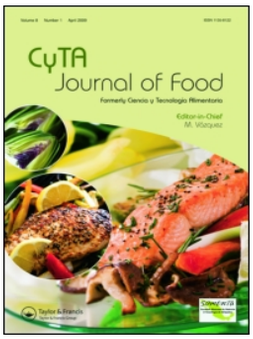

CyTA - Journal of Food

ISSN: 1947-6337 (Print) 1947-6345 (Online) Journal homepage: https://www.tandfonline.com/loi/tcyt20

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To cite this article: Attila Kiss, Zoltán Naár, Lajos Daróczi, Sándor Kukovics, Ferenc Kukovics, Iman Mirmazloum & Erzsébet Némedi (2019) Changes of free fatty acid composition and number of lactic acid bacteria in three functional goat and sheep milk products fortified with inulin or fish oil, CyTA - Journal of Food, 17:1, 51-59, DOI: 10.1080/19476337.2018.1551936

To link to this article: https://doi.org/10.1080/19476337.2018.1551936

Published online: 19 Apr 2019.

CYTA – JOURNAL OF FOOD
2019, VOL. 17, NO. 1, 51–59
https://doi.org/10.1080/19476337.2018.1551936

Changes of free fatty acid composition and number of lactic acid bacteria in three functional goat and sheep milk products fortified with inulin or fish oil

Attila Kiss[a], Zoltán Naár[b], Lajos Daróczi[c], Sándor Kukovics[d], Ferenc Kukovics[d], Iman Mirmazloum [a,e] and Erzsébet Némedi[f]

[a]Food Science Innovation Centre, Kaposvár University, Kaposvár, Hungary; [b]National Agricultural Research and Innovation Centre, Food Science Research Institute, Budapest, Hungary; [c]Y-FOOD Ltd., Berettyóújfalu, Hungary; [d]Hungarian Sheep and Goat Dairying Public Utility Association, Herceghalom, Hungary; [e]Department of Plant Physiology and Plant Biochemistry, Szent István University, Budapest, Hungary; [f]Expedit Nodum Ltd., Budapest, Hungary

ABSTRACT

Inulin and fish oil rich in omega-3 fatty acid were applied to yoghurt, kefir, and smearcase made from goat and sheep milk, and their impact on fatty acid composition was investigated at 8°C throughout 40 days. The number of lactic acid bacteria (LAB) was significantly diminished within 16 days, and by the end of the storage period the population size was decreased by 3 orders of magnitude in fish oil fortified samples. In inulin-fortified samples, a significant decrease in LAB number (by 2.5–3 orders of magnitude) was observed just by the end of the storage time, while the amount of unsaturated free fatty acids was increased. The extent of lipolysis and the resulted amount of free fatty acids exhibited varying feature depending on the composition of the product. Goat and sheep milk products exhibit analogous trends in terms of change in fatty acid composition with one exception: the ratio of free C16:0 was increased by 10% for goat milk products, while for the sheep milk products only by 3%. Ratio of C16:0 did not change during storage of sheep yoghurt as a result of inulin fortification. In contrast, 13% increase of this parameter was observed in case of the product variant prepared with fish oil addition. It might be stated that fortification with prebiotics can be regarded as a better way to improve the biological value of dairy products than that with fish oil.

ARTICLE HISTORY
Received 6 June 2018
Accepted 10 October 2018

KEYWORDS
Functional dairy products; Fortification with inulin and fish oil; Fatty acid composition; LAB number; Diverse packaging methods

Cambios en la composición de ácido graso libre y en el número de bacterias lácticas en tres productos funcionales de leche caprina y ovina fortificados con inulina o aceite de pescado

RESUMEN

En la presente investigación se aplicó inulina y aceite de pescado rico en ácidos grasos omega 3 a muestras de yogurt, kéfir y queso cottage elaboradas con leches caprina y ovina, para indagar su impacto en la composición de ácidos grasos de estos productos al ser almacenados durante 40 días a 8°C. Se constató que al cabo de 16 días de almacenamiento, en las muestras fortificadas con aceite de pescado disminuyó significativamente el número de bacterias lácticas (LAB) y que al finalizar el periodo de almacenamiento el tamaño de esta población de bacterias se había reducido en tres órdenes de magnitud. A su vez, se comprobó que, al finalizar el periodo de almacenamiento, en las muestras fortificadas con inulina se redujo significativamente el número de LAB (en 2.5 a 3 órdenes de magnitud), produciéndose también un aumento en la cantidad de ácidos grasos libres insaturados. El grado de lipólisis y la cantidad de ácidos grasos libres resultantes presentaron distintos aspectos, dependiendo de la composición del producto. Los productos elaborados con leche caprina u ovina muestran tendencias análogas en términos de los cambios en la composición de ácidos grasos, con una excepción: la ratio de C16:0 libre en los productos de leche caprina se incrementó en 10%, mientras que en los elaborados con leche ovina lo hizo solo en 3%. Por otra parte, durante el almacenaje de yogurt ovino fortificado con inulina no se produjeron variaciones en la ratio de C16:0. En cambio, se constató un aumento de 13% en este parámetro en una variante del producto preparada con la adición de aceite de pescado. Lo anterior permite concluir que para mejorar el valor biológico de los productos lácteos es más adecuada su fortificación con la adición de prebióticos que con aceite de pescado.

PALABRAS CLAVE
Productos lácteos funcionales; Fortificación con inulina y aceite de pescado; Composición de ácido graso; Número de bacterias lácticas (LAB); Diversos métodos de empaque

1. Introduction

Products made of goat and sheep milk have been an important food source for the mankind for millennia and their popularity is continuously rising nowadays. Further to this trend, increasing demand for functional foods induced a more extensive use of special milk products containing agents such as pre- and probiotics, or considerable content of unsaturated fatty acids (Rodrigues, Rocha-Santos, Gomes, Goodfellow, & Freitas, 2012).

