

# Quality control of alimentary animal fats under different storage conditions

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## Abstract

Physicochemical characteristics of alimentary beef tallow during refrigeration and freezing storage were studied. To establish the freshness indicators in various stages of alterative processes were determined: iodine value (IV), peroxide value (PV), thiobarbituric acid reactive substances (TBARS), the presence of epyhidrinic aldehyde (Kreis reaction) and acidity index for measuring the degree of oxidation and lipolysis. Appreciation of oxidation process installation was performed also by microscopic examination. Iodine value of fresh tallow had a value of  $45.6 \pm 0.07$  g I<sub>2</sub>/100 g, during refrigerated storage was registered a very significant decrease ( $P \leq 0.001$ ) in the 5th month due to the reduction of unsaturation degree by unsaturated fatty acids oxidation. Between PV and IV there was determined an inverse correlation, the correlation coefficient was  $R = -0.915$ . Between PV and storage time there was a linear relationship until the 5th month of storage, the correlation coefficient was  $R = 0.964$ , and from the 6th month of refrigeration there was established an inverse correlation ( $R = -0.936$ ).

Storage temperature had a very significant effect ( $P \leq 0.001$ ), and storage time had a significant effect ( $P \leq 0.05$ ) on alterative processes installation, the shelf life of tallow stored under freezing was almost twice from that of tallow stored under refrigeration.

**Keywords:** quality control, alimentary beef tallow, oxidation, refrigeration, freezing

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## 1. Introduction

Alteration (hydrolysis and oxidation) of food is responsible for the degradation of sensory quality, nutritional value and even formation of toxic substances such as peroxides, requiring intimate knowledge of the process and consequently taking appropriate measures to avoid losses that can be registered.

Lipids entered in the composition of living matter from the very beginnings of life on Earth. It is recognized that life appeared on Earth about 3,7 million years ago, being composed of single-cell organisms of bacterial type, type of life that dominated the planet over one billion years. These

early forms of lipids have evolved with animal and plant species leading to different forms of animal and vegetable fats, used by people in ancient times. If prehistoric people knew animal fats and used them in different ways, not only as food, vegetable fats began to be produced and used about 8.000 years ago [1,2]. Excessive consumption of mostly fat foods, especially saturated fats had led to health problems: increase blood cholesterol levels [3], forming atheromatose plates [4], increase blood pressure, increase the number of patients with cardiac and circulatory diseases [5,6]. Unsaturated lipids are less dangerous and contain significant amounts of fat-soluble vitamins useful to the body, which function as an antioxidant both in mostly fat foods and in

human body, preventing many diseases due to oxidative stress [7].

Oxidative rancidity takes place under oxygen action and consists in the formation of free radicals and hydroperoxides, the transformation of unstable hydroperoxides in stable peroxides, and finally, the split into aldehydes and acids with characteristic odor [8]. Involves the oxidation of unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA) and generates compounds that affect food quality by altering of color, flavor, texture, nutritional value and food safety [9,10].

Lipid oxidation proceeds through a typical self-propagating free radical mechanism where oxygen attack occurs mainly at the allylic positions adjacent to double bonds [11]. The photosensitized route is an alternative oxidative pathway that involves the direct reaction of excited singlet oxygen ( $^1O_2$ ) to unsaturated lipids in the presence of sensitizers [11,12]. In the peroxidation of unsaturated fatty acids, lipid hydroperoxides form during the propagation phase. These compounds are unstable and decompose rapidly, giving rise to a range of new free radicals and other non-radical compounds, including alkoxy and alkyl radicals, aldehydes, ketones, as well as variety of carboxyl compounds that form a complex mixture of secondary lipid oxidation products. Volatiles such as hexanal and pentanal have been associated with the development of undesirable flavours and have been proposed as potential markers of fresh product quality [13-16].

Free radicals in lipid peroxidation have been associated with the etiology of degenerative diseases such as atherosclerosis, rheumatoid arthritis, degeneration of the retina, tumor stimulation and aging. MDA is suspected to be carcinogenic because it can react with DNA and cause mutagenic compounds [17,18].

