

## THE THRESHOLD EMBEDDING ESSENTIAL FATTY ACIDS IN RIPENED CHEESE IN BRINE

Hilma Elena\*

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

### **Abstract**

*This study aims to analyze the incorporation of essential fatty acids in matured cheese in brine made from sheep's milk. To enrich the product in the component to fish oil used is composed of essential fatty acids and is a product of optimum physical-chemical as well as in the incorporation of the fat globules in the milk. It intended to obtain thus a product identical to the natural in terms of digestibility in the human body but with a high biological value. To achieve the objectives described above has matured cheese in brine made from sheep's milk without added fish oil with the addition of 0.05%; 0.10% and 0.15%. The inclusion of fish oil was accomplished by splitting the fat globules to 70 ° C and a pressure of 200 bar and such fish oil is intended to adhere to milk fat. Afterwards the fat globules are recovering with incorporating fish oil rich in essential fatty acids. To protect essential fatty acids to cheese ripening temperature increased by 2 ° C and thus reduced maturation time of 14 days. From the matured product were analyzed by gas chromatography three essential fatty acids (linoleic acid, linolenic and  $\gamma$ -linolenic acid), which are both in the milk of sheep and fish oil. The aim was to limit the incorporation of the three unsaturated fatty acids in milk fat globule and the following values were obtained the theoretical threshold concentrations in the milk added fish oil: linoleic acid-0.286%; linolenic acid-0.318%; and  $\gamma$ -linolenic acid-0.345%.*

**Key words:** ripened cheese in brine essential fatty acids

### **INTRODUCTION**

The maturation period of 16-20 weeks did not cause significant changes in indices NPU and PER protein cheese, in fact, in most cases, the qualitative indicators of the level of these proteins nutritional are even higher than those of the milk proteins (Costin, 2003).

A number of recent studies bring into question the existence of the fat milk of unsaturated fatty acids including the home "Conjugated linoleic acid" (CLA) which possess specific physiological (MierlițăDaniel, 2010) and whose concentration is influenced by diet (MierlițăDaniel, 2009). As a result because presence CLA in milk fatty acids confer nutritional and biological facets special cheeses that challenge the opportunity behavior of dairy food too restrictive with higher fat content. Apparently cheeses are produced which gives oils rich in essential fatty acids and nutritional value superior stability ( Bodkowski R, 2015).

The beneficial role of essential fatty acids in the body alations is increasingly emphasized in the contemporary period. Results show that the deficit membrane unsaturated fatty acids in case of schizophrenia occur early in the disease, before treatment and also the analysis suggests low concentrations of PUFA (Ravinder ş.a., 2004). The beneficial effect of these unsaturated fatty acids was observed in case of Parkinson's disease. The experiment was carried out in mice (Bousquet, 2009). It has been shown that fatty acids  $\omega$ -3 and  $\omega$ -6 to help with depression and mental disorders. Study was done on 150 patients divided in 4 groups. It was concluded that the fatty acids are involved in different biological mechanisms through membrane fluidity and changes in membrane receptors modulating the activity of enzymes and neuronal broadcaster (Riemer, 2010). Therefore, intake of essential fatty acids has beneficial effects in cases of Alzheimer's disease and mild cognitive decline and prevention of dementia (Solfrizzi ş.a., 2010). For accelerating the ripening of cheeses was investigated  $\beta$ -cyclodextrin treatment ( $\beta$ -CD). Was obtained accelerate the maturation process to yield the short chain fatty acids and amino acids. At the same time obtained reducing cholesterol in the cheese by about 90%. They have improved sensory characteristics while reducing maturation period of avoiding risk rancid and bitter taste (K.H. Seon, şii alţii, 2009).

Increasing the temperature to 18 ° C has been shown to accelerate the ripening cheese with increased lipolysis process and increase the proportion of free fatty acids without leading to their degradation (Guillermo A. şii alţii, 2007).

