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DEVELOPMENT OF FUNCTIONAL SHEEP AND GOAT MILK PRODUCTS AND THE INVESTIGATION OF FUNCTIONALITY IN VARIOUS PACKAGING MATERIALS

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Abstract

Small mammalian's milk products can serve as the basics of development of functional foodstuffs whose bioactive compounds are well preserved and the biological uptake is promoted in these media.

Sheep and goat milk products (yogurt and smearcase) amended with omega-3 containing oil and inulin were developed in this study. Besides different packaging means we were investigating hygienic status and plausible changes in functionality throughout storage. Yogurt products were filled in polystyrene or polypropylene bottles with in-bottle or in-tank congealing. Smearcase products were packed in shrink foil or in modified atmosphere. Products were amended with 3% inulin and 0.9% omega-3 fatty acid containing oil. Prototypes were stored for 35 days at 5-8 ^{0}C and samples were taken weekly to follow the changes of 6 microbes (*Escherichia coli*, coliforms, *Enterococcus faecalis, Staphylococcus aureus,* moulds, lactic acid bacteria) by cultivation techniques. The amounts of 11 fatty acids were assessed at the start and the end of storage by using gas chromatography.

Significant differences were found in hygienic parameters of products made from the same milk-type, but the amount of functional compounds remained almost unchanged. The proliferation of budding yeasts was predominant in the microbiota of yogurt but the addition of omega-3 containing oil decreased it to tenfold lower value. Inulin amendment did not influence the composition of the microbiota. Fatty acid composition of products did not change markedly during the storage, just tridecane and docozane acids were not detectable after 35 days. Snap closured cup gave 6-times higher microbial count in yogurt than the welded one, but it caused no difference in the presence of risk indicator microbes. In case of smearcase application of modified atmosphere did not result in any benefit compared to the shrink foil packaging.

Major outcome of study was the development of two different functional milk based product with monitored preserved bioactive compound content. Amounts of beneficial compounds were not decreased significantly throughout 35 days of storage, thus potential physiological impact should be retained. Welded cup closure was more favourable for storage as it promotes the proliferation of bacteria to the least extent.

***Key words**: Sheep and goat milk products, Added omega 3 fatty acid, Increased functional values, Various packaging materials.*

1. Introduction

Small ruminant's milk products are well known alternatives to cow milk as they have high digestibility and nutritional value (Jandal, [1]). Furthermore goat milk is very useful for people who are suffering from different problems such as acidity, eczema, asthma, migraine, colitis, stomach ulcer, etc. (Silanikove *et al*. [2]). They can serve as the basics for development of functional foodstuffs whose bioactive compounds are well preserved and the biological uptake is promoted in these media. Research has been carried out to develop goat yogurt comprising plant-based material as components of high added value. Zare *et al*. [3] argued that products like yogurt provide good opportunities for the development of fiber-enriched foods whose acceptability by consumers is mainly based on satisfactory textural and sensory quality parameters. However, inulin as a soluble fiber is applied not only for sensory but for functional reasons because it is among the most frequently applied prebiotic compounds in the formulation of functional foods (Buriti *et al*. [4]). 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*. [5]).

There have been some attempts dealing with incorporation of fish oil into the feed of ruminants for increasing the unsaturated fatty acid content of milk resulted in poor transformation ratios varying between 0.3 - 1,12% (Kitessaa *et al*. [6]). Recently a new trend appeared to enhance the milk lipid profile that has prompted the dairy industry to develop new products enriched with omega-3, omega-6 fatty acids and other components with potentially positive effects on human health. Such is the case with some new dairy products, in which milk fat is partly replaced with vegetable fat or a mixture containing fish oil. This would increase the levels of omega-3 fatty acids, with a consequent benefit of prevention of cardiovascular disease (Li *et al*. [7]).