Prebiotics are non-digestible dietary components that can reach intactly the colon, where they stimulate the proliferation and activity of desirable bacteria in situ (Mattila-Sandholm et al., 2002). The utilization of the rich nutrient stock of milk products may be promoted by sufficiently high

CONTACT Attila Kiss kiss.attila@ke.hu Food Science Innovation Centre, Kaposvár University, Guba Sandor. str. 40. 7400 Kaposvár, Hungary

number of probiotic bacteria in the intestine. Various kinds of materials could be used to support the growth of probiotic bacteria (fructo-oligosaccharides/FOS/, inulin or resistant starch), but the application of inulin added to the milk before processing seems to be the most adequate method. Inulin amendment of milk increased the number of probiotic bacteria during the maturation of products, but the inulin content practically remained unchanged (Gustaw, Kordowska-Wiater, & Kozioł, 2011; Modzelewska-Kapituła, Kłębukowska, & Kornacki, 2007). At the same time, the sensory traits as well as the texture and the organic acid contents of the products were improved (Seckin & Ozkilinc, 2011), and the potential prebiotic effect of the final product was increased (Vlaseva, Ivanova, Petkova, Todorova, & Denev, 2014).

Recently, a new trend appeared related to the modification of milk's lipid profile, in accordance with the enhanced demand for the development of new products fortified with omega-3, omega-6 fatty acids and other components with potentially positive effects on the human health (Saini & Keum, 2018). In case of some new dairy products fat is partly replaced with vegetable fat or a mixture containing fish oil. This would increase the level of omega-3 fatty acids, with a consequent benefit of prevention of cardiovascular diseases (Li, Bode, Drummond, & Sinclair, 2003).

The functional component added to customary foods may itself influence the stability of the products. Health promoting bioactive agents may affect biochemical processes occurring throughout storing, and the activity of microorganisms present in the products for technological purposes. Kiss, Naár, Daróczi, Némedi, and Kukovics (2014) added inulin to goat and sheep milk products and observed no significant change of their microbiota during 35 days of cold storing. A marked effect of the mode of packaging was observed because the number of lactobacilli decreased significantly faster in the snap-cap container than in the weld-cap one. No significant changes were detected in the composition of total fatty acids in goat and sheep milk products containing inulin, although the composition of free fatty acids was not investigated.

There are some research data on the potential beneficial effect of prebiotic amendment on the technological and sensory features of fermented sheep milk products as well as on the fatty acid profile (Balthazar et al., 2017). However, the way how inulin amendment may influence the fatty acid composition of fermented milk products is not clearly understood. Balthazar et al. (2016) found that inulin can slow down the post-acidification during 28-day-long cold storage, suggesting that inulin may alter the metabolism of lactic acid bacteria used for processing. However, inulin did not protect them from being declined during this period. Although addition of inulin did not lead to influencing the fatty acids, a correlation was found between the metabolic activity of starter bacteria and the changes of fatty acid composition. Considering the role of microbes in lipolytic processes and metabolism of fatty acids in cheese, as it was comprehensively described by Thierry et al. (2017), one may suppose that factors like inulin amendment that influences the physiology of microbes may result in changes of the fatty acid profile.

To our knowledge, no study was conducted involving different goat and sheep milk products together. Apart from this, the novelty of our investigations is supported by the fact that no relevant studies were accomplished in order to prove the impact of inulin or oil amendment and different packaging methods on the free fatty acid content of the fortified product. In our paper, revealing alterations in free fatty acid composition by fortification goat and sheep milk products with inulin might fill a presently existing gap in the relevant knowledge being necessary for characterization biological and technological properties of such functional foodstuffs.

There were no studies performed so far with respect to revealing the necessity of intensive homogenization in the course of addition of functional components such as inulin or fish oil rich in omega-3 fatty acid. It is also to be investigated, how such treatments would increase lipolysis, or as a consequence of changing the composition of free fatty acids in the products.

It was also of keen interest to obtain new results on the impact of fish oil addition on the extent of lipolysis, as no relevant information can be found in previous studies. Novelty of our studies is also strengthened by the lack of former researches on revealing the differences in the behaviour of fortified sheep and goat milk products.

Accordingly, the aim of this work was to investigate whether the lactobacillus population of the examined products would change due to the effect of addition of inulin or omega-3 rich oil. Assessment of the impact of packaging technology on the microbial and chemical composition of the products was also set as one of our major objectives.

2. Materials and methods

2.1. Origin and pre-treatment of milk

The raw sheep and goat milk used in the experiments originated from several producers. As having mixed breed background, these collected milk bulks represented the average domestic quality of sheep and goat milk. These collected sheep and goat milk bulks with mixed breed background gave the basis of various products (yoghurt, kefir, and smearcase) after well-tailored heat and homogenization treatments. The processing and admixture experiments were carried out in the milk processing manufactory of TEBIKE Ltd. (Győr, Hungary).

2.2. Product preparation

During the production of kefir, the raw milk, pre-warmed in a heat exchanger, was homogenized at ~150bars pressure. It was followed by pasteurization at 83°C with 30-s heat holding. High omega-3 containing fish oil was dissolved in the existing 24–28°C milk. CHN-22, XPL-1 and LAF-4 starters were added following this process. After portioning into the cups, sealing was achieved by snap-caps or by welding of the sealing aluminium foil. Congelation was taken place for approx. 10 hours at 24–28°C. The production was completed by cold maturation at 6°C for 12 h. The inulin was added to milk before pasteurization.

Yoghurt products were made in a similar way. Slightly different circumstances were applied in this case: the exiting temperature was 41–42°C, the congelation temperature 42°C, and the congelation time 6 h. These parameters were selected and applied in accordance with the relevant industrial practice, and suggested by the manufacturer partner of the project. ABY-12 starter was used for inoculation.

For the production of smearcase, pasteurization has taken place at 73°C with 30–40-second-long heat holding. CHN22 culture was used to inoculate the milk cooled to 30°C. Omega-3 containing oil product was added following the congelation at 32–34°C for 20–25 minutes. In the next step 20–minute-long pre-pressing was performed before 90 min of form pressing. Salting (90 min.), drying (18–24 hours) packaging and ripening (21 days) were made prior to cold storage test.

