INCORPORATING ESSENTIAL FATTY ACIDS FROM FISH OIL IN YOGURT

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Abstract

In this research was made yogurt from sheep's milk with added fish oil. To embed essential fatty acids in milk with added fish oil increasing percentage of 0.05%, 0.10% and 0.15% was subjected to homogenization at a pressure of 200 bar. and temperature of 70 ° C. Thus making the embedding fat fish oil in milk fat globule. Thereafter the process was the same as that for obtaining classic yogurt. Fatty acids of the control sample without addition and three samples with fish oil were analyzed by gas- chromatography. Evolution of fatty acids from samples was statistically analyzed by ANOVA method. Domain analysis and optimal concentration at which the incorporation of essential fatty acids in fat globule from product comparison method was performed by ROC curves (Receiver Operator Characteristic = Operating Characteristic) and were obtained following threshold values of the embedding theory three essential fatty acids studied in yogurt: 0.729% linoleic acid, linolenic acid and 1.382% and for γ-linolenic acid is not potting reaches concentrations up to 0.15% fish oil added to milk.

Key words: yogurt, sheep milk, fatty acids

INTRODUCTION

In recent years more and more sheep milk is used to manufacture acids products due to high fat and protein content (Ag-Ventures, 2000). This has a beneficial effect on consistency of product (A. Mohameed et al, 2004).

Sheep's milk yogurt has a significant biological value due to compounds that are formed from the fermentations taking place but also because it contains lactic acid bacteria.

Several studies have shown that were isolated from sheep milk bioactive peptides with inhibitory (ACE) (Hernandez-Ledesma et al, 2005), and the acidic dairy products (Gobbetti et al, 2000; Muguerza et al, 2006; Nakamura et al 1995).

Consumption of yogurt fermented with bacteria of the genus Lactobacillus helveticus containing tripeptide Ile-Pro-Pro and Pro-Val-Pro, the inhibitory role, has the effect of lowering blood pressure (Tuomilehto et al., 2004).

Were studied lately combination of lactic acid bacteria to increase the concentration of bioactive peptides from whey acidic dairy products (Jenn-Shou Tsai at al 2008) and due to the concentration of whey lactoglobulin were obtained in higher concentrations in bioactive peptides with enzyme

inhibitory role and effect of blood pressure lowering and beneficial role in gastrointestinal tract.

Enriching foods with essential fatty acids from fish oil (Alair Alfredo Berbert, 2005) decreased the risk of inflammatory diseases. Consumption of fish oil, rich in essential fatty acids increase the body's resistance against cardiovascular disease (Ka He, 2009) and against infections (Chen Wei, MD, 2010). ω-3 fatty acids and ω-6 from fish oil have role in preventing depression and other neural diseases (Sabine Riemer at al, 2010).

MATERIALS AND METHODS

To obtain yogurt enriched in essential fatty acids using mixed sheep from 3000 sheep in the first period of lactation, April. Added to milk in increasing percentages of 0.05%, 0.10% and 0.15% fish oil (purchased from the company "Hofigal"). To embed fatty acids from fish oil, milk was homogenized in three steps at a pressure of 20 bar. and temperature range 70 ° C. It was further made yoghurt after the classic technology (Costin GM, 2005, 2007), using selected lactic cultures manufacturing Ch Hansen, type YCX 11. 4 samples were obtained yogurt coded as follows: blank without fish oil: I_0, with the addition of 0.05%: $I_{0,\ 05}$, with the addition of 0.10% $I_{0,\ 10}$, and with the addition of 0.015% : $I_{0,\ 15}$. Technological process was considered completed after 7 days of storage under refrigeration at 4 ÷ 8 ° C. Samples were analyzed in terms of organoleptic seen by unauthorized persons 5. Fizco-chemically analyzed the evolution of acidity (according to ISO 6091/2008), fat percentage (according to S.T.A.S. 6352/2-87).