## **MATERIALS AND METHODS**

Sensory analysis: according STAS 66345-95;

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

Analysis fish oil: aspect is determined by visual inspection; the color is carried out in accordance with the European Pharmacopoeia, current edition, monograph „IECORIS ASELLI OLEUM A”; relative density is carried out in accordance with the European Pharmacopoeia, current edition, chapter 2.2.5;

Gas chromatographic analysis of fatty acids: Separation of fatty acid methyl esters was done by gas chromatography using a Shimadzu GC-17 equipped with a capillary column Chrompack with a length of 25 m and a diameter of 0.25 mm, stationary phase (a derivative of polyethylene glycol)

is done inside the column as a thin film of 0.2  $\mu\text{m}$ . FID detector and using, a mobile phase was 99.9% pure helium. The operating parameters of the gas chromatograph: injector and detector temperature: 260°C at a flow rate of carrier gas: 1.9 ml / min, a plate for 5 min at 70 ° C initial temperature, the temperature gradient of 4 ° C per minute up to 235°C: Report split: 1: 19, the total analysis time: 39.25 minutes. After the gas chromatograph has reached the programmed parameters were injected 0.5 ml hexane solution of methyl esters of fatty acids with the aid of a Hamilton syringe. As a preliminary there were retention times short chain fatty acids and long in minutes: Butyric Acid Methyl Ester, 2.0614; Caproic Acid Methyl Ester, 3.8596; Caprylic Acid Methyl Ester, 6.7105; Capric Acid Methyl Ester, 9.6491; Lauric Acid Methyl Ester, 13.1140; Myristic Acid Methyl Ester, 17.8863; Myristoleic Acid Methyl Ester, 19.5481; Pentadecanoic Acid Methyl Ester, 20.3939; Cis-10-Pentadecanoic Acid Methyl Ester, 21.5317; Palmitic Acid Methyl Ester-22.9759; Palmitoleic Acid Methyl Ester-23.5449; Heptadecanoic Acid Methyl Ester-25.3497; Stearic Acid Methyl Ester-27.7972; Elaidic Acid Methyl Ester-28.1906; Oleic Acid Methyl Ester-28.5402; Linoleic Acid Methyl Ester-29.2395; Linolenic Acid Methyl Ester-30.6550;  $\gamma$ -Linolenic Acid Methyl Ester-31.2664; Arahidic-32.190; Cis-5,8,11,14,17-Eicosapentanoic-35.870; Cis-4,7,10,13,16,19-Docosahexanoic-36.735.

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; Teuşdea A, 2009).

## RESULTS AND DISCUSSION

To determine the optimal dose of fish oil from organoleptic and technological point of view, we have experienced three amounts of added fish oil: 0.05%; 0.10%; 0.15%. They were produced and analyzed three variants of ripened cottage cheese with added fish oil compared with a probe made from sheep's milk without added fish oil. Samples were coded according to Table 1.

Table 1

Experimental variants ripened cheese from sheep's milk cheese			
No.	Addition of fish oil %	Sample code	
		Raw milk	Ripened cheese in brine
1	0	LT <sub>0</sub>	Tm <sub>0</sub>
2	0,0,5	LT <sub>0,05</sub>	Tm <sub>0,05</sub>
3	0,10	LT <sub>0,10</sub>	Tm <sub>0,10</sub>

4	0,15	LT <sub>0,15</sub>	Tm <sub>0,15</sub>
---	------	--------------------	--------------------

Sensory analysis was performed by five approved persons. It was found that the organoleptic characteristics of the product is not affected by the addition of fish oil in the raw milk in concentrations of 0.055, 0.10% and 0.15%. Does not feel the taste of fish oil. The cottage cheese matured so has the same sensory qualities when milk is produced without the addition of fish oil when and where milk is added fish oil to 0.15%.

To assess variation ripened cheese fatty acids compared to their proportion in raw milk were analyzed 19 fatty acids with short chain and long, monounsaturated and polyunsaturated. To determine the limit enclosing the fish oil globule of milk fat were considered three fatty acids representative for sheep milk and fish oil: linoleic aci; linolenic and  $\gamma$ -linolenic acid. Chromatographic surfaces of the representation of surfaces is observed that fish oil samples exceed those of mature white cheese samples are greater than milk. It aims to demonstrate that essential fatty acids from samples of cheese and are in higher concentration than in milk, milk coming from oil added cheese and obtained (Figure 1).

