Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară Vol. XIII/A, 2014

# THE OPTIMAL RANGE OF EMBEDDING THE ESSENTIAL FATTY ACIDS FROM FISH OIL IN THE FAT GLOBULE OF FRESH CHEESE IN BRINE

#### Hîlma Elena\*

#### \*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania, e-mail: hilma\_elena@yahoo.com

#### Abstract

For the determination of the optimal percentage of fish oil is necessary to be added to the milk to set the limit of the optimal concentration of embedding three essential fatty acids which are representative of both the composition of the milk and fish oil. He watched the fish oil incorporation of milk fat globule so as to avoid loss of essential fatty acids in whey and better assimilation of their body, being known that milk fat is assimilated to 98% by it.

For incorporating fish oil into sheep's milk, raw material, was homogenisation 70 ° C and a pressure of 200 bar., When scission occurs fat globule membrane and the oil adheres to milk fat. Later on fat globule membrane is restored and includes essential fatty acids with fish oil.

For research we used four samples of cheese made from milk with added fish oil with added progressively as a percentage of 0.05%, 0.10% and 0.15%. Fatty acids analysis was performed by gas chromatography and their embedding degree globule of fat in cheese was determined statistically by Anova method and comparison method ROC curves (Receiver Operator Characteristic = Operating Characteristics). The following values were obtained for enclosing the theoretical the threshold: linoleic acid- 0.1600%; linolenic acid- 0.1716%; and for  $\gamma$ -linolenic acid- 0.4668%.

Key words: fat milk essential fatty acids

#### **INTRODUCTION**

For cheese in brine is used as raw milk, sheep's milk. Within fat globule of sheep milk were incorporated essential fatty acids from fish oil. Sheep's milk is considered healthy because of the high content of orotic acid. Orotic acid compounds have antitumor activity (Butour et al, 1997). Orotic acid is associated with potential liver cell recovery and prevention of liver lipolytic effect. It also has a role in decreasing body fat (Versiani others, 2008).

It is known that sheep milk is not only important for the metabolism of the liver but also to cover the magnesium. The present study aims to enrich the product in essential fatty acids from fish oil. In the modern world is putting increasing emphasis on the role of essential fatty acids in the human body. The two key functions of the pineal gland, melatonin synthesis and oxygenation lipolytic, can be adjusted to  $\omega$ -3 essential fatty acids (Catala, 2010). The beneficial effect of these unsaturated fatty acids was observed in the case of Parkinson's disease. The experiment was carried out in mice (Bousquet, 2009). It was concluded that the fatty acids are involved in various biological mechanisms through changes in membrane fluidity and membrane receptors modulating the activity of enzymes and neuronal transmitters (Riemer, 2010). Therefore, intake of essential fatty acids may be beneficial in cases of mild Alzheimer's disease and prevent cognitive decline and dementia (Solfrizzi et al, 2010). High levels of  $\omega$ -3 fatty acids and  $\omega$ -6 in the tissues of the heart are associated with reduced mortality because heart disease (Nippon et al, 2009). Fatty acids can affect immune cell functions through a variety of complex mechanisms and these mechanisms now beginning to be revealed (Calder, 2008). One optimal level of long chain unsaturated fatty acids is considered diet, high levels of these components are associated with diabetes, obesity and polycystic metabolic (Kruger et al, 2010). Also these essential fatty acids are protective and level of liver cells by direct effect on macrophages and hepatocytes and indirect anti-inflammatory role of liver cells (Wei Hao et al, 2010). Fat globule from the sheep's milk has a diameter of 2-3 times lower than that of cow's milk and thus increase the number of cells in the sheep's milk in comparison with that of the cow milk.

The threshold embedding fatty acids it is important to maintain the characteristics of the cheese and the percentage of fatty acids needed to obtain the body of the food. The report  $\omega$ -6 /  $\omega$ -3 in the diet should not exceed 4:1 (Simopoulos, Leaf, and Salem, 1999). The new dietary guidelines issued by the Joint Health Claims Initiative (2004) suggest a minimum contribution of 0.45 g EPA + DHA / day / person.

## MATERIALS AND METHODS

Organoleptically examination: according STAS 66345-95;

Physico-chemical analysis: determination of titratable acidity, according to STAS 6353-85; determination of fat content according S.TA.S. 6352 / 2-87; determining the percentage of NaCl in cheese STAS 6354-84, determination of protein substances, according to STAS 6355-89.