2.3. Packaging of products

The yoghurt and kefir were portioning partly into PET flacon with PE closure (Snap) and partly into PS food industry plastic cap with aluminium food industrial closing foil with PP lake cover (Welded). The sheep and goat milk smearcases were partly packaged into vacuum foils (Vacuum) and partly under MAP (70% N_2 + 30% CO_2) (MAP).

2.4. Storage

Changes in hygienic status and the content of functional compounds were monitored through a 40-day-long period of storage at 8°C in the dark. Three packaged units were opened for sampling for each treatment combinations on 0., 8., 16., 24., 32. and 40. days of cold storage. Further 3 units were used on days 1 and 40 for chemical analysis.

2.5. Investigations

Chemical analysis was carried out by using gas-chromatography for fatty acids. C16:0 was investigated as the most abundant fatty acid in the milk of small ruminants, while C18:3 (n-3), C18:2 (n-6), and C18:1 trans-9 were measured as representatives of unsaturated fatty acids. C18:3 (n-3) (α-linolenic acid) and C18:2(n-6) (linoleic acid) are well-known representatives of omega-3 and omega-6 unsaturated fatty acids having protective role against series of degenerative chronic diseases (Saini & Keum, 2018). C16:0 (palmitic acid) is the major fatty acid component of milk fat (Srbinovska & Dushica, 2018), so it is suitable to follow the rate of lipolysis (Rodrigues et al., 2012). C18:1 trans-9 (elaidic acid) is also a major constituent of milk's trans fatty acids that may contribute to the development of inflammations, cardiovascular diseases, type 2 diabetes as well (Tardy, Morio, Chardigny, & Malpuech-Brugère, 2011).

Free fatty acids and conjugated linoleic acid isomers were extracted from cheese matrix according to methodology described by Silva et al. (2011). Extracted samples were analyzed with GC-MS technique (QP5000, Shimadzu, Kyoto, Japan) using a DB5 capillary column (0.25 µm film × 0.25 mm × 30 m; Agilent, Santa Clara, CA), operated in SIM mode. Helium was used as carrier gas, at a linear velocity of 35 cm/s. The sample (1 µL) was injected in splitless mode, at 250°C. The temperature program started at 80°C, and increased until 300°C at a rate of 8°C/min. The MS detector temperature was kept at 290°C.

Microbiological investigations were performed with cultivation on selective media to assess the population size of coliforms, *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus*, molds, and lactic acid bacteria in yogurt according to the following standards: coliform: MSZ 3640–17:1979, *Escherichia coli*: MSZ ISO 16649–2:2005; *Staphylococcus aureus*: MSZ EN ISO 6888–1:2008; mould and budding yeast: MSZ ISO 7954:1999; *Enterococcus faecalis*: MSZ EN ISO 7899–2:2000.

For statistical analysis, colony forming unit (cfu) data of microbes were normalized in logarithmic numbers, and ANOVA was applied to test the effect of the investigated factors, as it is generally acknowledged in this field and in case of such experiments. Multiple mean comparisons were made using Duncan's multiple range test in order to classify the effect of amendments and packaging methods on the microbial status and survival of lactic acid bacteria. Differences were considered at a significance level of $P < 0.05$. Samples fortified with diverse functional components and those exposed to different packaging methods were treated as separate blocks. To check the changes in fatty acid profile during the 40day-long cold storage, the ratios of the specific investigated fatty acid compounds were calculated on the basis of the free fatty acid content, the and Student's t-tests were performed to test the significance levels of the differences between the starting and the ending date of storage. Mean values and SEM data are presented in the relevant tables. SPSS 22 software was applied for all statistical analyses. Organoleptic examinations of the products were not carried out in this study, as our target was to reveal the scientific background of enrichment of specific milk products with some relevant bioactive components, in order to lay the foundation of prospective functional food manufacturing. It is up to the decision of the manufacturers, which line of the displayed product development suits better their market strategy.

3. Results

3.1. Changes occurring in the number of probiotic bacteria

Gradual decrease in the number of lactobacteria (LAB) was observed as a general tendency whose intensity was affected by all examined factors. The feature of the product exerted the largest impact on the number of lactobacteria, while the least changes in this parameter were experienced in case of varying the animal species (Tables 1 and 2).

LAB number of the yoghurt throughout 40 days was reduced from 10^9 to 10^6, while that of the kefir from 10^8 to 10^5, and that of the smearcase from 10^3 to 10^2. As a result of inulin addition, the extent of cell devastation was decreased by two orders of magnitude in the cases of yoghurt and kefir, whilst this phenomenon did not prove to be so pronounced for smearcase made from goat milk.

Generally, the addition of fish oil did not affect the survival of LAB, except for kefir with welded closure. In this case significantly higher bacterium number was observed by the end of the 40-day-long storage period in comparison with the control sample ($P < 0.05$). Application of different package patterns led to considerable distinctions just in case of these conditions: despite the previous finding, when using snap closure, the same bacterium number was observed as for the control. Alterations in the milk composition of the two animal species did not influence the decrease of LAB number to a considerable extent. It is important to note that the number of surviving LAB in yoghurt made of sheep milk exceeds that of yoghurt manufactured from goat milk by one order of magnitude.

Effect of packaging methods did not prove to be consistent and considerable. Based on the data received we might establish that in case of the snap-closed control sample the

Table 1. Population size of lactic acid bacteria in sheep milk yoghurt, kefir, and smearcase of different amendments and packaging.

Tabla 1. Tamaño de la población de bacterias lácticas en el yogurt, kéfir y queso cottage elaborados con leche ovina con distintas adiciones y distintos empaques.