Lipolytic degradation occurs due to hydrolytic degradation of lipids in fat composition. Hydrolysis is catalyzed by lipase and produces free fatty acids that can cause defects to butter like the taste of rancid, butyric, bitter or astringent [19]. Lipases which hydrolyze fatty acid glycerides are esterases, and those that hydrolyzes glycerides are lipases. The first are active in aqueous solutions, and the latter are more active at lipid-water

interface. The specificity of lipolytic enzymes is controlled by three factors: molecular properties of the enzyme, substrate structure and factors that affect the relationship between enzyme and substrate [20,21].

The objective of this study was to monitor the sensory and physicochemical parameters during refrigerated (2 ... 4°C) and frozen (-15 ... - 18°C) storage of alimentary beef tallow to determine its freshness and validity.

## 2. Material and methods

*2.1. Samples.* Beef tallow was obtained by melting of raw tallow collected from "Baltata Romaneasca" race and was stored under refrigeration (2...4°C) and freezing (-15...- 18°C) conditions, the aim of the research was to study the sensory and physicochemical changes that occur during storage and the time when alterative processes (hydrolysis and oxidation) are installed.

### 2.2. Chemical analysis

*2.2.1. Iodine value (IV).* Iodine value was determined using Hanus method. Approximately, 0.5 g sample (dissolved in 15 mL  $CCl_4$ ) was mixed with 25 mL Hanus solution (IBr) to halogenate the double bonds. After storing the mixture in dark for 30 min., excess IBr was reduced to free  $I_2$  in the presence of 20 mL of KI (100 g/L) and 100 mL distilled water. Free  $I_2$  was measured by titration with 24.9 g/L  $Na_2S_2O_3 \cdot 5H_2O$  using starch (1.0 g/100 mL) as an indicator. IV was calculated as g  $I_2$ /100 g sample [22].

*2.2.2. Peroxide value (PV).* Peroxide value was determined using UV-VIS spectrophotometer. This protocol was based on the spectrophotometric determination of ferric ions ( $Fe^{3+}$ ) derived from the oxidation of ferrous ions ( $Fe^{2+}$ ) by hydroperoxides, in the presence of ammonium thiocyanate ( $NH_4SCN$ ). Thiocyanate ions ( $SCN^-$ ) react with  $Fe^{3+}$  ions to give a red-violet chromogen that can be determined spectrophotometrically, the absorbance of each solution was read at 500 nm. To quantify PV, a calibration curve (absorbance at 500 nm vs.  $Fe^{3+}$  expressed in  $\mu g$ ) was constructed and peroxide value was expressed as meq  $O_2$ /kg sample [23].

*2.2.3. TBARS determination .* TBARS determination was carried out as follows: TBA Reagent (0.02 M 2-

thiobarbituric acid in 90% glacial acetic acid) was prepared, then 1 g of sample was weight into a glass-stoppered test tube and 5 mL of TBA reagent was added. Then, the tube was immersed in a boiling water bath for 35 min. A distilled water-TBA reagent blank was also prepared and treated like the sample. A portion was transferred to a cuvette and the optical density of the sample was read against the blank at a wavelength of 538 nm [23].

**2.2.4. Acid value (AV).** Determination of acidity is the basic criterion for assessing the installation and intensity of hydrolysis. The method consists in neutralizing acidity with sodium hydroxide 0.1 N, using phenolphthaleine, as an indicator. Acidity was expressed as oleic acid grams to 100 grams sample [22].

**2.2.5. Kreis reaction.** Epyhidrinic aldehyde, formed during advanced oxidation of fats, released in an acid environment, reacts with phluoroglucine, giving a colored compound. Color intensity is proportional to the quantity of epyhidrinic aldehyde, and so with the oxidation process [22].

**2.2.6. Statistical analysis.** All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean  $\pm$  standard deviation ( $X \pm SD$ ). Significant differences between mean were determined by using "Student" ("t") distribution.

### 3. Results and discussion

The determined acidity for fresh beef tallow was  $0.16 \pm 0.007\%$  (g oleic acid), during refrigerated storage it presented an increasing evolution, there were no significant differences reporting to control sample ( $P > 0.05$ ) until the 15th day, acidity exceed the maximum permissible limit of 1% (g oleic acid) for fresh tallow in the 115th day of storage ( $P \leq 0.001$ ), between acidity values and storage time there was a strong positive correlation ( $R = 0.992$ ). At this time for tallow were not found essential changes in color, only the present of a slightly acid taste and smell.