Gas chromatographic analysis of fatty acids: 1 g of the sample prior to macerated in 10 ml of distilled water and then added 0.6% ammonia solution, 2 ml of ethanol, 4 ml 4 ml of ethyl ether and hexane and then the mixture agitated 3 minutes. After that the lower layer has been removed ammonia and the mixture was filtered through a cellulose filter, and sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) and dried. The fatty acids were converted to methyl esters by reaction with boron trifluoride / methanol at 80 ° C for two hours in a closed tube of Pyrex glass. The contents were transferred to a separating tube. Extraction of methyl esters was performed using 10 ml of hexane. Hexane fractions collected were dried using anhydrous sodium sulfate, filtered, concentrated under a stream of nitrogen and finally taken up in 1 ml of hexane. Analysis of fatty acids methyl esters was performed using a gas chromatograph Shimadzu GC-17A equipped with a Chrompack capillary column with a length of 25 m and a diameter of 0.25 mm, stationary phase (a derivative of polyethylene glycol) being deposited inside the column in the form of a thin film of 0.2  $\mu$ m.

Methods of statistical analysis: for multiple comparisons were used Tukey's test, Fisher and Duncan and for comparison with the control using Dunnett test. For the determination of the optimum concentrations of fish oil that are incorporated in fat globule used statistical analysis method comparing ROC curves, Receiver Operator Characteristic = Operating Characteristics (Teuşdea A, et al, 2008; Teusdea A, 2009).

## **RESULTS AND DISCUSSION**

To determine the optimal dose of fish oil in terms of Organoleptically and technological, have experienced three concentrations of added fish oil: 0.05%; 0.10%; 0.15% in the raw sheep milk. Were produced and analyzed three variants of cottage cheese with added fish oil compared with a probe made from sheep's milk without the addition of fish oil. Samples were coded according to Table 1.

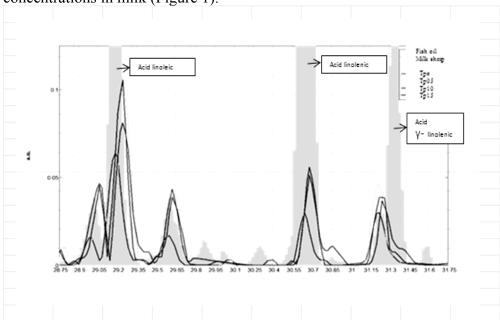
T	able	1	

No.	Addition of fish oil	Sample code	
	%	raw milk	Fresh cheese in brine
1	0	LT <sub>0</sub>	$Tp_0$
2	0,0,5	$LT_{0,05}$	Tp <sub>0,05</sub>
3	0,10	$LT_{0,10}$	Tp <sub>0,10</sub>
4	0,15	$LT_{0,15}$	Tp <sub>0,15</sub>

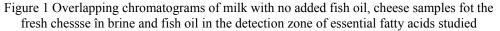
Experimental variants of cheese in brine from sh	neep's milk
--	-------------

After analyzing sensory it was found that only the taste and aroma of cheese are affected by the addition of fish oil and the weak sensed in adoas 0.05% and is increasing with increasing concentration of fish oil in the raw milk.

The physico-chemical properties of the fresh cheese in brine is not affected by the addition of fish oil. For the determination of optimal the threshold potting fish oil in globule fat cheese were considered representative of three essential fatty acids composition of sheep's milk and fish oil: linoleic acid, linolenic and  $\gamma$ -linolenic acid. As a result of the overlapping of the chromatograms, retention times in the three essential fatty acids can be seen that the fall in concentration between the strand fish



oil and milk samples are approximately in the order of added fish oil concentrations in milk (Figure 1).



After analyzing the threshold potting linoleic acid in the fresh cheese in brine can be observed that it is theoretically 0.16% (Figure 2, Table 2).

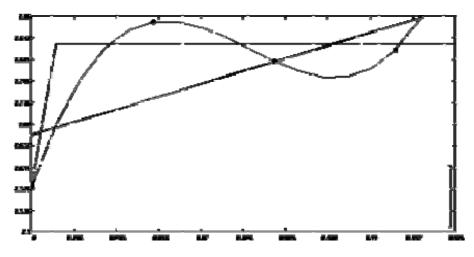


Figure 2 The threshold of linoleic acid incorporation in fresh cheese in brine

#### Table 2

			Tuble 2	
Values of embedding threshold for linoleic acid in the fresh cheese in brine				
Precision	Saturation threshold	Saturation threshold	Saturation threshold	
	(from regression)	(the derivative regression)	(theory)	
0.0001	0.015	0.025	0.16	

For optimum limit linolenic acid concentration of fish oil is 0,17% of the theoretical, estimated (Table 3, Figure 3).