			Days of incubation at 8 °C*					
Product	Amendment	Closure	0	8	16	24	32	40
Yogurt	Control	Snap	9.59 ± 0.52	8.58 ± 0.49	8.10 ± 0.32*	7.87 ± 0.28**	7.24 ± 0.12***	6.45 ± 0.32***a
		Welded	9.69 ± 0.30	8.98 ± 0.34	8.75 ± 0.57	8.38 ± 0.36*	7.46 ± 0.22***	6.14 ± 0.26***a
	Inulin	Snap	9.53 ± 0.33	9.56 ± 0.27	9.03 ± 0.36	8.84 ± 0.45	8.70 ± 0.37	8.15 ± 0.12*b
		Welded	9.34 ± 0.37	9.49 ± 0.23	9.30 ± 0.14	9.07 ± 0.22	8.58 ± 0.42	8.26 ± 0.17*b
	Omega-3 fish oil	Snap	9.45 ± 0.46	8.63 ± 0.48	8.35 ± 0.15*	7.65 ± 0.28**	7.08 ± 0.26**	6.70 ± 0.22***a
		Welded	9.29 ± 0.34	8.84 ± 0.16	8.71 ± 0.26	7.21 ± 0.32**	7.40 ± 0.14**	6.21 ± 0.32***a
Kefir	Control	Snap	8.34 ± 0.48	7.33 ± 0.52	6.85 ± 0.28**	6.12 ± 0.20**	5.95 ± 0.28**	5.28 ± 0.26***a
		Welded	8.44 ± 0.22	7.73 ± 0.36	6.50 ± 0.22**	6.13 ± 0.23**	6.01 ± 0.22***	5.69 ± 0.22***a
	Inulin	Snap	8.28 ± 0.36	8.31 ± 0.26	8.08 ± 0.14	7.89 ± 0.24	7.85 ± 0.42	7.76 ± 0.22 c
		Welded	8.09 ± 0.25	7.94 ± 0.27	8.07 ± 0.15	7.92 ± 0.22	7.73 ± 0.17	7.71 ± 0.19 c
	Omega-3 – fish oil	Snap	8.20 ± 0.47	7.38 ± 0.36	6.90 ± 0.15*	6.30 ± 0.28**	5.55 ± 0.28***	5.45 ± 0.16***a
		Welded	8.04 ± 0.47	7.59 ± 0.42	7.46 ± 0.42	6.96 ± 0.16*	6.15 ± 0.12**	5.96 ± 0.17**b
Smearcase	Control	Vacuum	3.84 ± 0.42	3.43 ± 0.26	3.12 ± 0.42	2.75 ± 0.57	2.68 ± 0.12*	2.58 ± 0.22*a
		MAP	3.88 ± 0.46	3.59 ± 0.42	3.50 ± 0.26	3.35 ± 0.32	2.98 ± 0.42	2.86 ± 0.16*ab
	Inulin	Vacuum	3.81 ± 0.16	3.66 ± 0.16	3.51 ± 0.15	3.72 ± 0.22	3.48 ± 0.26	2.86 ± 0.17*ab
		MAP	3.73 ± 0.31	3.52 ± 0.12	3.64 ± 0.22	3.47 ± 0.26	3.43 ± 0.22	3.09 ± 0.17 b
	Omega-3 –fish oil	Vacuum	3.78 ± 0.42	3.45 ± 0.34	3.26 ± 0.35	2.86 ± 0.45	2.72 ± 0.17*	2.68 ± 0.16*a
		MAP	3.72 ± 0.36	3.54 ± 0.21	3.48 ± 0.22	3.28 ± 0.46	2.96 ± 0.38	2.88 ± 0.16*ab

Data are mean of lg values of colony forming units counted on agar plates in triplicates. SEM values indicate the error of trial.
* Marks significant difference (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) from the starting control within a row.
Different letters mark significant differences ($P < 0.05$) among the variants of a given product after 40 days of storage.

Los datos son las medias de los valores lg de las unidades que forman colonias, contadas en placas de agar por triplicado. Los valores SEM [error estándar de la media] indican el error de la prueba. * Indica una diferencia significativa (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) a partir del control de inicio, en una misma fila.
Las distintas letras indican diferencias significativas ($P < 0.05$) entre las variantes de determinado producto después de 40 días de almacenamiento.

Table 2. Population size of lactic acid bacteria in goat milk yoghurt, kefir, and smearcase of different amendments and packaging.

Tabla 2. Tamaño de la población de bacterias lácticas en el yogurt, kéfir y queso cottage elaborados de leche caprina con distintas adiciones y distintos empaques.