PV of fresh tallow had a value of  $0.7 \pm 0.08$  meq  $O_2$ /kg. During the first three month of refrigerated storage, PV increase significantly ( $P \leq 0.05$ ), from  $1.1 \pm 0.07$  meq  $O_2$ /kg in the first month to  $2.3 \pm 0.14$

meq  $O_2$ /kg in the 3th month ( $P \leq 0.01$ ), followed by a marked increase in the 4th month, in the 5th month the increase was very significant reported to the first month ( $P \leq 0.001$ ), and in the 6th month the value decreased due to the splitting of hydroperoxides in secondary compounds. It can be concluded that from the 6th month the oxidative status passed from primary to secondary status.

IV of fresh tallow was  $45.6 \pm 0.07$  g  $I_2$ /100 g, during refrigerated storage was registered a very significant decrease ( $P \leq 0.001$ ) in the 5th month due to the reduction of unsaturation degree by unsaturated fatty acids oxidation (Figure 1). Between PV and IV there was an inverse correlation, the correlation coefficient was  $R = -0.915$ . Between PV and storage time there was a linear relationship until the 5th month of storage, the correlation coefficient was  $R = 0.964$ , and in the 6th month of refrigeration there was an inverse correlation ( $R = -0.936$ ).

MDA was detected early in the storage period with the value of  $0.19 \pm 0.007$  mg/kg, increasing its value very significantly ( $P \leq 0.001$ ) in the 6th month of storage at 2...4 °C, of 10.84 times higher than in the first month (Figure 2). There was observed an inverse correlation between MDA and PV in the 6th month ( $R = -0.758$ ), when PV registered a decline MDA value recorded the highest growth, indicating the presence of secondary oxidation compounds with toxic effects on the body. In the 6th month was also registered the presence of epihydric aldehyde, whose amount is proportional to the intensity of developed color, and regardless of the intensity of reaction tallow should be excluded from the food chain. It may be established a maximum TBARS value of about 1.5 mg MDA/kg, value to which tallow presented a relative freshness, and above this value was installed a rancid taste and odor. Between TBARS and storage time was established a positive linear correlation with a correlation coefficient of  $R = 0.994$ . Based on these results it can be concluded that tallow stored under refrigeration was fresh up to 3 months, until the 5th month was relatively fresh showing slightly acidic taste and smell and a high value of PV, and from the 6th month was installed the advanced alteration due to the formation of secondary oxidation compounds that change the organoleptic properties in rancid taste and smell but the color remained unchanged.