Table 3

1	Threshold values linolenic acid embedding in the fresh cheese in brine			
Precision	Saturation threshold	Saturation threshold	Saturation threshold	
	(from regression)	(the derivative regression)	(theory)	
0.0001	0.035	0.035	0.171617884	

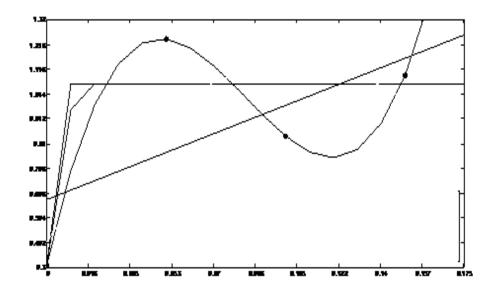


Figure 3 The threshold of linolenic acid incorporation in fresh cheese in brine

In the case of  $\gamma$ -linolenic acid, the optimum concentration limits of fish oil is added to milk is about 0.47% theory (Table 4, Figure 4).

## Table 4

Threshold values $\gamma$ - linolenic acid embedding in the fresh cheese in brine			
Precision	Saturation threshold	Saturation threshold	Saturation threshold
	(from regression)	(the derivative regression)	(theory)
0.0001	0.145	0.185	0.466845504

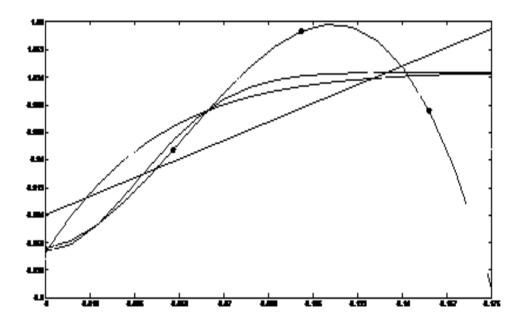


Figure 4 The threshold of  $\gamma$ - linolenic acid incorporation in fresh cheese in brine

## CONCLUSIONS

To obtain fresh cheese in brine enriched in essential fatty acids, the role of functional product by adding fish oil in milk is recommended in technologically addition of  $0.10 \div 0.15\%$ .

This is the conclusion of the analysis incorporation linoleic acid (0.16%), linolenic acid (0.17%) and  $\gamma$ -linolenic acid (0.46%) in the fat globule from the fresh cheese in brine.

From the technological point of view, the theoretical concentration of added fish oil sheep's milk for cheese in brine is necessary to be greater than the practical use.

### REFERENCES

- Bouattour, M.A., Casals, R., Albanell, E., Such, X., Caja, G. 2007. *Milk CLA and fatty acids profile in milk from Lacaune ewes fed whole sufflower grains*. The 5<sup>th</sup> International Symposium on The Challenge to Sheep and Goats milk, Sardinia, Italia from 18-20 april, I-P014.
- Bousquet M., C. Gibrat, M. Saint-Pierre, C. Julien, F. Calon, F. Cicchetti. 2009. Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. Progress in Neuro-Psychopharmacology & Biological Psychiatry 33 1401–1408.