			Days of incubation at 8 °C					
Product	Amendment	Closure	0	8	16	24	32	40
Yogurt	Control	Snap	8.91 ± 0.52	7.90 ± 0.47	7.12 ± 0.22*	6.79 ± 0.29**	6.02 ± 0.32**	5.78 ± 0.28***ab
		Welded	9.01 ± 0.46	8.30 ± 0.46	8.07 ± 0.52	7.20 ± 0.17*	6.28 ± 0.17**	5.46 ± 0.17*a
	Inulin	Snap	8.85 ± 0.36	8.88 ± 0.22	8.35 ± 0.47	8.36 ± 0.29	8.03 ± 0.42	7.67 ± 0.27*d
		Welded	8.66 ± 0.38	8.11 ± 0.27	7.98 ± 0.42	7.79 ± 0.57	7.90 ± 0.46	7.58 ± 0.23*d
	Omega-3-fish oil	Snap	8.77 ± 0.48	7.95 ± 0.45	7.47 ± 0.26*	6.47 ± 0.28**	6.12 ± 0.26***	6.03 ± 0.28***b
		Welded	8.61 ± 0.32	8.16 ± 0.27	8.03 ± 0.44	7.53 ± 0.15*	6.72 ± 0.17**	6.53 ± 0.15**c
Kefir	Control	Snap	7.66 ± 0.44	7.25 ± 0.35	6.87 ± 0.38	6.24 ± 0.27*	5.77 ± 0.26**	5.23 ± 0.27***ab
		Welded	7.76 ± 0.33	7.05 ± 0.42	6.82 ± 0.52	6.45 ± 0.16*	5.53 ± 0.22**	5.21 ± 0.15***ab
	Inulin	Snap	7.60 ± 0.56	7.63 ± 0.17	7.10 ± 0.25	6.81 ± 0.52	6.48 ± 0.55	6.22 ± 0.23*c
		Welded	7.41 ± 0.58	7.26 ± 0.15	7.08 ± 0.17	6.84 ± 0.56	6.25 ± 0.54	6.33 ± 0.22*c
	Omega-3-fish oil	Snap	7.52 ± 0.38	6.70 ± 0.47	6.22 ± 0.28*	5.22 ± 0.26**	4.87 ± 0.16***	4.78 ± 0.26***a
		Welded	7.36 ± 0.36	6.91 ± 0.25	6.78 ± 0.18	6.28 ± 0.15*	5.47 ± 0.12**	5.28 ± 0.15**b
Smearcase	Control	Vacuum	3.16 ± 0.46	2.75 ± 0.32	2.44 ± 0.32	2.07 ± 0.46	2.00 ± 0.47	1.90 ± 0.17*a
		MAP	3.20 ± 0.52	2.92 ± 0.17	2.82 ± 0.23	2.67 ± 0.35	2.31 ± 0.46	2.18 ± 0.12*a
	Inulin	Vacuum	3.13 ± 0.57	2.95 ± 0.15	2.93 ± 0.16	2.74 ± 0.26	2.40 ± 0.46	2.58 ± 0.35 b
		MAP	3.06 ± 0.32	3.14 ± 0.16	3.16 ± 0.22	2.89 ± 0.17	2.65 ± 0.42	2.52 ± 0.41 b
	Omega-3-fish oil	Vacuum	3.10 ± 0.53	2.77 ± 0.15	2.58 ± 0.17	2.18 ± 0.51	2.04 ± 0.17*	2.00 ± 0.14*a
		MAP	3.04 ± 0.46	2.86 ± 0.22	2.81 ± 0.16	2.60 ± 0.14	2.28 ± 0.55	2.21 ± 0.12 ab

Data are mean of lg values of colony forming units counted on agar plates in triplicates. SEM values indicate the error of trial.
* Marks significant difference (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) from the starting control within a row.
Different letters mark significant differences ($P < 0.05$) among the variants of a given product after 40 days of storage.

Los datos son las medias de los valores lg de las unidades que forman colonias, contadas en placas de agar por triplicado. Los valores SEM indican el error de la prueba.
* Indica una diferencia significativa (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) a partir del control de inicio, en una misma fila.
Las distintas letras indican diferencias significativas ($P < 0.05$) entre las variantes de determinado producto después de 40 días de almacenamiento.

decrease of LAB number started earlier than for the welded closed one. Similar phenomenon was observed in the case sheep milk yoghurt and kefir, as well as in goat milk yoghurt and kefir fortified with fish oil. It seems that the welded closure could slow down the reduction of LAB numbers and the fish oil fortification had no effect on this process. At the same time, the added inulin could protect the number of LAB for a much longer period independently from the type of closure. There should be smaller amount of air below the snap closure than that of the welded one in the background of this phenomenon, which could be exploited by LAB much quickly.

3.2. Alterations of fatty acid content during the storage

Significant changes occurred in the composition of free fatty acids during the 40-day-long storage in terms of all the examined fatty acids and the factors. Addition of inulin or

fish oil influenced the ratio of the investigated fatty acids to the largest extent, while pattern of packaging exerted the least impact on this parameter. As a result of addition fish oil, the ratios of the given (examined) fatty acids in terms of the fat content have been significantly raised in nearly all cases. Largest increase was observed in case of C18:2(n-6) in yoghurt and kefir whose ratio was doubled. This parameter exhibited considerable increase in the control sample throughout the storage (Tables 3 and 4).

Smaller alterations were detected for cheese manufactured with the addition of fish oil. Ratio of C18:3(n-3) was raised to smaller extent compared to that of C18:2(n-6). The ratio of C18:1trans-9 fatty acid was also considerably enhanced in yoghurt and kefir, while the ratio C16:0 changed notably just as a result of treatment with fish oil. Addition of inulin led to enhancement of the ratio of C18:2(n-6) independently of both the kind of the animal species and the packaging method. It is noteworthy that in cases of yoghurt and kefir greater changes occurred due to the influence of inulin than as a result of fish oil addition. Analogous changes were observed for yoghurt and kefir, while in case of smearcase the same tendencies prevailed with smaller quantity changes. In case of the previous product the extent of C18:2(n-6) fatty acid's quantity enhancement never reached 100%.

Impact of difference between milks deriving from the examined two distinctive animal species could be experienced in the free fatty acid composition of the smearcase variants, although the tendency and the extent of changes in yoghurt and kefir were similar. It is noteworthy that the ratio of C16:0 was increased by 3% in cases of sheep yoghurt and kefir fortified with fish oil, while analogous products made from goat milk displayed 10% enhancement of the same parameter. Ratio of C18:2(n-6) fatty acid was significantly increased in cases of products manufactured from fortified sheep milk, whilst statistically interpretable alterations could be observed in cases of the goat milk-based products just for the MAP-packaged control sample and the MAP-packaged experimental sample with fish oil addition.