However, the supplements applied may interact with the microbiota of the products that may result in decreased amount of functional compounds. Although the addition of prebiotic compounds FOS alone or combined with inulin was not found to affect significantly the probiotic strain growth and viability during the manufacturing or the 60-day ripening period, the fatty acid content of the products was significantly enhanced during the ripening (Rodrigues *et al*. [12]).

The dominant part of goat or sheep milk produced is used in the production of cheese (Milani and Wendorff [8]. While application of goat or sheep milk for cheese making is well known, few attempts have been made to systematically study the use of goat or sheep milk to manufacture milk beverages, ice cream, butter, milk powder, condensed milk, traditional products, even yoghurt, etc. (Pandya and Ghodke [9]). Thus the increasing production of goat milk by around 70% between 1991 and 2011 (FAOSTAT, [10]) points to a promising future for this sector only if it is supported by increasing scientific research efforts in the field of small ruminant's milk processing for products other than cheese.

Piredda and Pirisi [11] have shown that it is difficult to describe the gross and detailed composition of sheep and goat milks. The great variability in milk quantity and composition is caused by genetic and physiological factors such as breed, individual characteristics, lactation stage, etc. Further elements of variability are linked to flock management, climate, altitude, the botanical composition of pasture, etc. Therefore we think that the detailed monitoring of changes in product composition with special emphasis on functional amendments during production and storage is very important.

To our knowledge, there is no such goat or sheep products on the market that have inulin or omega-3 containing oil as functional compounds, and the effect of packaging material and techniques on the functionality of milk products would have been assessed.

Therefore the aims of our work were to develop new sheep or goat milk based functional products amended with inulin or omega-3 containing oil, and to follow the quantitative changes of the functional compound and microbiota as well as the influence of different packaging on the functionality of food prototypes.

In the present study, sheep and goat milk products (yogurt and smearcase) amended with omega-3 containing oil or inulin were developed. Besides different packaging means were investigated for hygienic status and plausible changes in functionality throughout storage. Yogurt products were filled in snap closed polystyrene or weld closure polypropylene bottles with in-bottle or in-tank congealing. Smearcase products were packed in shrink foil or in modified atmosphere. Products were amended with 3% inulin and 0.9% omega-3 fatty acid containing oil. Prototypes were stored for 35 days at 5 - 8 ^{0}C and samples were taken weekly to follow the changes of 6 microbes (*Escherichia coli, coliforms, Enterococcus faecalis, Staphylococcus aureus*, moulds, lactic acid bacteria) by cultivation techniques. The amounts of 11 fatty acids were assessed at the start and the finish of storage by gas chromatography.

Major outcome of study was the development of two different functional milk based product with monitored and stable bioactive compound content. According to the results beneficial impact will not decreased significantly throughout 35 days of storage. Welded cup closure was more favorable for storage as it promotes the proliferation of bacteria to the least extent.

2. Materials and Methods

<u>Product development</u>

Raw sheep or goat milk was pasteurized and used for yogurt or smearcase processing with regular technologies. Inulin (Synergie-1 from Orafti) was applied as prebiotics at 30.0 g/L for two kinds of congealing. In-bottle fermentation was performed with amended milk whereas not amended milk was used for tank fermentation after inulin was mixed into the finished yogurt before cooling. Omega-3 fatty acid containing fish oil was applied in similar manner in 0.9 %.

The abovementioned functional bioactive compounds were mixed into the clot in the same ratios for smearcase production.

Bottle fermented yogurt variants were sealed with snap closure while variants produced in tank got welded closure. Smearcase products were stored in shrink foil with vacuum packaging or under modified atmosphere.

Storage

Changes in hygienic status and the content of functional compounds were monitored through a 35-day-long period at 5 - 8 ^{0}C storage in the dark. Samples were taken at the start and at the end of the experiments for chemical and weekly for microbiological investigations.