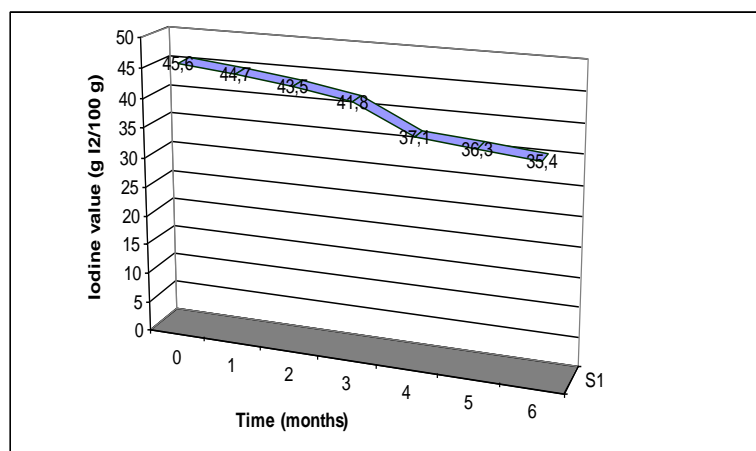


Figure 1. Iodine index variation of beef tallow during refrigerated storage

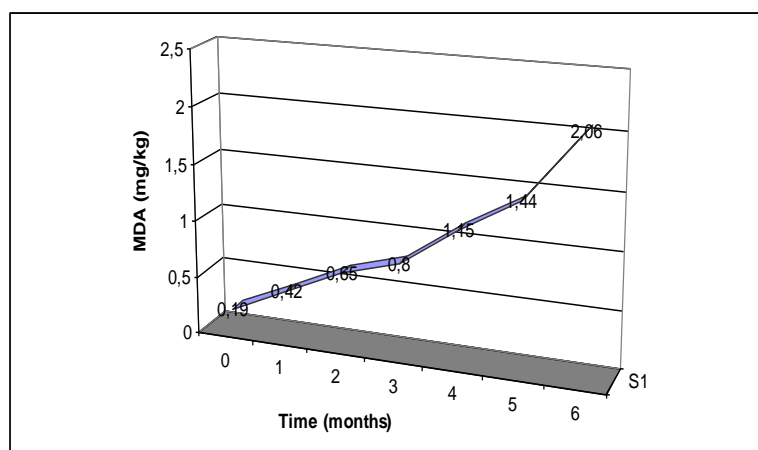


Figure 2. MDA content variation of beef tallow during refrigerated storage

Table 1. Physicochemical indices values of frozen beef tallow

Fat tipe	Peroxide value (meq O <sub>2</sub> /kg)	Iodine value (g I <sub>2</sub> /100 g)	TBARS test (MDA mg/kg)	Kreis reaction
T0	0.7±0.08	45.6±0.14	0.19±0.007	negative
Tf1	0.9±0.06 <sup>NS</sup>	45.4±0.11 <sup>NS</sup>	0.22±0.005 <sup>NS</sup>	negative
Tf2	1.2±0.07 <sup>a</sup>	45.1±0.15 <sup>a</sup>	0.45±0.006 <sup>a</sup>	negative
Tf3	1.5±0.14 <sup>a</sup>	44.6±0.10 <sup>a</sup>	0.60±0.011 <sup>a</sup>	negative
Tf4	1.9±0.13 <sup>a</sup>	43.7±0.08 <sup>b</sup>	0.77±0.014 <sup>b</sup>	negative
Tf5	2.3±0.15 <sup>b</sup>	42.6±0.14 <sup>b</sup>	0.94±0.010 <sup>b</sup>	negative
Tf6	2.8±0.14 <sup>b</sup>	41.6±0.09 <sup>b</sup>	1.16±0.014 <sup>b</sup>	negative
Tf7	3.2±0.07 <sup>c</sup>	40.5±0.16 <sup>c</sup>	1.32±0.009 <sup>c</sup>	negative
Tf8	6.1±0.09 <sup>c</sup>	36.8±0.07 <sup>c</sup>	1.49±0.007 <sup>c</sup>	negative
Tf9	6.7±0.14 <sup>c</sup>	35.7±0.13 <sup>c</sup>	1.63±0.013 <sup>c</sup>	negative
Tf10	7.4±0.14 <sup>c</sup>	34.3±0.08 <sup>c</sup>	1.94±0.007 <sup>c</sup>	negative
Tf11	6.5±0.07 <sup>c</sup>	33.6±0.07 <sup>c</sup>	2.74±0.015 <sup>c</sup>	positive

Values are expressed as the mean ± standard deviation of three determinations, significant differences: NS (P>5%); <sup>a</sup>(1%<P≤5%); <sup>b</sup>(0.1%<P≤1%); <sup>c</sup>(P≤0.1%)

T0 –fresh beef tallow, Tf1 –tallow to 1 month freezing, Tf2 – tallow to 2 months freezing, Tf3 – tallow to 3 months freezing, Tf4 – tallow to 4 months freezing, Tf5 – tallow to 5 months freezing, Tf6 – tallow to 6 months freezing, Tf7 – tallow to 7 months freezing, Tf8 – tallow to 8 months freezing, Tf9 – tallow to 9 months freezing, Tf10 – tallow to 10 months freezing, Tf11 – tallow to 11 months freezing