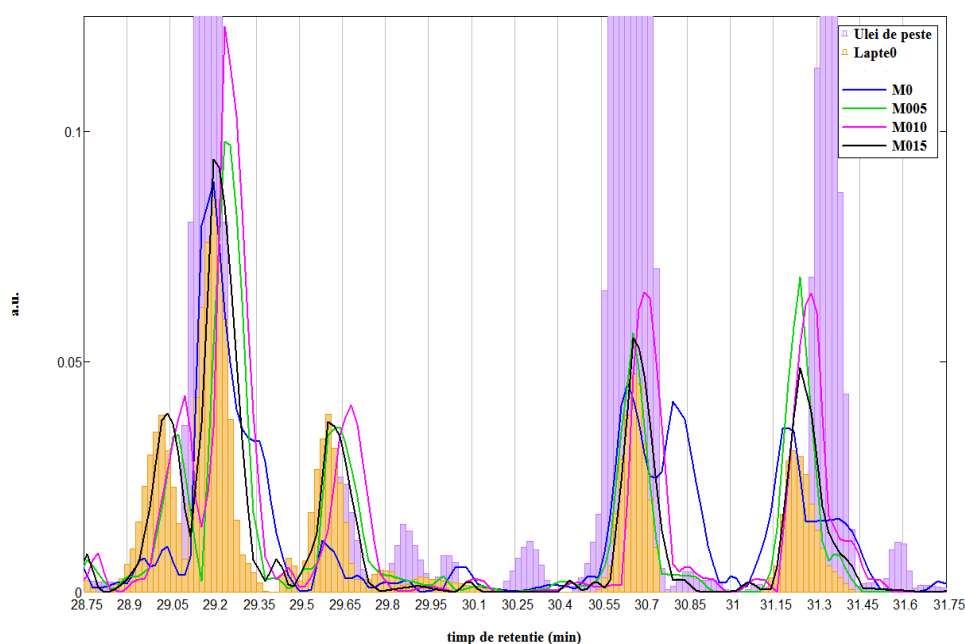


Figure 1 Overlapping chromatograms of milk with no added fish oil, cheese samples for the ripened cheese in brine and fish oil in the detection zone of essential fatty acids

Analyzing potting limits of linoleic acid in milk fat globule of using logistic regression function, resulting limits 0.063% and ranging from 0.163% fish oil added to milk (figure 2).

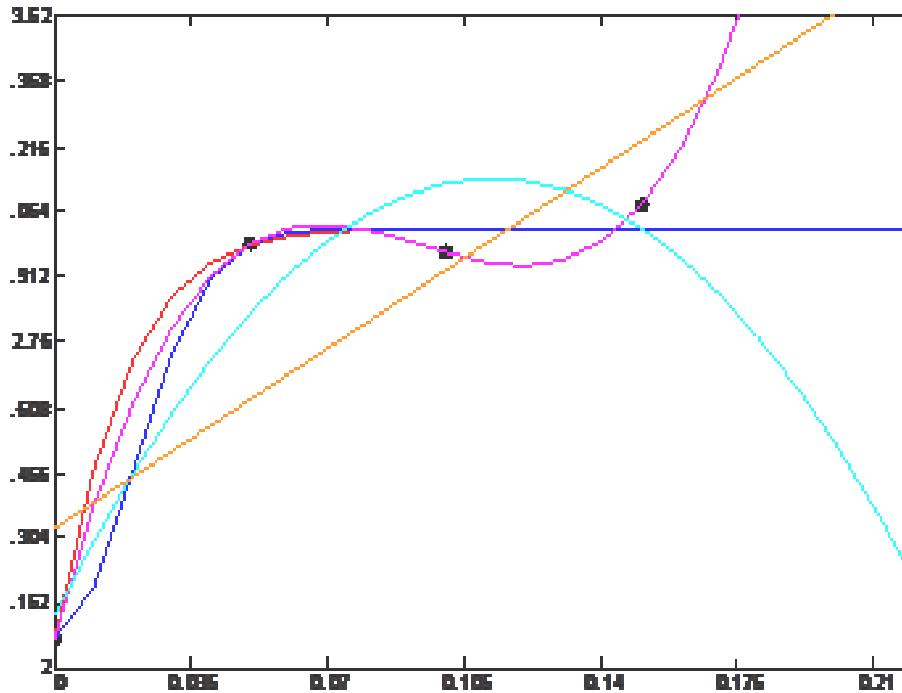


Figure 2 The threshold of linoleic acid incorporation in ripened cheese in brine

Analyzing the threshold values of embedding linoleic acid results in a theoretical amount of 0.286% added fish oil in milk (table 2).