The focus was on the analysis of fatty acids were determined by gas-chromatography: milk fat was extracted by using the following protocol: about 1ml of milk samples were mixed with 0,6 ml ammonia 25%, 2ml EtOH, 4ml Ethyl ether and 4 ml hexane and then agitated for 2-3min. After this process the lower layer (the ammonia layer) was discarded. Following this step the mixture was passed through a cellulose filter with Na_2SO_4 and then brought to dryness; transesterification: fatty acids were converted to methyl esters by reaction with boron trifluoride/methanol at 80ºC for two hours in a closed Pyrex glass tube. The content was transfered into a separatory funell; the methyl ester extraction: the extraction was made using 10 ml hexane, the hexanic fractions collected were dried using anhydrous sodium sulfate, filtered, concentrated under a nitrogen stream and finally re-eluted in 1 mL hexane. Fatty acids were analyzed by gas chromatography (GC) with flame ionization detection (FID). A 1µL sample was injected into the Shimadzu GC-17A series gas-chromatograph, equipped with a 30m polyethylene glycol coated column (Alltech AT-WAX, 0.25mm I.D., 0.25µm film thickness). Helium was used as the carrier gas at a pressure of 147 kPa. The injector and detector temperatures were set

at 260ºC. For the oven temperature the following program was used: 70ºC for 2 min. then raised to 150ºC at 10ºC/ min. rate and held at 150ºC for 3min., then further raised up to 235ºC at a 4ºC/min; fatty acids were analyzed by gas chromatography (GC) with flame ionization detection (FID). A 1µL sample was injected into the Shimadzu GC-17A series gas-chromatograph, equipped with a 30m polyethylene glycol coated column (Alltech AT-WAX, 0.25mm I.D., 0.25µm film thickness). Helium was used as the carrier gas at a pressure of 147 kPa. The injector and detector temperatures were set at 260ºC. For the oven temperature the following programe was used: 70ºC for 2 min. then raised to 150ºC at 10ºC/ min. rate and held at 150ºC for 3min., then further raised up to 235ºC at a 4ºC/min.

Statistical validation fatty acids variation was made to the method ANOVA tests Tukey, Duncan and Dunnet, ROC curves comparison method (Receiver Operator Characteristic = Operating Characteristic) and Fourier analysis of the correlation type (Sipos, etc. C. 2004; Teuşdea A, et al, 2008; Teuşdea A., et al, 2008; Teusdea A, 2009).

RESULTS AND DISCUSSION

Organoleptic point of view in the blank were not identified foreign smell and taste samples and fish oil taste and smell of fish completely disappeared after 7 days at refrigerator temperature maintenance.

Physico-chemical results are not significantly different between samples.

Table 1.

Multiple comparisons tests I_0; $I_{0.05}$; $I_{0.10}$; $I_{0.15}$ yogurt by Tukey algorithm (HSD) / 95.00% for fatty acids detected chromatographic

Fatty acid	I_0~ $I_{0.05}$	I_0~ $I_{0.10}$	I_0~ $I_{0.15}$	$I_{0.05}$ ~ $I_{0.10}$	$I_{0.05}$ ~ $I_{0.15}$	$I_{0.10}$ ~ $I_{0.15}$
Caproic	0.00222646	0.00222646	0.00222646	1	1	1
Caprilic	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Capric	0.00222646	0.00222646	0.00222646	0.00222646	0.01076372	0.00222646
Lauric	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Miristic	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Miristoleic	0.01127178	0.00222646	0.00683047	0.00222646	0.00222646	0.00222646
Pentadecanoic	1	0.00222646	0.00327910	0.00222646	0.00327910	0.00222646
Palmitic	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Palmitoleic	0.00222646	0.00222646	0.00222646	0.00236522	0.00222646	0.00236522
Heptadecanoic	0.00222646	0.00222646	0.00222646	0.01275078	0.23156576	0.23156576
Cis-10 heptadecanoic	0.01491550	0.01491550	0.01491550	1	1	1
Stearic	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Oleic	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Elaidic	0.93426349	0.94377894	0.89738694	0.68040860	0.999506162	0.619804769
Linoleic ω6	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
Linolenic ω3	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646	0.00222646
γLinolenic ω6	0.00222646	0.0022264	0.00222646	0.00222646	0.00222646	0.00222646

Statistical analysis of samples using Tukey test for comparison between samples, caused significant differences in the proportion of 85.4% (Table 1) and using Dunnet test, comparison with the control sample, significant differences are at 100%, unless consider fatty acids that are very poorly detected and no interest for research (Table 2).