4. Discussion

The effect of the studied factors exhibited considerable alterations regarding both the microbial and the chemical composition of the test products. Amongst the investigated microbes, lactic acid bacteria (LAB) were detectable in the products during the storage period supporting the need for a stronger pasteurization. It was apparent, however, that the number of LAB was significantly decreased by the end of the storage in cases of the examined products. Although the positive influence of inulin on the survival of probiotic bacteria in milk products has been authentically confirmed, its effect on the starter microbes is rarely studied. In our trial, we found that no significant changes occurred in LAB numbers till day 8. The cold storage temperature (8°C) and the applied packaging materials led to strong retardation of the metabolism, as the water loss steming from the packaging process and the deficiency of oxygen supply prevented both the proliferation and the decline of the starter microbes (Kiss et al., 2014). From day 16 of the storage a moderate, gradual decrease was observed in terms of the number of LAB, that was considerably retarded by the addition of inulin; however, the presence of fish oil did not much affect the process. Inulin apparently acts as a beneficial nutrient for LAB in these products leading to their enhanced survival. In an earlier work, we observed the fast proliferation of yeasts coupled with a rapid decrease of LAB population in similar functional foods made from moderately heated milks (Kiss et al., 2014). Fermented products and cheese showed marked loss of LAB with 2–5 days earlier than in the present study. Now we observed a slower decrease of LAB number in the control of all the three products. It became significant at day 16 in yoghurt and at day 24 in kefir. At day 40 the population of LAB was lowered by 2.5–3 orders of magnitude compared to the starting day. The number of LAB decreased somewhat slower in cheese, which showed markedly different population at day 32.

Earlier it was detected (Kiss et al., 2014), that the snap closure of plastic cups supported the proliferation of contaminant yeasts causing increased devastation of LAB due to better oxygen supply compared to the welded closure of cups. We suppose that the stronger pasteurization might perish the contaminant bacteria and decrease the importance of the oxygen supply.

On the other hand, we did not notice the positive effect of inulin in cheese, in accordance with the examination of Rodrigues et al. (2012), in which they found no significant correlation between the added inulin to cow cheese and the survival of *Lactobacillus casei*. In both cases, the primary reason might be that these bacteria were able to maintain the initial germ numbers in the control, but they were unable to utilize inulin, therefore the population could not increase.

Lipid content of milk and milk products is an important source of fatty acids. Among them, linoleic acid and α-linolenic acid are considered essential because human body cannot synthesize them. These fatty acids are precursors of eicosapentaenoic acid and docosahexaenoic acid that play key role in homeostasis regulation. During our work, we investigated the changes in four fatty acids. C18:3(n-3) (α-linolenic acid) and C18:2(n-6) (linoleic acid) are well-known representatives of omega-3 and omega-6 unsaturated fatty acids having protective role against a series of degenerative chronic diseases (Saini & Keum, 2018). To follow the rate of lipolysis, C16:0 (palmitic acid) was measured as it is the major fatty acid component of milk fat (Srbinovska & Dushica, 2018). Milk products are among the sources of trans fatty acids that may contribute to the development of inflammations, cardiovascular diseases, type 2 diabetes as well (Tardy et al., 2011). C18:1 trans-9 (elaidic acid) content of test products was also followed to check the potentially adverse changes in fatty acid composition.

Addition of fish oil used for increasing the amount of omega-3 fatty acids may play a crucial role in the process leading to changes in the fatty acid composition of milk products. The importance of lactic acid bacteria is confirmed by the experimental evidence that in cases of yoghurt or kefir with LAB numbers of 10^7 and 10^9, respectively, made with fermentation technology, significant changes in fatty acid composition can more frequently be observed than for the smearcase. Lactic acid bacteria are capable of synthesizing unsaturated fatty acids as described by Rodríguez-Alcalá, Braga, Malcata, Gomes, and Fontecha (2011). Rodrigues et al. (2012) found that the addition of inulin can lead to even a multiple elevation of the concentration of free CLA in symbiotic cheese.

If we compare the effect of the difference between the two investigated animal species, similar trends are observed for the goat milk products and the sheep's milk products. As

Table 3. Rate of four free fatty acids in the sheep milk products after 40 days of storage.

Tabla 3. Tasa de cuatro ácidos grasos libres en la leche ovina después de 40 días de almacenamiento a 8°C.

			Rate of free fatty acids at the start (Day 1) and the end (Day 40.) of storage at 8 °C (% of fat w/w)							
			C16:0		C18:3(n-3)		C18:2(n-6)		C18:1 trans-9	
Product	Amendment	Closure	1.	40.	1.	40.	1.	40.	1.	40.
Yogurt	Control	Snap	16.3 ± 0.77	14.1 ± 0.63*	4.50 ± 0.76	6.77 ± 0.49**	3.22 ± 0.36	4.01 ± 0.29	2.23 ± 0.19	2.46 ± 0.18
		Welded	16.4 ± 0.76	14.5 ± 0.84	4.60 ± 0.49	6.48 ± 0.53*	3.19 ± 0.33	3.97 ± 0.37	2.25 ± 0.17	2.50 ± 0.19
	Inulin	Snap	15.9 ± 0.69	14.6 ± 0.79	4.66 ± 0.55	8.14 ± 0.69***	3.31 ± 0.35	6.73 ± 0.32***	2.19 ± 0.15	1.93 ± 0.18
		Welded	15.7 ± 0.68	14.4 ± 0.62	4.71 ± 0.57	8.93 ± 0.71***	3.28 ± 0.29	6.89 ± 0.34**	2.22 ± 0.21	1.95 ± 0.16
	Omega-3- fish oil	Snap	16.8 ± 0.58	19.1 ± 0.69*	6.73 ± 0.70	8.45 ± 0.76*	4.11 ± 0.34	8.23 ± 0.32***	2.30 ± 0.16	3.13 ± 0.17***
		Welded	16.4 ± 0.81	18.6 ± 0.89*	6.54 ± 0.47	8.14 ± 0.51**	4.29 ± 0.38	7.89 ± 0.21***	2.27 ± 0.12	3.42 ± 0.26***
Kefir	Control	Snap	15.8 ± 0.64	15.2 ± 0.53	4.34 ± 0.56	6.52 ± 0.52**	3.11 ± 0.24	4.04 ± 0.42	2.17 ± 0.16	2.34 ± 0.17
		Welded	16.0 ± 0.86	14.3 ± 0.55	4.39 ± 0.50	6.77 ± 0.59**	3.21 ± 0.43	4.12 ± 0.39	2.21 ± 0.13	2.42 ± 0.23
	Inulin	Snap	16.9 ± 0.89	15.0 ± 0.98	4.23 ± 0.61	8.42 ± 0.60***	3.02 ± 0.37	7.65 ± 0.32***	2.30 ± 0.12	1.93 ± 0.12*
		Welded	16.5 ± 0.85	15.2 ± 0.66	4.37 ± 0.57	8.33 ± 0.67***	3.17 ± 0.43	7.42 ± 0.32***	2.28 ± 0.09	1.95 ± 0.12*
	Omega-3- fish oil	Snap	16.3 ± 0.68	19.9 ± 0.72*	6.49 ± 0.47	8.01 ± 0.62*	4.30 ± 0.39	8.27 ± 0.42***	2.30 ± 0.11	3.29 ± 0.24***
		Welded	16.4 ± 0.57	20.3 ± 0.62**	6.44 ± 0.49	8.14 ± 0.68*	4.27 ± 0.34	8.17 ± 0.38***	2.26 ± 0.18	3.40 ± 0.25***
Smearcase	Control	Vacuum	17.4 ± 0.64	18.3 ± 0.68	5.10 ± 0.57	6.01 ± 0.53	3.87 ± 0.42	4.14 ± 0.33	2.42 ± 0.16	2.59 ± 0.19
		MAP	17.8 ± 0.59	18.3 ± 0.72	5.03 ± 0.61	5.87 ± 0.44	3.93 ± 0.32	4.63 ± 0.41	2.40 ± 0.09	2.55 ± 0.12
	Inulin	Vacuum	17.0 ± 0.85	18.4 ± 0.73	5.22 ± 0.55	6.16 ± 0.67	3.77 ± 0.35	4.97 ± 0.31*	2.47 ± 0.18	2.29 ± 0.17
		MAP	16.8 ± 0.75	18.1 ± 0.69	5.28 ± 0.58	6.23 ± 0.67	3.68 ± 0.37	5.13 ± 0.42**	2.40 ± 0.09	2.36 ± 0.15
	Omega-3- fish oil	Vacuum	16.5 ± 0.59	21.3 ± 0.54**	7.02 ± 0.72	10.0 ± 0.89*	4.22 ± 0.33	7.14 ± 0.35***	2.36 ± 0.11	2.21 ± 0.18
		MAP	16.5 ± 0.79	22.4 ± 0.86***	6.95 ± 0.69	10.8 ± 0.84**	4.34 ± 0.42	6.89 ± 0.47**	2.33 ± 0.12	2.18 ± 0.18

