ± 0.44	0.449 5
HFA	41.31 ± 4.18	46.85 ± 1.85	0.004 1
TFA	3.16 ± 1.00	1.88 ± 0.40	0.004 7
SFA	63.37 ± 3.23	66.87 ± 1.42	0.014 0
MUFA	26.62 ± 2.39	25.35 ± 0.98	0.185 7
PUFA	4.83 ± 0.94	3.75 ± 0.46	0.011 2
UFA	31.76 ± 2.84	29.24 ± 1.12	0.035 3
SCFA	10.46 ± 1.51	11.24 ± 0.67	0.199 6
MCFA	45.25 ± 3.39	50.25 ± 2.12	0.003 3
LCFA	44.29 ± 4.80	38.50 ± 2.23	0.007 9

CLA = conjugated linoleic acid, isomer C18:2 *cis*-9, *trans*-11; FA – fatty acid; GHe = grazing herd; HFA = hypercholesterolemic FAs; LCFA = long-chain FAs; MCFA = medium-chain FAs; MUFA = monounsaturated FAs in *cis* configuration; n = number of cases; P = probability value; PUFA = polyunsaturated FAs; SD = standard deviation; SCFA = short-chain FAs; SHe = stabled herd; SFA = saturated FAs; TFA = *trans* isomers of unsaturated FAs; UFA = unsaturated FAs; VFA = volatile FAs

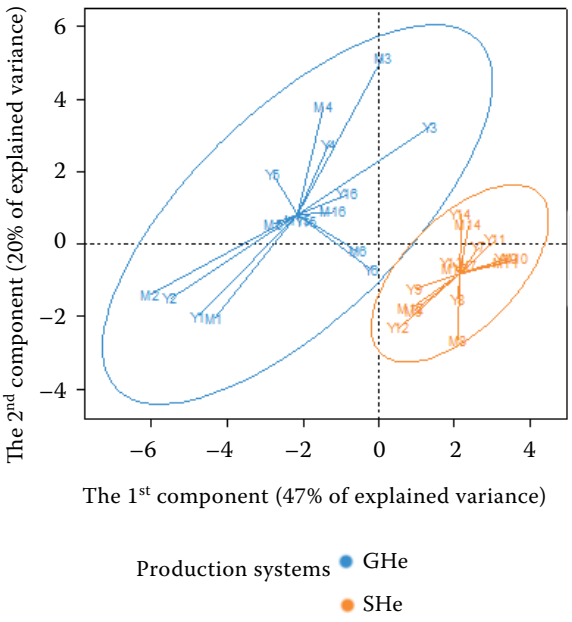

Figure 1. Sample plots from sPLS-DA performed on the selected fatty acid data content including 95% confidence ellipses

Samples are projected into the space spanned by the first two components and colored according to their production systems (SHe, GHe) and indicated with milk (M) and yoghurt (Y)

GHe = grazing herd; SHe = stabled herd

Differences and relationships between the FAPM of milk and yoghurt

The possible effect of milk fermentation on the FAPM is summarised in Table 7. The differences between the FAPM of milk and yoghurt samples were negligible (in relative values from 0.04% to 5.21%), including C14:0, C16:0, C18:0, and C18:3n-3, although average values of these FAs between milk and yoghurt samples differed statistically significant. It results in a minimal effect of milk fermentation on the original FAPM, and then the conclusion that systematic selection of raw material (GHe) or change of DPS (in the sense of GHe) can ensure a more beneficial FAPM in yoghurts. It is supported by the results of correlation and regression analysis, where the correlation coefficients (r) between milk and yoghurt for nutritionally beneficial FAs range from 0.925 0 (C18:2n-6) to 0.998 8 (CLA; Figure 2; both $P < 0.001$). In general, all correlation coefficients were statistically significant ($P < 0.001$) except for C4:0 and C6:0 ($P > 0.05$) and consequently for VFA ($P < 0.05$). Then for C18:3 n-3 and CLA, 100% of their variability (R^2) in yoghurt can be explained by their variations in milk. In this case, it means the dependence on DPS (GHe) and thus on the nutrition of the dairy cows. For SFAs, MUFAs and PUFAs (Figure 2), coefficients of determination were 94%, 93%, and 97%.