Investigations

Chemical analysis was carried out by HPLC with ELS detector for inulin content, and by gaschromatograpy for fatty acids (decane-, dodecane, tridecane-, pentadecane-, palmitoleic, hexadecane-, linolenic-, oleic-, T-9-octadecane-, stearic-, and docozane acid). 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. The cfu data were expressed in logarithmic numbers and ANOVA was applied for statistical test of the effect of packaging on the microbial status and the quantity of functional compounds.

3. Results and Discussion

3.1 Results

The products were made from the same milk sample, but significant differences appeared in two microbiological parameters mainly due to the packaging. Among the investigated microbes, only budding yeasts showed marked proliferation during the 35-day-long cold storage both in yogurt and smearcase (Fig. 1-2). It started from 1,75 lg cfu/g and continuously increased to 5,26 lg cfu/g in the snap closed control as a maximum.

In case of yogurt no difference was observed between the applied two production technologies, bottle and tank fermentation. Among the different packaging manners snap sealed closure facilitated the proliferation of yeasts to greater extent. In this respect no alterations were experienced when comparing sheep and goat milk based products. Number of germs in cases of the snap sealed closed products was found to be 6-times larger for the control, 7-time bigger for the inulin containing product, 3-times larger for the omega-3 amended product than in cases of application of welded closure. Hence it might be concluded that amendment with omega-3 rich oil led to the slight diminishment of proliferation of yeasts.

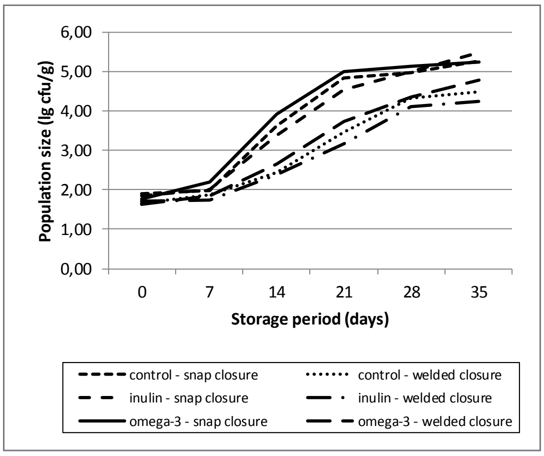

Figure 1. Yeast population size of sheep yogurt of different amendments and packaging

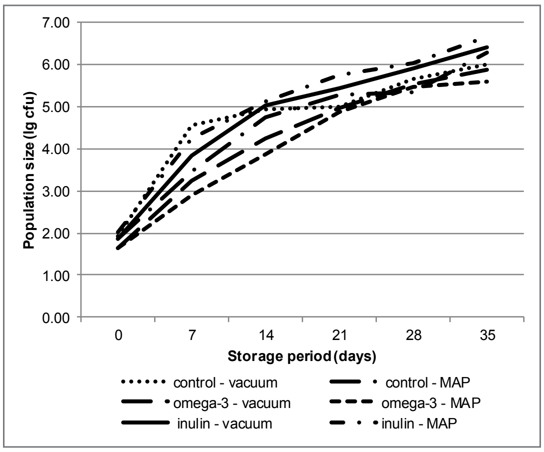

Figure 2. Yeast population size of differently amended and packed goat smearcase

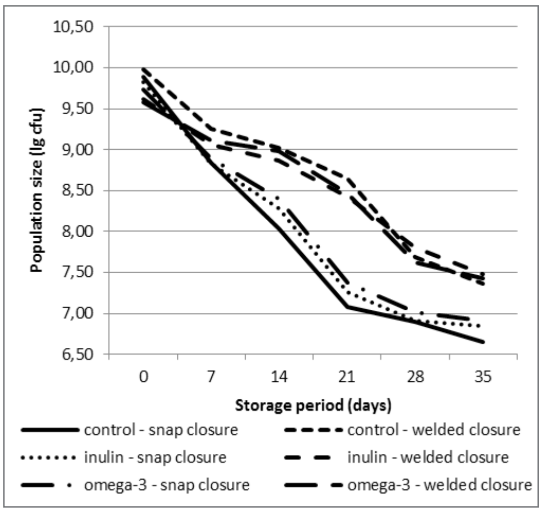

Figure 3. Lactic acid bacterial counts of sheep yogurt during 35-day-long storage at 4 ^{0}C