Values obtained at day 40. Marked with asterisk are significantly (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) different from values of day 1. of the same treatment [Valores obtenidos en el día 40. Los valores marcados con asterisco son significativamente diferentes (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) de los obtenidos el día 1 del mismo tratamiento].

Table 4. Rate of four free fatty acids in goat milk products after 40 days of storage at 8°C.

Tabla 4. Tasa de cuatro ácidos grasos libres en leche caprina después de 40 días de almacenamiento a 8°C.

			Rate of free fatty acids at the start (Day 1) and the end (Day 40.) of storage at 8 °C (% of fat w/w)							
			C16:0		C18:3(n-3)		C18:2(n-6)		C18:1 trans-9	
Product	Amendment	Closure	1.	40.	1.	40.	1.	40.	1.	40.
Yogurt	Control	Snap	14.8 ± 0.77	13.2 ± 0.64	8.62 ± 0.74	10.8 ± 0.46*	3.42 ± 0.38	4.17 ± 0.39	2.37 ± 0.29	2.43 ± 0.18
		Welded	14.3 ± 0.74	13.5 ± 0.74	8.67a ± 0.43	11.2 ± 0.52**	3.43 ± 0.38	3.81 ± 0.39	2.45 ± 0.17	2.50 ± 0.29
	Inulin	Snap	13.9 ± 0.69	12.6 ± 0.79	8.17a ± 0.51	11.4 ± 0.65**	3.19 ± 0.32	6.78 ± 0.32***	2.29 ± 0.15	2.03 ± 0.21
		Welded	14.5 ± 0.61	12.2 ± 0.42*	8.41a ± 0.54	10.9 ± 0.72*	3.44 ± 0.26	6.84 ± 0.32***	2.28 ± 0.11	1.95 ± 0.12*
	Omega-3- fish oil	Snap	15.8 ± 0.53	24.1 ± 0.62***	10.6 ± 0.72	12.8 ± 0.76*	4.73 ± 0.32	9.14 ± 0.35***	2.36 ± 0.18	3.83 ± 0.17***
		Welded	15.7 ± 0.71	24.9 ± 0.9***	10.2 ± 0.44	12.3 ± 0.56*	4.69 ± 0.36	9.69 ± 0.26***	2.29 ± 0.12	3.92 ± 0.26***
Kefir	Control	Snap	14.0 ± 0.61	13.2 ± 0.51	7.34 ± 0.51	10.7 ± 0.52*	3.62 ± 0.26	4.43 ± 0.48	2.27 ± 0.16	1.64 ± 0.15**
		Welded	14.0 ± 0.82	12.7 ± 0.57	7.77 ± 0.54	11.6 ± 0.53*	3.51 ± 0.43	4.35 ± 0.59	2.21 ± 0.13	1.66 ± 0.11**
	Inulin	Snap	13.7 ± 0.79	12.0 ± 0.97	8.13 ± 0.64	12.4 ± 0.65*	4.02 ± 0.34	7.35 ± 0.32**	2.34 ± 0.12	2.03 ± 0.09*
		Welded	13.5 ± 0.84	12.2 ± 0.68	8.37 ± 0.54	13.0 ± 0.62**	4.17 ± 0.45	7.71 ± 0.39**	2.26 ± 0.09	2.05 ± 0.27
	Omega-3- fish oil	Snap	13.5 ± 0.66	22.3 ± 0.72***	8.49 ± 0.41	15.1 ± 0.67***	4.00 ± 0.32	8.72 ± 0.41***	2.39 ± 0.15	3.79 ± 0.27***
		Welded	13.0 ± 0.55	23.0 ± 0.66***	7.94 ± 0.44	14.8 ± 0.62***	4.27 ± 0.32	8.37 ± 0.38***	2.29 ± 0.18	3.71 ± 0.22***
Smearcase	Control	Vacuum	14.2 ± 0,61	14.3 ± 0,64	6.10 ± 0,54	6.00 ± 0,58	3.66 ± 0,41	4.10 ± 0,43	2.48 ± 0,14	2.59 ± 0,19
		MAP	14.8 ± 0,54	15.3 ± 0,63	6.00 ± 0,62	5.83 ± 0,41	3.30 ± 0,31	4.63 ± 0,31*	2.50 ± 0,19	2.50 ± 0,17
	Inulin	Vacuum	15.0 ± 0,65	15.4 ± 0,72	6.55 ± 0,55	6.33 ± 0,61	3.11 ± 0,55	4.38 ± 0,61	2.37 ± 0,16	2.39 ± 0,17
		MAP	14.4 ± 0,71	16.1 ± 0,81	6.28 ± 0,52	6.23 ± 0,69	3.83 ± 0,37	4.13 ± 0,32	2.38 ± 0,19	2.46 ± 0,15
	Omega-3- fish oil	Vacuum	14.1 ± 0,55	18.3 ± 0,52**	6.32 ± 0,22	7.10 ± 0,74	4.14 ± 0,53	5.14 ± 0,55	2.36 ± 0,15	2.21 ± 0,16
		MAP	14.5 ± 0,72	19.1 ± 0,81**	6.64 ± 0,61	7.81 ± 0,80	4.37 ± 0,32	5.69 ± 0,27*	2.38 ± 0,12	2.13 ± 0,11*

