JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2013, 19(1), 37-42

Journal of Agroalimentary Processes and Technologies

Changes in the quality of alimentary animal fats due to storage conditions

Pop Flavia*, Voşgan Zorica

Technical University of Cluj-Napoca, North University Center of Baia Mare, Chemistry and Biology Department, 76A Victoriei Str., 430122, Baia Mare, Romania,

Received: 17 January 2013; Accepted: 13 Feruary 2013

Abstract

Physicochemical parameters and freshness indicators of alimentary poultry fat during refrigeration (2 ... 4°C) and freezing (-15 ...- 18°C) storage were studied. In assessing the various stages of oxidative changes, peroxide value (PV), iodine value (IV), thiobarbituric acid reactive substances (TBARS) and the presence of epyhidrinic aldehyde (Kreis reaction) were determined to identify and measure the primary and secondary oxidation compounds, fatty acid profile and acidity index determination for measuring the degree of lipolysis. Appreciation of oxidation process installation was performed also by microscopic examination. PV registered an increased durin storage at -15...-18°C, a very significant increase was observed at 90 days when riched the value of 7.9±0.14 meq O_2/kg due to the formation of a large amount of peroxides, at 120 days the value increased relatively slow and after 150 days it was registered a decrease of PV to 7.7±0.09 meq O_2/kg, which corresponds to the interruption stage and the split in secondary compounds. Based on the obtained results it can be concluded that poultry fat stored under freezing was fresh up to 2 months, up to 4 months the freshness was relatively due to the slightly acidic taste and smell and the high value of PV, and in the 5th month the oxidative spoilage was installed also highlighted by the color, taste and smell deffects. Storage temperature had a very significant effect (P≤0.001) and storage time a significant effect (P≤0.05) on the installation of advanced processes of hydrolysis and oxidation, the acceptability tolerance of fat stored under freezing was almost twice for that of fat stored under refrigeration.

Keywords: alimentary animal fats, hydrolysis, oxidation, refrigeration, freezing, fatty acid profile

1. Introduction

Lipids are abundantly represented in animal tissues, having in living organisms in especially energy role. They can be considered as the main form of energy storage. Also, they meet plastic role because they enter in the structure of cellular components and also protective role [1]. Food lipids and that used for meals preparing, improve their sensory properties, because they have own taste and smell and serve as solvents for the fragrance ingredients, stimulates digestion and absorption, and the use of troffines from food improve them. Lipids are very susceptible to the prooxidant factors, this process once started can be slowed, but can not be stopped, lowering the shelf life of the food [2,3].

Off-flavorings, nutritional losses and other deteriorative changes in animal fats are concerned with the changes that result from reaction with atmospheric oxygen, i.e., oxidative rancidity, or by hydrolytic reactions catalyzed by lipases from food or from microorganisms. However, oxidative rancidity or autoxidation cannot be stopped by lowering the temperature of storage since it is a chemical reaction with low activation energy [4,5].

Animal fats contain polyunsaturated fatty acids that are exposed to autoxidation during storage [6-8], so that the shelf life depends on polyunsaturated fatty acids content, temperature and storage time.

Packaging plays an important role in controlling and minimizing side effects that may occur during storage [9].

Corresponding author: *e-mail: flavia_maries@yahoo.com*

It has been reported that a number of physical and environmental factors, chemical compounds and enzymes, processing and storage conditions influence the emergence and expansion of fat oxidation. Among these are mentioned: oxygen, light, metals, antioxidants, carotenoids, proteins and enzymes, storage temperature and water activity [11-14]. During storage or food processing, lipid autoxidation is the main reaction for organoleptic and nutritional (loss of essential fatty acids, nutritional losses) deterioration [15].

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. 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 [16, 17].

During peroxidation of unsaturated fatty acids, a complex of secondary oxidation compounds is generated (alkanes, alkenes, aldehydes, ketones, etc.). Of aldehydes and ketones that are formed there are include pentanal, hexanal, 4-hidroxinonenal and malondialdehyde (MDA) [18]. These compounds cause loss of flavor and nutritional value, and limited the shelf life of the product. Secondary oxidation compounds, saturated or unsaturated, including aldehydes, ketones, hydrocarbons, and alcohols may be percepted by consumers even at very low concentrations. Fatty acids with three or more double bonds produce malondialdehyde (MDA), a major compound of lipids autoxidation [19].

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 [20-23]. Of the chemical, specific reaction for aldehydes identification (Kreis) will be positive and regardless of the intensity of the reaction (weak positive, positive or mostly positive), fat should be excluded from the food circuit. Peroxide index provides us information on the incipient oxidation, and Kreis reaction illustrates advanced oxidation.