In cases of smearcase products the quality of the animal species did not exert impact on the proliferation of yeasts. No alterations were observed in the size of yeast populations of the products in comparison of application of vacuum shrink foil packaging and packaging under modified atmosphere. Yeast population of more than 10^6 cfu/g was formed in case of smearcase, which is larger by one order of magnitude than that of the yogurt products.

Number of lactic acid bacteria in yogurts decreased significantly subsequent to 35 days of storage at lowered temperature. The extent of decrease is largely affected by the type of packaging. Application of welded closure proved to be favorable, as resulted in germ number bigger by approximately one order of magnitude.

Number of indicator microbes designating the hygienic state (*Escherichia coli, coliforms, Enterococcus faecalis, Staphylococcus aureus*) did not change suggesting food safety problems regarding the examined products.

Quantity of inulin as functional additive was not changed in any of the products throughout the processing and the storage. Omega-3 content of the products displayed small, not significant fluctuations. Change of the relative quantity of linoleic acid is the consequence of modification of the amounts of other fatty acids, primarily hexadecanic acid (Table 1 - 2). It is to be noted that there is a great difference in this respect between the milk of the two animal species. Ratio of hexadecanic acid in yogurt manufactured from sheep milk increased by 9.07 - 10.39% throughout the storage, while in case of yogurt manufactured from goat milk opposite tendency was observed recording -8.79 – -9.87% change. As significant change was found just in case of application of snap closure, the alteration observed might possibly be explained by the proliferation of yeast germs. Further studies might provide us with interpretation of the diversity in terms of animal species.

3.2 Discussion

In the present study new sheep and goat milk based functional food prototypes were developed by amending with inulin as prebiotic or omega-3 fatty acid containing fish oil as an anti-atherosclerotic compound. The changes in microbiological status and in the amount of functional additives were followed through 35 days long cold storage. It was stated that neither inulin nor omega-3 components did interact with microbial or chemical components of the products, as their amounts remained at the initial level till the end of the trial. Rodrigues *et al*. [11] found similar tendency during symbiotic product development for inulin. Furthermore they found that the prebiotic amendments may serve as preserving agents against the lipolysis of the conjugated linoleic acid (CLA) in cheese. Contrary, Raynal-Ljutovaca *et al*. [13] stated that ratios of C16:0, C18:1trans, C18:3n–3 and total fatty acids were similar for milk and cheese of sheep and goat. The changes in fatty acid composition during the storage can be different in sheep yogurt. Serafeimidou *et al*. [14] found that CLA content of sheep yogurt increased during 14 days of storage at 5 ^{0}C.

Table 1. Changes in the fatty acid composition of sheep milk yogurt during 30 days of storage*

Fatty acids	Control		Inulin amendment		Fish oil amendment	
	Snap closure	Welded closure	Snap closure	Welded Closure	Snap closure	Welded Closure
C10:0	0.38	-0.59	0.4	-0.62	0.51	0.63
C12:0	0.66	-1.18	0.7	-1.25	0.32	0.71
C13:0	0.01	1.35	0.02	1.43	0.02	-2.47
C15:0	-3.38	-1.35	-3.58	-1.48	-3.68	-2.52
C16:1	-0.06	-1.8	-0.07	-1.68	-0.07	-1.53
C16:0	9.07**	3.19	9.61**	2.27	10.39**	2.33
C18:3(n-3)	0.47	1.98	0.76	2.09	0.78	2.15
C18:2(n-6)	-5.35	-0.91	-5.8	-0.02	-5.97	0
18:1 trans-9	0.01	-0.01	0	-0.02	0	-0.05
C18:0	-3.02	-1.23	-3.2	-1.3	-3.49	1.34
C22:0	1.21	0.55	1.16	0.58	1.19	-0.59

*Values are differences between the percent rates of the given fatty acid measured at day 0 and 30 within the fat content.
**Data of which absolute value were above 7,63 are treated as significant changes (P < 0.05).