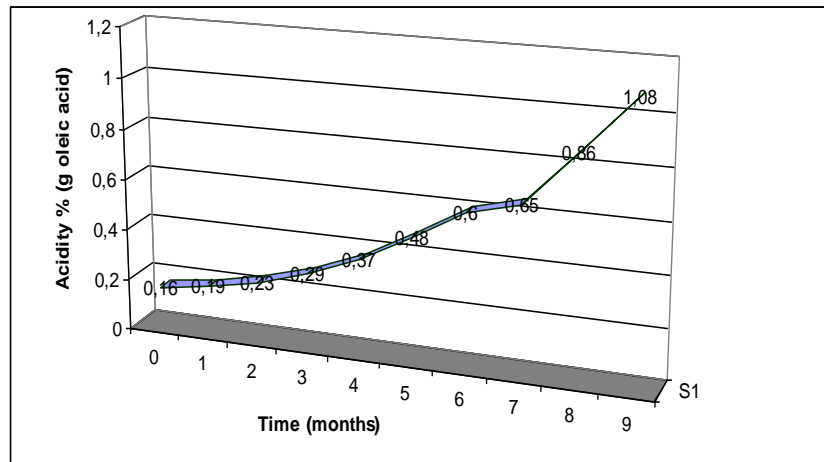


Figure 3. Acidity variation of beef tallow during frozen storage

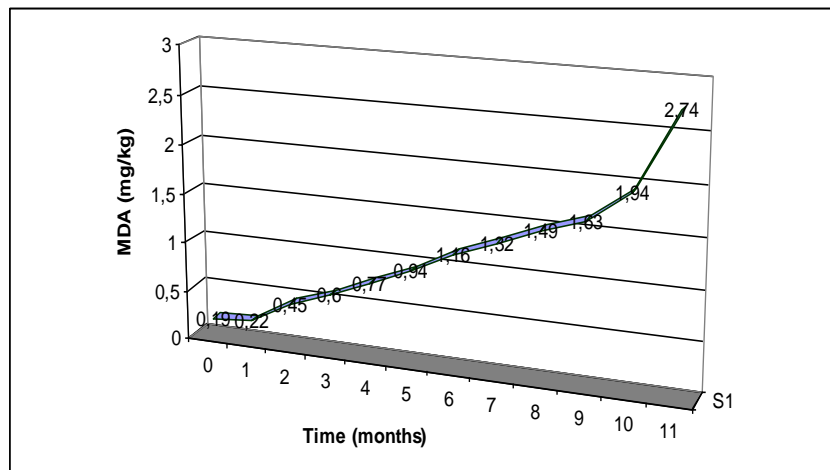


Figure 4. MDA content variation of beef tallow during frozen storage

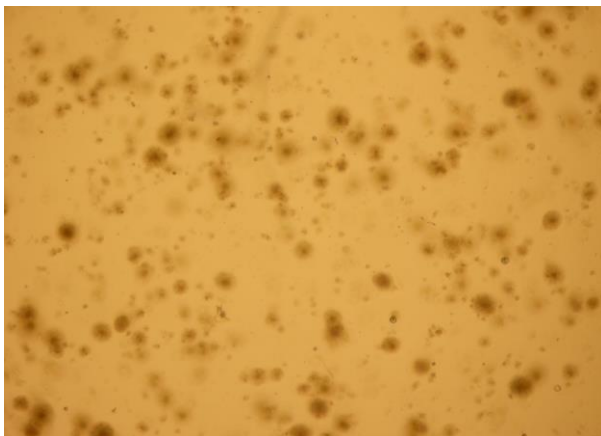


Figure 5. Microscope view for fresh beef tallow

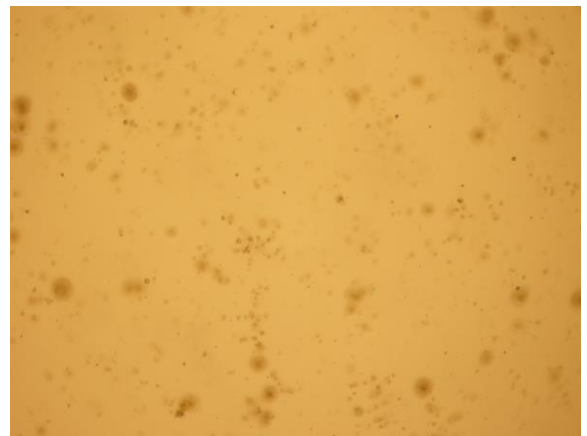


Figure 6. Microscope view for oxidised beef tallow

The variation of physicochemical parameters for frozen beef tallow is presented in Table 1.