Values obtained at day 40. marked with asterisk are significantly (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) different from values of day 1. of the same treatment.

Valores obtenidos en el día 40. Los valores marcados con asterisco son significativamente diferentes (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$) de los obtenidos el día 1 del mismo tratamiento.

one of the distinctions, it was found that in yogurt and kefir enriched with fish oil the ratio of free C16:0 increased to a greater extent: in the case of goat milk products the growth rate was almost 10%, while for the sheep milk products only 3%. Based on the comparison of goat and sheep milks' physico-chemical characteristics by Park, Juárez, Ramos, and Haenlin (2007) there might be two reasons for assuming this.

On the first hand, the goat milk has somewhat higher C16: 0 fatty acid content, than the sheep milk. On the second hand the fat drops of goat milk are significantly smaller than that of the sheep milk, therefore they are much more exposed to damages generated by homogenization and other physical effects. Thus the induced lipolysis can be more pronounced in goat milk products. In this process, microbes play an important role, as in case of cheese of less germ number this difference was not to be observed. Interestingly, this difference was not noticed for the growth rate of the three other studied fatty acids. Conversely, in case of inulin treatment it was observed that the amount of C18: 3 (n-3) in kefir made of sheep milk was considerably less increased compared to the goat milk product. It is a noteworthy phenomenon that in kefir's control sample a significant decrease was experienced in the quantity of C18:1 (t9) free fatty acid. Changes in the fatty acid composition of smearcase made from sheep milk display some differences in comparison with that of the goat cheese. In the previous case, a slight increase in the proportion of unsaturated fatty acids was observed just for the product manufactured with fish oil and MAP packaging method. However, these differences cannot be unambiguously explained by the well-known differences between the milks of the two animal species. Additional tests need to be performed for the exploration of the background of these differences.

In case of treatment with fish oil, the increased extent of lipolysis could result in the enhancement of the free unsaturated fatty acid content. Ratio of C16:0, representing the amount of measured saturated fatty acids to follow the rate of lipolysis, did not change during storage of sheep yoghurt control and its inulin containing variant. Similar experiences were reported by Rodrigues et al. (2012). In contrast, approximately 13% increase was observed in case of the product variant prepared with fish oil addition. Nouira, Park, Guler, and Terrill (2011) found that in the cheese made from milk of reduced-fat content, proportionately more free fatty acids were formed during the storage than in the full fat cheese variant. This finding was explained by the possibly damaged membrane of the fat droplets as a result of the milk fat separation and the subsequent technological factors.

As a consequence the milk's own lipolytic enzymes and the similar microbial enzymes in the product had a better access to the lipid molecules, thus the extent of the lipolysis was significantly higher. Authors (Akoh & Min, 2002; Dhankhar, 2014; Elias-Argote, 2011; Kiełczewska, Kruk, Czerniewicz, & Haponiuk, 2006; Thierry et al., 2017) agree in the conclusion that the higher the pressure and the temperature are applied during the homogenization, the smaller fat droplets are formed. Along with this, the amount of the fatty acids released from the membrane of fat droplets will be higher in the milk, which is more exposed to enzymatic effects during the lipolysis. These observations support our experiences, but also raise the need to study the fatty acid composition in the samples after homogenization and before the addition of the extra components as well throughout our next experiments. This might be a relevant intermediate step in terms of success of further product development activities.

5. Conclusions

Free fatty acids play an important role in both utilization prospects of sheep and goat milk for functional foodstuffs and tailoring their organoleptic quality. Application of inulin and fish oil comprising omega-3 fatty acid exerted much influence on the free fatty acid composition of the products. Fortification of the products with inulin resulted in an enhanced survival of lactic acid bacteria and facilitated production of unsaturated fatty acids providing an excellent opportunity to increase the functionality of fermented milk products.

Addition of fish oil comprising omega-3 contributed to the enhancement of the extent of lipolysis during storage. Based on these facts we think that it is more favourable to supplement the fermented products with prebiotics, than to apply oils containing unsaturated fatty acid. It requires further investigations to reveal the background of the differences in the behaviour of sheep and goat milk products in some cases, and at the same time our next studies will be finished with organoleptic examinations of the products.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The present work was funded by the National Research, Development and Innovation Office within the FUNKMILK project no. [TECH_08_A3/2-2008-0431].

ORCID

Iman Mirmazloum http://orcid.org/0000-0002-1461-0382

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