Some publications have assessed the effects of irradiation and light exposure on lipid stability in beef, pork and turkey [24, 25].

The purpose of this study was to follow the stability in refrigerated (2 ... 4^{o}C) and frozen (-15 ...- 18^{o}C) storage of alimentary poultry fat by quality parameters monitoring to determine its validity.

2. Materials and methods

2.1. Samples.

Poultry fat was obtained by melting of raw material fat collected from broilers, male and female, packed in unvacuumate plastic bags, and stored under refrigeration (2...4^{o}C) and freezing (-15- 18^{o}C), the research aim was to study the organoleptic and physicochemical changes, and the installation of alterative processes (hydrolysis and oxidation).

2.2. Chemical analysis

2.2.1. Peroxide value (PV). Peroxide value was determined using UV-VIS spectrophotometer: operating temperature 5 – 45^{o}C; field wavelength 190 - 1100 nm; wave length accuracy 0.1 nm. 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 µg) was constructed and peroxide value was expressed as meq O_2/kg sample [26].

*2.2.2. 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 [27].

*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 oil sample was weight into a glass-stoppered test tube and 5 mL of TBA reagent

was added. The tube was stoppered and the contents were mixed. 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. After heating, the sample was cooled in tap water for 10 min. 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 in a UV-VIS spectrophotometer. The optical density value was converted to the moles of malondialdehyde per gram of fat sample by using a standard curve [26].

*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 [27].

*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 [27].

*2.2.6. Sensory analysis.*To assess the state of freshness we used descriptive sensory analysis which is an ideal technique to identify flavors in a product and to distinguish between these products, using tasters familiar with scoring methods and sensory language. Taster is presented with a single sample, and is required to assess the intensity of preselected attributes, this test was applied to the poultry fat throughout storage. The selected attributes were: smell, taste, color, appearance and consistency, their intensity was evaluated on an 1 to 5 scale and performed with star-shaped diagrams.

For sensory analysis the descriptive scale is the next: 1 = very little normal, 2 = less than normal, 3 = moderately normal, 4 = almost normal, 5 = normal

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

3. Results and discussion

3.1. Physicochemical examination of refrigerated poultry fat.

The determined acidity for fresh poultry fat was 0.29±0.007% (g oleic acid), during the first 10 days, the increase was insignificant ($P>0.05$), exceeding the maximum allowed limit of 1% (g oleic acid) for fresh fat in the 40th day of storage ($P\leq0.001$). At this time there were no essential changes of color, only the presence of a slightly acidic taste and smell, because the fatty acids released by hydrolysis are not volatile and do not affect the organoleptic properties, between acidity values and storage time there was a strong positive correlation (R=0.973).

The variation of physico-chemical parameters for refrigerated poultry fat is presented in Table 1.

Peroxide value (PV) determined for fresh poultry fat was 1.1±0.07 meq O2/kg, oxygen absorption can occur during the melting process. After 30 days of storage at 2...4°C, PV increased significant ($P\leq0.01$) up to the value of 3.0±0.14 meq O_2/kg, after 60 days the increase was very significant ($P\leq0.001$) up to the value of 6.8±0.14 meq O_2/kg, but after 90 days the value decrease to 5.9±0.09 meq O_2/kg, due to the split of hydroperoxides in secondary compounds. It can be concluded that after 60 days of refrigerated storage the oxidative process passed from primary to secondary status.

Iodine value (IV) determined for fresh poultry fat was 74.8±0.07 g I_2/100 g, during storage the value gradually decreased, a very significant decrease was registered after 60 days of storage ($P\leq0.001$). There was observed an inverse correlation between IV and PV (R=-0.897), due to the reduction of unsaturated degree by fatty acids oxidation. Between PV and storage time there was a positive correlation up to 60 days (R=0.975), after 60 days of refrigeration an inverse correlation (R=-0.946) and between IV and storage time was determined an inverse correlation (R=-0.984)

MDA has been detected since the beginning of the period of storage with a value of 1.24±0.006 mg/kg, MDA content increase during storage, a statistical significance ($P\leq0.01$) was registered after 60 days of storage at 2...4°C. There was observed an inverse correlation between MDA and PV after 60 days of storage (R=-0.679), when PV recorded a decline MDA value recorded the highest growth, indicating the presence of secondary oxidation compounds with toxic effects on the body.