Table 2.

Multiple comparisons tests I_0; $I_{0.05}$; $I_{0.10}$; $I_{0.15}$ yogurt
by algorithm Dunnet / 95.00% for the chromatographic fatty acids detected.
Critical range test Dunnet: [-2.89061, +2.89061]

Fatty acids	$I_{0.05} \sim I_0$	$I_{0.10} \sim I_0$	$I_{0.15} \sim I_0$
Caproic	-354.37040	-354.37040	-354.37040
Caprilic	139.94668	169.31821	133.03574
Capric	-593.22740	-580.23702	-597.55753
Lauric	-223.32896	-205.17213	-283.24649
Miristic	-137.30381	-90.41958	-232.74670
Miristoleic	4.29478	-14.05564	-4.68521
Pentadecanoic	0.00000	8.81591	-5.28954
Palmitic	185.93630	57.88583	149.09986
Palmitoleic	17.05606	-6.39602	-12.79204
Heptadecanoic	12.60252	16.80336	14.70294
Cis-10-heptadecanoic	4.08248	4.08248	4.08248
Stearic	93.83149	202.25898	150.13038
Oleic	533.68924	502.41075	604.06584
Elaidic	0.58361	-0.55066	0.69186
Linoleic (ω6)	18.18275	56.56854	66.67007
Linolenic (ω3)	7.27607	24.25356	43.65641
γ-Linolenic (ω6)	7.94719	25.82838	65.56435

The analysis of chromatograms surface samples of yogurt and fish oil results in their growth with increasing concentration of fish oil samples, which shows that the percentage of fatty acids to total fatty acids, increase margins on oil samples fish, mainly (Table 3).

Table 3.

Comparison of chromatography surface (ua) of essential fatty acids in yoghurt

$I_{0,05}$	S(u.a.)	$I_{0,10}$	S(u.a.)	$I_{0,15}$	S(u.a.)	Ulei_peste	S(u.a.)
Linoleic	44.0512	**Linoleic**	46.0073	**Linoleic**	41.3040	**Linoleic**	64.2705
Linolenic	12.4874	**Linolenic**	16.0480	**Linolenic**	13.7005	**Linolenic**	178.0618
γ-Linolenic	11.6731	**γ-Linolenic**	14.0242	**γ-Linolenic**	11.0440	**γ-Linolenic**	40.2639

ROC curves use genuine comparison method, we determined the optimal threshold potting essential fatty acids in yogurt: Figure 1, linoleic acid; Figure 2, linolenic acid; Figure 3, γ-linolenic acid.

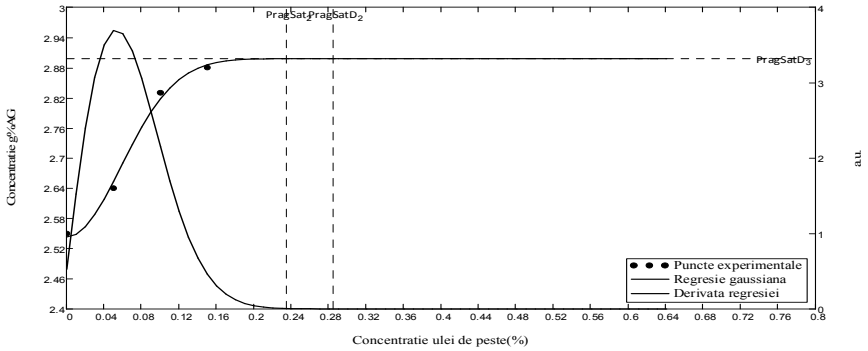

Figure 1. Asymptotic threshold determination linoleic acid potting in yogurt with transformed Gaussian function