These results were also confirmed by Bodnar et al. (2021). Authors observed FA composition

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Table 7. Comparison and relationship of individual fatty acids (FAs) and FA groups (g/100 g of FAs) in bulk tank milk samples and model yoghurt samples (n = 16)

FAs	Milk	Yoghurt	Differences			Correlation and regression analysis							
	mean		abs	rel	P	r	SE (r)	A	SE (A)	P	B	SE (B)	P	R^2
C4:0	1.70	1.61	+0.09	+5.21	0.188 9	0.319 8	0.25	1.05	0.45	0.034 0	0.33	0.26	0.227 2	10
C6:0	1.51	1.48	+0.03	+1.69	0.460 9	0.276 7	0.26	1.06	0.39	0.016 3	0.28	0.26	0.299 6	8
C8:0	1.14	1.14	0.00	+0.19	0.898 2	0.857 5	0.14	0.24	0.14	0.121 1	0.79	0.13	< 0.001	74
C10:0	2.69	2.74	–0.05	–1.67	0.103 8	0.966 1	0.07	0.16	0.19	0.186 1	0.96	0.07	< 0.001	93
C12:0	3.21	3.26	–0.05	–1.55	0.069 0	0.983 8	0.05	0.07	0.16	0.679 7	0.99	0.05	< 0.001	97
C14:0	10.69	10.81	–0.12	–1.08	0.025 3	0.987 7	0.04	0.47	0.44	0.302 3	0.97	0.04	< 0.001	98
C16:0	30.17	29.99	+0.18	+0.61	0.032 3	0.994 7	0.03	1.21	0.80	0.149 9	0.95	0.03	< 0.001	99
C18:0	11.08	10.80	+0.28	+2.48	0.000 1	0.992 1	0.03	0.68	0.35	0.070 0	0.91	0.03	< 0.001	98
C18:1 *cis*-9	20.64	20.60	+0.04	+0.19	0.707 7	0.973 4	0.06	0.92	1.24	0.468 9	0.95	0.06	< 0.001	95
C18:2n-6	2.29	2.30	–0.01	–0.51	0.716 9	0.925 0	0.10	0.47	0.20	0.036 7	0.80	0.09	< 0.001	86
C18:3n-3	0.62	0.61	+0.01	+2.00	0.039 1	0.998 4	0.01	0.03	0.01	0.007 6	0.93	0.01	< 0.001	100
CLA	0.71	0.71	+0.01	+0.74	0.578 7	0.998 8	0.01	0.05	0.01	0.000 3	0.93	0.01	< 0.001	100
FA groups														
VFA	7.04	6.97	+0.07	+1.01	0.592 2	0.601 9	0.21	2.94	1.43	0.059 8	0.57	0.20	0.013 6	36
HFA	44.08	44.06	+0.02	+0.04	0.872 7	0.994 9	0.03	1.53	1.15	0.204 1	0.96	0.03	< 0.001	99
TFA	2.52	2.45	+0.07	+2.75	0.689 4	0.786 7	0.16	0.30	0.48	0.543 4	0.85	0.18	0.000 3	62
SFA	65.12	64.83	+0.29	+0.44	0.131 0	0.971 1	0.06	1.72	4.15	0.685 7	0.97	0.06	< 0.001	94
MUFA	25.98	26.17	–0.19	–0.73	0.162 0	0.965 7	0.07	4.26	1.58	0.017 3	0.84	0.06	< 0.001	93
PUFA	4.29	4.37	–0.08	–1.83	0.065 4	0.987 4	0.04	0.49	0.17	0.012 1	0.90	0.04	< 0.001	97
UFA	30.50	30.81	–0.31	–1.01	0.055 2	0.970 9	0.06	2.73	1.86	0.163 2	0.92	0.06	< 0.001	94
SCFA	10.85	10.84	+0.01	+0.09	0.949 5	0.873 7	0.13	1.60	1.38	0.266 1	0.85	0.13	< 0.001	76
MCFA	47.75	47.98	–0.22	–0.47	0.102 0	0.990 9	0.04	1.98	1.67	0.255 1	0.96	0.03	< 0.001	98
LCFA	41.40	41.18	+0.21	+0.51	0.228 3	0.989 5	0.04	0.36	1.60	0.823 8	0.99	0.04	< 0.001	98

Correlation analysis is expressed by r = correlation coefficient; regression analysis is expressed by regression equation $Y = A + B \times X_1$; where A = intercept; B = slope coefficient; X_1 (individual FAs or FA groups in milk)

abs = absolute difference in g/100 g of FAs; CLA = conjugated linoleic acid, isomer C18:2 *cis*-9, *trans*-11; FA = fatty acid; HFA = hypercholesterolemic FAs; LCFAs = long-chain FAs; MCFAs = medium-chain FAs; MUFA = monounsaturated FAs in *cis* configuration; P = probability value; PUFA = polyunsaturated FAs; R^2 = coefficient of determination (%); rel = relative difference (%) calculated as: [absolute difference × 100/FAs (milk)]; SCFAs = short-chain FAs; SE = standard error; SFA = saturated FAs; TFA = *trans* isomers of unsaturated FAs; UFA = unsaturated FAs; VFA = volatile FAs

in goat's milk and semi-hard cheeses depending on different feeding diets (as the experimental diet, extensive pasture was used). While favourable FA composition in the milk of grazing goats was confirmed, non-significant differences between FAs in goat milk and cheese were found. In a similar comparison (Pecova et al. 2019) in goat yoghurts (from individual milk samples, n = 40, Anglo-Nubian breed), the corresponding correlation coefficient for CLA was 0.981 1 (P < 0.001), i.e. only slightly lower. For MUFAs and PUFAs, the correlations were 0.930 3 and 0.938 6 (P < 0.001). Sampling here was random, without a targeted selection of raw milk, performed in one farm according to the type of nutrition. The results for other FAs were similar and justified similar conclusions for goat breeding in the sense of guaranteeing the invariance of the FAPM during milk fermentation.