- 3. Butour J.L, S Wimmer, F Wimmer, P Castan. 1997. *Palladium(II) compounds with potential antitumour properties and their platinum analogues: a comparative study of the reaction of some orotic acid derivatives with DNA in vitro.* Chemico-Biological Interactions, Volume 104, Issues 2-3, 2 May Pages 165-178.
- Calder Philip C. 2008. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins, Leukotrienes and Essential Fatty Acids, Volume 79, Issues 3-5, September-November, Pages 101-108
- Catalá Angel. 2010. A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochemical and Biophysical Research Communications, Volume 399, Issue 3, 27, Pages 318-323
- Costin, G. M., Florea, T., Popa, C., Rotaru, G., Segal, R., Bahrim, G., Botez, E., Turtoi, M., Stanciu, S., Turtoi, G. 2003. *Ştiinţa şi ingineria brânzeturilor*. pag. 29-214, 458-564, Ed. Academica, Galaţi.
- Hao Wei 1, Olive Y. Wong, Xuelai Liu, Puiyan Lee, Yan Chen, Kenneth K.Y. Wong. 2010. ω-3 fatty acids suppress inflammatory cytokine production by macrophages and hepatocytes. Journal of Pediatric Surgery 45, 2412–2418.
- Kruger M.C., M. Coetzee, M. Haag, H. Weiler. 2010. Long-chain polyunsaturated fatty acids: Selected mechanisms of action on bone. Progress in Lipid Research, Volume 49, Issue 4, October, pages 438-449.
- 9. Mierliță D., St. Dărăban, F. Lup, C. Maerescu, 2010. *The Influence of Bypass Fats Used in Ewes' Diet on the Productive Performances and on the Fatty Acids Profile From Milk*. Lucrari stiintifice, seria Agronomie, vol. 53.
- Mierliță D., St. Daraban, F.G. Lup. 2010. Influence of Bypass Fats Used in Ewes' Diets on Intake and on Rumenal Metabolism. Bulletin of University of Agricultural sciences and Veterinary Medicine Cluj-Napoca; Animal Science and Biotechnologies, 67(1-2), p. 468, Print ISSN 1843-5262, Electronic ISSN 1843-536X.
- Mierliță D., C. Maerescu, St. Daraban, F. Lup. 2009. Effects of energy and protein content in the diet on milk yield and milk fatty acid profile in dairy ewes. Bulletin USAMV Cluj-Napoca, Animal Science and Biotechnologies, 66(1-2), ISSN 1843-5262, p: 67-73.
- Mierliță D. 2009. The effect of energy and protein levels of feeding on milk yield and rumen fermentation in dairy ewes. Analele Univversitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, ISSN 1583-4301.
- Mierliță D., F. Lup, C. Maerescu. 2009. Nutritional and technological factors inorder to obtain functional food enriched with PUFA Omega 3 and CLA at sheep: a review. Analele Univ. din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, ISSN 1583-4301.
- 14. Nipon Chattipakorna, Jongkolnee Settakornc, Petnoi Petsophonsakula, Padiphat Suwannahoid, Pasuk Mahakranukrauhd, Somdet Srichairatanakoole, Siriporn C. Chattipakornb. 2009. Cardiac mortality is associated with low levels of omega-3 and omega-6 fatty acids in the heart of cadavers with a history of coronary heart disease. Nutrition Research, 29, 696–704.
- 15. Riemer Sabine , Michael Maes , Armand Christophe , Winfried Rief. 2010. Lowered  $\omega$ -3 PUFAs are related to major depression, but not to somatization syndrome. Journal of Affective Disorders, 123, 173–180.
- Simopoulos, A. P., Leaf, A., & Salem, N. 1999. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Annals of Nutrition and Metabolism, 43, 127–131.

- Solfrizzi Vincenzo, Vincenza Frisardi, Cristiano Capurso, Alessia D'Introno, Anna M. Colacicco, Gianluigi Vendemiale, Antonio Capurso, Francesco Panza. 2010. Dietary fatty acids in dementia and predementia syndromes: Epidemiological evidence and possible underlying mechanisms. Ageing Research Reviews, Volume 9, 184-199.
- Teusdea, A.C. & Gabor, G. 2009. *Iris Recognition with Phase-Only Correlation*. Annals of DAAAM for 2009 & Proceedings of the 20th International DAAAM Symposium, ISBN 978-3-901509-68-1, ISSN 1726-9679, pp 690-691, Editor B. Katalinic, Published by DAAAM International, Vienna, Austria.
- Teuşdea, A. C., Modog, T., Mancia A., Drăgan Dan. 2008. *Deformations analisys with fourier correlation*. Annals of DAAAM for 2008 & Proceedins of the 19th International DAAAM Symposium, "Intelligent Manufacturing & Automation: Focus on Next Generation of Intelligent Systems and Solutions", 22-25th October, ISBN 978-3-90150-958-X (ISI Proceedings M/IT);
- Versiani Matos Ferreira Adaliene, Gleydes Gambogi Parreira, Laura Cristina Jardim Porto, Érica Guihen Mario, Helen Lima Delpuerto, Almir Sousa Martins, Leida Maria Botion. 2008. *Fenofibrate prevents orotic acid—Induced hepatic steatosis in rats*. Life Sciences, Volume 82, Issues 15-16, 876-883.