During frozen storage, acidity increased gradually, reaching the value of  $1.08 \pm 0.014\%$  (g oleic acid) ( $P \leq 0.001$ ) in the 9th month, exceeding the maximum allowable limit of 1% (g oleic acid) for fresh tallow (Figure 3). From this moment was installed a slightly acidic taste and smell, but the color remained unchanged.

PV recorded an increase during storage at  $-15...-18^\circ\text{C}$ , a very significant increase ( $P \leq 0.001$ ) was observed in the 8th month of storage, reaching the value of  $6.1 \pm 0.09$  meq  $\text{O}_2/\text{kg}$  due to the formation of large quantities of peroxides, during the 9th and 10th months value increased relatively slowly compared to the 8th month, and in the 11th month there was a decrease that corresponded to disruption phase and the splitting of peroxides in secondary compounds. It can be concluded that the induction period of tallow stored under freezing conditions was about 7 months, the propagation period was about 3 months up to the 10th months, and the period of decline began in the 11th month when the secondary oxidation compounds were formed, ie after 10 months the oxidative status of fat sample goes from primary to secondary status. Between PV and storage time there was a positive correlation ( $R=0.959$ ) until the 10th month, and from the 11th month there was an inverse correlation ( $R=-0.927$ ).

IV decreased during frozen storage, a very significant decrease ( $P \leq 0.001$ ) was recorded in the 8th month, there was an inverse correlation between IV and PV ( $R=-0.749$ ) due to the scindation of unsaturated bonds from fatty acids structure.

MDA content registered a very significant increase ( $P \leq 0.001$ ) in the 11th month of storage, up to  $2.74 \pm 0.015$  mg/kg (Figure 4), in this month was also identified the presence of epihidrynic aldehyde and of the other secondary oxidation compounds that are volatile and give tallow bitter taste and pungent odor, yellow color became more intense. It can be established a maximum value of TBARS of about 2 mg MDA/kg, value up to the frozen tallow presents a relative freshness, and above this value the rancid taste and odor are installed.

It was found that TBARS values were in positive correlation with storage time ( $R = 0.996$ ).

Based on the obtained results it can be concluded that tallow stored under freezing was fresh up to 8 months, in the 9th and 10th months the freshness was relatively because of the sour taste and smell and the high value of PV, and from the 11th month oxidative alteration was installed, also highlighted by the color, taste and smell defects. In the literature there were no found studies on storage stability of beef tallow.

The purpose of microscopic examination was to follow changes at microscopic level that occur when advanced oxidation process was installed in animal fats. In the case of fresh beef tallow examination there were observed molecules of different sizes, irregularly scattered among portions of compact fat (Figure 5). Microscopic examination of oxidized beef tallow showed a more compact image with rare molecules, irregularly dispersed in the mass of fat (Figure 6).

In another study it has been reported that butter was resistant to oxidation, advanced oxidation was installed after 6 months in chilled butter and after 9 months in frozen butter.

#### 4. Conclusions

Storage temperature had a very significant effect ( $P \leq 0.001$ ), and storage time had a significant effect ( $P \leq 0.05$ ) on alterative processes instalation, the shelf life of tallow stored under freezing was almost twice from that of tallow stored under refrigeration.

The time of changes occurring in hydrolysis and oxidation processes of beef tallow has particular importance in assessing the quality and its preservation.

At microscopic examination of altered fat there was observed differences from the fresh fat, noticing that microscopic examination can be a method for assessing the quality of fats.

Hydrolysis processes were installed earlier than oxidative processes in both refrigerated and frozen storage, oxidation was prevented by limiting the contact with atmospheric oxygen and light intensity and also through the packaging.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