Table 2

Values of embedding threshold for linoleic acid in the ripened cheese in brine

Precision	Saturation threshold (from regression)	Saturation threshold (the derivative regression)	Saturation threshold (theory)
0.0001	0.095	0.115	0.286

In the case of linoleic acid, embedding it in the limit of the fat globule, analyzed using logistic regression function, the proportions range between 0.05% and 0.17% fish oil added to the milk (figure 3).

In the theoretical threshold of saturation of fat globule of this is achieved, if linolenic acid to a value of 0.32% added fish oil in raw milk (table 3).

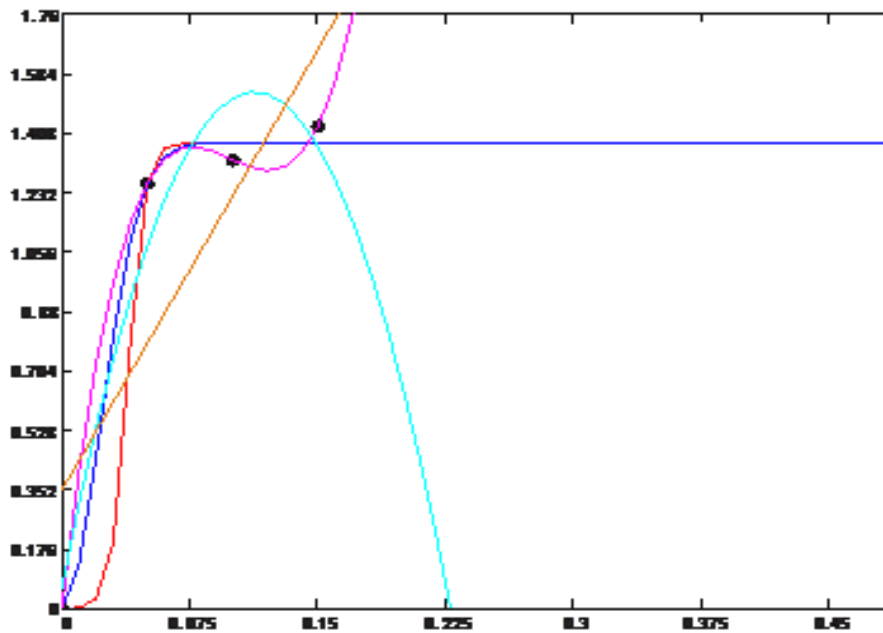


Figure 3 The threshold of linolenic acid incorporation in ripened cheese in brine

Table 3

Threshold values linolenic acid embedding in the ripened cheese in brine

Precision	Saturation threshold (from regression)	Saturation threshold (the derivative regression)	Saturation threshold (theory)
0.0001	0.105	0.125	0.318510059

For determining the optimum concentration of fish oil were added to the milk, taking into account the incorporation of essential fatty acids of milk fat globule is analyzed centrally three essential fatty acids in the study. (table 5).

Table 5

Concentrations in fish oil sheep milk to a maximum of embedding samples of cheese in brine matured a three-essential fatty acids

Fatty acids	Percentage concentration of fish oil in sheep milk	Limit potting
Linoleic	0.0950	from regression values
Linoleic	0.1150	regression of the values of the derivative
Linoleic	0.2868	theoretical
Linolenic	0.1050	from regression values
Linolenic	0.1250	regression of the values of the derivative
Linolenic	0.3185	theoretical
$\gamma$ -Linolenic	0.1250	from regression values

$\gamma$ -Linolenic	0.1450	regression of the values of the derivative
$\gamma$ -Linolenic	0.3494	theoretical

Average concentrations of fish oil added to the milk of sheep, for the three fatty acids, is presented in table 6.