Table 2. Changes in the fatty acid composition of goat milk yogurt during 30 days long storage

Fatty acids	Control		Inulin amendment		Fish oil amendment	
	Snap closure	Welded closure	Snap closure	Welded Closure	Snap closure	Welded Closure
C10:0	-0.37	-0.57	-0.38	-0.58	-0.48	-0.59
C12:0	-0.64	1.14	-0.66	1.18	-0.34	-0.67
C13:0	0	-1.31	-0.19	-1.35	-0.02	2.34
C15:0	3.27	1.3	3.04	1.4	3.49	2.39
C16:1	0.05	1.74	7	1.59	0.06	1.45
C16:0	-8.79**	-3.09	-9.12**	-2.15	-9.87**	-2.21
C18:3(n-3)	-0.46	-1.9	-0.72	-1.98	-0.78	-2.04
C18:2(n-6)	5.18	0.88	5.51	0.02	5.67	0
C18:1 trans-9	0	0.87	-2.18	0.09	0	0.04
C18:0	2.92	1.47	0.04	1.23	3.31	-1.27
C22:0	-1.16	-0.53	-2.34	0.55	-1.04	0.56

*Values are differences between the percent rates of the given fatty acid measured at day 0 and 30 within the fat content.
**Data of which absolute value were above 6,89 are treated as significant changes ($P < 0.05$).

We could not detect similar changes in fatty acid composition in cases of neither sheep nor goat milk yogurt. Weak, not significant variability was observed in C18:3n–3 rate, but it was mainly caused by the shift of the rate of C16:0 fatty acid. This phenomenon appeared only in case of applying the snap closed bottles in which budding yeasts became predominant microorganisms during the storage. However opposite tendency was found for the yogurt of the investigated two animal species: yeasts increased in sheep milk yogurt but significantly decreased in the goat milk one. Changes in CLA content of sheep and goat yogurt exhibited variations. The latter did not show any alterations during the storage, whilst in case of goat yoghurt slight modifications were observed in the CLA content. We assume that the distinct lipid composition of sheep and goat milk could be the main reason for this phenomenon. The budding yeast are the putative agents contributing to the changes in fatty acid composition because Boutrou and Gueguen [15] found that C18:1 is preferentially liberated by lipase of *Geotrichum candidum* which is frequent spoilage fungus of milk products and no other microbes did proliferate to the similar high rate than yeasts. Further research is needed to clarify the mechanisms through yeast can generate changes of fatty acid profile of small ruminant's milk products.

4. Conclusions

- New sheep and goat milk based yogurt and smearcase products, amended with addition of inulin and omega-3 containing oil were developed and examined for storage behavior and preservation of functional character. The prototypes were packed in two different manners to check the effect of packaging on the microbiological status and the amount of bioactive compounds of the milk products.

- It was substantiated that the beneficial impact is not significantly decreased throughout the storage period of 35 days. Inulin and omega-3 components did not affect the microbial and chemical composition of the yoghurt products.

- Storage may have crucial impact on the fatty acid composition in sheep yogurt. For yoghurt, welded cup closure proved to be more favorable because it preserved the hygienic status of the product. For smearcase product, modified atmospheric packaging did not provide any benefit over the application of shrink foil storage in vacuum.

- Further research is nedded to clarify the background of the differences appeared in the fatty acid composition changes between sheep and goat milk yogurt, and the putative role of budding yeasts in this phenomenon.

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