#### References

1. Simion, C.; Albu, H.; Simion Alina, *Quality and food control* **2007**, Printech Press, Bucharest
2. Ubbink, J.; Krüger, J., Physical approaches for the delivery of active ingredients in foods, *Trends in Food Science and Technology* **2006**, *17*, 244–254
3. Din, J.N.; Newby, D.E.; Flapan, A.D., Science, medicine, and the future – Omega 3 fatty acids and cardiovascular disease– fishing for a natural treatment, *British Medical Journal* **2004**, *328*, 30–35
4. Banu, C.; Vizireanu C., *Lipids role in nutrition. Aspects of lipid use in food industry* **1996**, Academic Press, Galati
5. Frank, B.; Joann, E.; Manson, W.C.; Willett, C., Types of Dietary Fat and Risk of Coronary Heart Disease, *Journal of the American College of Nutrition* **2001**, *20*, 15-19
6. Miyamoto, S.; Miyake, Y.; Sasaki, S.; Tanaka, K.; Ohya, Y.; Matsunaga, I.; Yoshida, T.; Oda, H.; Ishiko, O.; Hirota, Y., Fat and fish intake and asthma in Japanese women: baseline data from the Osaka Maternal and Child Health Study, *International Journal of Tuberculosis and Lung Disease* **2007**, *11:1*, 103-109
7. Moller, P.; Wallin, H., Adduct formation, mutagenesis and nucleotide excision repair of DNA damage produced by reactive oxygen species and lipid peroxidation product, *Mutation Research* **1998**, *410:3*, 271–290 Nawar, W.W., Biochemical processes: *Lipid instability*. In: *Food storage stability*, Boca Raton, Washington, 2001, pp. 89–103
8. Frankel, E.N., Lipid oxidation, *Progress in Lipid Research* **1997**, *19*, 1–22
9. Hearn, T.L.; Sgoutas S.A.; Sgoutas D.S.; Hearn, J.A., Stability of polyunsaturated fatty acids after microwave cooking of fish, *Journal of Food Science* **1997**, *52:2*, 1430–1431
10. Tofană Maria, *Food additives. Interaction with food* **2006**, Academic Press, Cluj-Napoca
11. O'Connor, T.P.; O'Brien, N.M., *Lipid oxidation* **2006**, In: *Advanced dairy chemistry*, vol. 2, Lipids, Third Edition, Springer, New York, pp. 557-600
12. Pérez-Alonso, F.; Arias, C.; Aubourg, S.P., Lipid deterioration during chilled storage of Atlantic pomfret (*Brama brama*), *European Journal of Lipid Science and Technology* **2003**, *105*, 661–667
13. Rawls, H.R.; Van Santen, P.J., A possible role for singlet oxygen in the initiation of fatty acid autoxidation, *Journal of the American Oil Chemistry Society* **2003**, *47*, 121–125
14. Soyer, A.; Hultin, H., Kinetics of oxidation of the lipids and proteins of cod sarcoplasmic reticulum, *Journal of Agriculture and Food Chemistry* **2000**, *48*, 2127–2134
15. Toso, B.; Procida, G.; Stefanon, B., Determination of volatile compounds in cows' milk using headspace GC–MS, *Journal of Dairy Research* **2002**, *69:4*, 569–577
16. Van de Voort, F.R.; Ismail, A.A.; Sedman, J.; Emo, G., Monitoring the oxidation of edible oils by Transform infrared spectroscopy, *Journal of the American Oil Chemists Society* **1994**, *71*, 243–253
17. Velasco, J.; Dobarganes, M.C.; Márquez-Ruiz, G., Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils, *Grasas y Aceites* **2000**, *51:6*, 439–446
18. Vito, V.; Ferioli, F.; Riciputi, Y.; Iafelice, G.; Marconi, E.; Caboni, M.F., Evaluation of lipid oxidation in spaghetti pasta enriched with long chain n-3 polyunsaturated fatty acids under different storage condition, *Food Chemistry* **2008**, *78*, 384-392
19. Laslo, C., *Quality control of meat and meat products* **1997**, ICPIAF Press, Cluj-Napoca
20. Marsili, R.T.; Miller, N., SPME-GC/MS testing for monitoring off-flavors in processed milk: Application of odor wheel plots, *Freshness and Shelf Life of Foods* **2003**, *836*, 80–94
21. Lozano, P.R.; Miracle, E.R.; Krause, A.J.; Drake, M.A.; Cadwallader, K.R., Effect of cold storage and packaging material on the major aroma components of sweet cream butter, *European Journal of Lipid Science and Technology* **2006**, *108*, 349-355
22. \*\*\*Romanian Standard SR EN 14082, **2003**, Bucharest
23. \*\*\* International Standard ISO 3976, **2006**