It seems that fermentation does not have a considerable effect on FAPM in yoghurt, both cow and goat. More extensive changes in yoghurts were detected by some authors rather during storage (Khan et al. 2020; Paszczyk et al. 2020).

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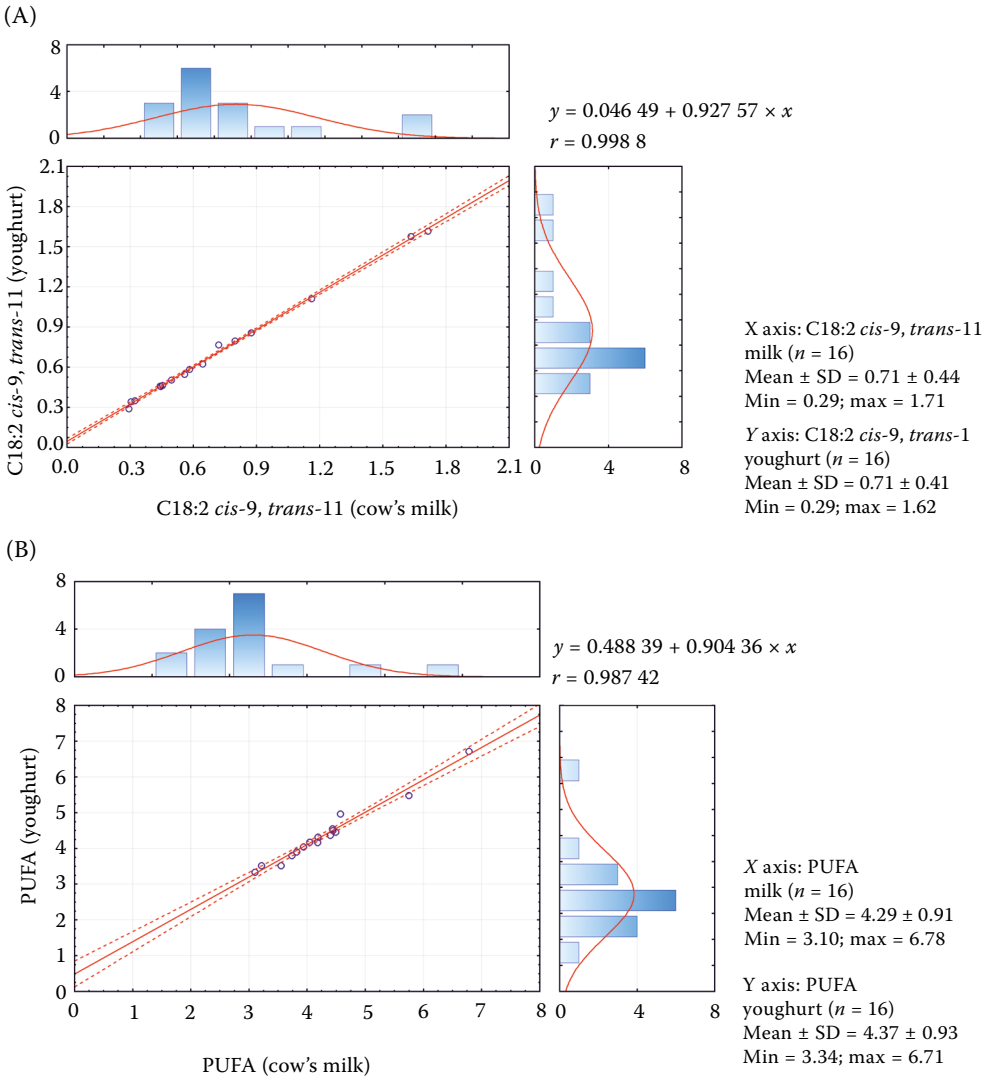

Figure 2. Regression analysis for proportion [g/100 g of fatty acids (FAs)] of (A) CLA; and (B) PUFA determined in cow's milk and yoghurt CLA = (C18:2 *cis*-9, *trans*-11); *r* = correlation coefficient; PUFA = polyunsaturated FAs; SD = standard deviation

CONCLUSION

The results of our experiment with a systematic selection of cow's milk sources according to the dairy production system confirmed the possibility of obtaining raw milk with healthier milk fat composition. Fermentation did not considerably change this composition in model yoghurt, including fatty acids with presumed nutritional and health benefits. Evaluating the relationships between the milk fatty acid proportion of raw cow's milk and model yoghurt provided new insights into the stability of fatty acids during the fermentation process.

Conflict of interest

The authors declare no conflict of interest.

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