Table 6

Average concentration of fish oil added to the milk of sheep, for the three essential fatty acids

Sample	The concentration of fish oil (%)
Tm <sub>0</sub> -linoleic acid	0.1656
Tm <sub>0,05</sub> - linolenic acid	0.1828
Tm <sub>0,10</sub> - $\gamma$ linolenic acid	0.2065

## CONCLUSIONS

In this study made cheese in brine from sheep's milk with added 0.05%; 0.10% and 0.15% fish oil for the enrichment of product in essential fatty acids. They have analyzed three essential fatty acids which are representative of the milk from sheep and fish oil as the optimum concentration of fish oil added to the milk at the limit of the enclosing them in a milk fat globule. Averages for essential fatty acids are: linoleic acid-0.1656%; linolenic acid- 0.1828% and acid- $\gamma$ linolenic-0.2065. Average concentrations in fish oil used to prepare milk ripened cheese in brine, at the limit potting essential fatty acids, is 0.1850.

So that may be made cheese in brine rich in essential fatty acids by adding fish oil sheep milk 0.15% (suggested maximum concentration) even if it is slightly lower than that obtained by calculations (0.185%) taking into account that the industrial system must be a limit to manufacturing error.

## REFERENCES

1. Bodkowski R, K. Czyż , R. Kupczyński, B. Patkowska-Sokoła, P. Nowakowski, A. Wiliczekiewicz, 2015, *Lipid complex effect on fatty acid profile and chemical composition of cow milk and cheese*, Journal of Dairy Science
2. 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. Costin, G. M., Florea, T., Popa, C., Rotaru, G., Segal, R., Bahrim, G., Botez, E., Turtoi, M., Stanciu, S., Turtoi, G. 2003. *Știința și ingineria brânzeturilor*. pag. 29-214, 458-564, Ed. Academica, Galați.
4. European Pharmacopoeia, current edition, chapter 2.2.5;
5. European Pharmacopoeia, current edition, monograph „IECORIS ASELLI OLEUM A”;

6. Guillermo A., Susana E. Zorrilla, Diego J. Mercanti, María C. Perotti, Carlos A. Zalazar, Amelia C. Rubiolo. 2007. *The influence of ripening temperature and sampling site on the lipolysis in Reggianito Argentino cheese*. Food Research International. Volume 40. Issue 10. December Pages 1220-122
7. Mierliță D., C.M., Maurescu; St. Daraban; F., Lup. 2010. *Dietary effects on milk yield, rumen fermentation and milk fatty acid composition in the milk of Turcana ewes*. Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară, ISSN 1583-4301.
8. Mierliță D., F. Lup, C. Maurescu. 2009. *Nutritional and technological factors in order to obtain functional food enriched with PUFA Omega 3 and CLA at sheep: a review*. Analele Univ. din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară, ISSN 1583-4301.
9. Mierliță D., St. Dărăban, F. Lup, C. Maurescu, 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.
10. Ravinder D. Reddy, Matcheri S. Keshavan, Jeffrey K. Yao. 2004. *Reduced Red Blood Cell Membrane Essential Polyunsaturated Fatty Acids in First Episode Schizophrenia at Neuroleptic-Naive Baseline*. Schizophrenia Bulletin, Volume 30, Issue 4, 2004, Pages 901-911.
11. 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.
12. Seon K.H., J. Ahn, H.S. Kwak. 2009. *The accelerated ripening of cholesterol-reduced Cheddar cheese by crosslinked  $\beta$ -cyclodextrin*. Journal of Dairy Science, Volume 92, Issue 1, p. 49-57.
13. 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.
14. STAS 6353-85; S.T.A.S. 6352 / 2-87; STAS 6354-84, STAS 6355-89.
15. Teuşdea, 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.
16. 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).
17. Teuşdea, A.; Modog, T.. 2008. *Fourier correlations of dam horizontal movements time series*. Journal of Electrical and Electronics Engineering, ISSN 1844 – 6035, Editura Universităţii din Oradea, Oradea, pg. 267-270.