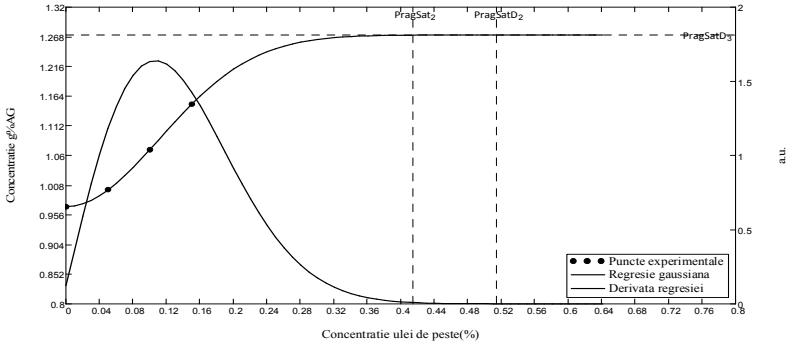

Figure 2. Asymptotic threshold determination linolenic acid potting in yogurt with transformed Gaussian function

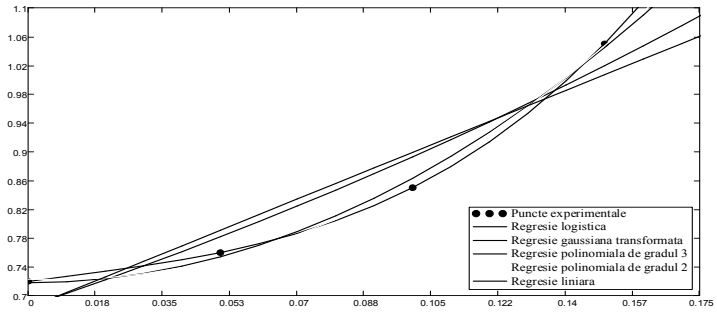

Figure 3. Regression functions studied for γ-linolenic acid of yogurt

In Figure 1 produces interval embedding linoleic acid in fat globule yogurt between 0.24 ÷ 0.28%, fish oil added to milk and linolenic acid (Figure 2) between 0.4 ÷ 0.5% fish oil added to the milk. For γ-linolenic acid was not reached maximum concentrations potting fish oil used. This anomaly is due to the warming milk homogenized at a temperature of 85 ° C. Fat globule membrane probably split heat and mergers fat globule of fat. After centralizing embedding threshold values of fatty acids in fat globule (Table 4), we see that they do not differ significantly depending on the function used.

Table 4.

Asymptotic threshold values potting essential fatty acids in yoghurt

Fatty acids	Embedding threshold (from regression values)	Embedding threshold (the derivative regression values)	Embedding threshold (theoretical)
linoleic	2.897	2.897	0.729
linolenic	1.270	1.271	1.382
γ-linolenic	threshold is not reached maximum embedding up to 0.15%		

By way ANOVA, Tukey test to determine the optimal concentration of fish oil that includes, in circumstances, in fat globule yogurt.

Table 5.

Fatty acid concentrations to a maximum of potting yogurt samples

Fatty acids	Conc. of fish oil(%)	Type asymptotic threshold
Linoleic	2.8978	the regression values
Linoleic	2.8979	regression of derivative values
Linoleic	0.7294	theoretical
Linolenic	1.2708	the regression values
Linolenic	1.2711	regression of derivative values
Linolenic	1.3825	theoretical

Table 6.

Number values, sample averages, standard deviation

Variable	No. value	No. valid values	No. invalid value	sum of weights	MD	SD
Conc. Fish oil (%)	6	6	0	6	1.742	0.924

Table 7.

Multiple comparisons of levels of essential fatty acids yogurt to embed the maximum produced by Tukey algorithm (HSD) / 95.00%. Tukey critical Vloarea statistics: 3927

Categories	Differences	Differences standardized	Critical value	Pr. > Dif.	significantly
Linoleic ~ Linolenic	0.867	1.198	2.776	0.297	No

Categories	MD	Groups
Linoleic	2.175	A
Linolenic	1.308	A

It is noted that there are significant differences between potting optimal thresholds, so they can consider the average concentration, 1.742% fish oil added to milk theoretical optimal limit, potting.

CONCLUSIONS

The study demonstrates that may be made from sheep's milk yogurt rich in essential fatty acids, the role of functional product. Additional studies are needed, it is recommended followed to maintain the high pasteurized and refrigerated for 24 hours to restore moisture and protein and fat globules in the end to carry incorporating essential fatty acids from fish oil in sheep milk with homogenization.

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