It can be established a maximum TBARS value of about 2.5 mg MDA/kg, value to which the chilled poultry fat presented a relative freshness, and over this value was installed the taste and smell of rancid. After 60 days of refrigerated storage was also indentified the presence of epyhidrinic aldehyde, whose amount is directly proportional to the intensity of the developed coloration and regardless of the intensity of the reaction (weak positive or positive), poultry fat should be excluded from the diet.

Based on this results it can be concluded that poultry fat stored under refrigeration was fresh up to 30 days, up to 60 days was relatively fress with slightly acidic taste and smell and high value of PV, and after 60 days the advanced alteration was installed due to the formation of secondary oxidation compounds that changed the organoleptic properties in rancid taste and odor.

Table 1. Physico-chemical indices values of refrigerated poultry fat

Fat tipe	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	TBARS test (MDA mg/kg)	Kreis reaction
PF0	1.1±0.07	74.8±0.05	1.24±0.006	negative
PFr30	3.0±0.14**	71.4±0.16**	1.70±0.007*	negative
PFr60	6.8±0.11***	65.9±0.14***	2.38±0.015**	negative
PFr90	5.9±0.09***	63.7±0.08***	3.86±0.014**	positive

Values are expressed as the mean ± standard deviation of three determinations, significant differences: NS (P>5%); *(1%<P≤5%); **(0.1%<P≤1%); ***(P≤0.1%)
PF0 –fresh poultry fat, PFr30 –poultry fat to 30 days of refrigeration, PFr60 – poultry fat to 60 days of refrigeration, PFr90 – poultry fat to 90 days of refrigeration

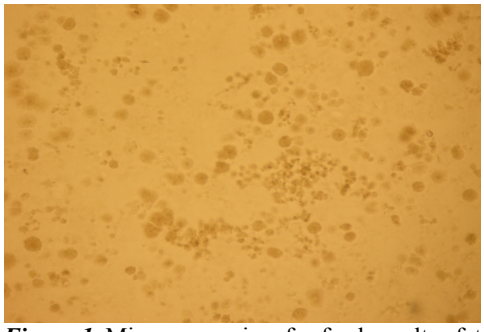

Figure 1. Microscope view for fresh poultry fat

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 poultry fat examination there were observed molecules of different sizes, irregularly scattered among portions of compact fat (Figure 1).

Microscopic examination of oxidized poultry fat showed a more compact image with rare molecules, irregularly dispersed in the mass of fat (Figure 2).

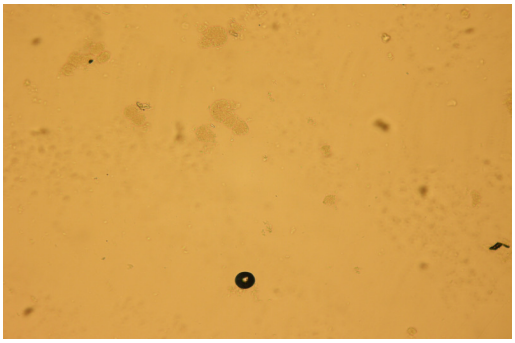

Figure 2. Microscope view for oxidized poultry fat

3.2. Physicochemical examination of frozen poultry fat

During frozen storage acidity increased significant (P≤0.001), at 90 days riched the value of 1.08±0.007% (g oleic acid), at this moment was installed a slightly acidic taste and smell, but the color remained unchanged. The variation of physico-chemical parameters for frozen poultry fat is presented in Table 2.

PV registered an increased durin storage at -15...-18^{o}C, a very significant increase was observed at 90 days when riched the value of 7.9±0.14 meq O_2/kg due to the formation of a large amount of peroxides, at 120 days the value increased relatively slow and after 150 days it was registered a decrease of PV to 7.7±0.09 meq O_2/kg, which corresponds to the interruption stage and the split in secondary compounds. It can be concluded that the induction period of poultry fat stored under freezing was about 60 days, the propagation period was also 60 days, and the period of decline started after 150 days of storage when the secondary oxidation compounds were formed, i.e. after 120 days the oxidative status passed from primary to secondary state. Between PV and storage time was observed a positive correlation up to 120 days (R=0.983), and up to 150 days an inverse correlation (R=-0.976). IV decreased during frozen storage, a very significant decrease (P≤0.001) was observed at 120 days of storage, between IV and PV there was an inverse correlation (R=-0.869) due to the split of unsaturated links from the fatty acids structure.

MDA content registered a very significant increase (P≤0.001) at 120 days of storage, the biggest increase was determined at 150 days riching the value of

4.82±0.015 mg/kg, also at this time was identify the epyhidrinic aldehyde presence and the formation of other secondary oxidation compounds that are volatile and gave pungent odor and bitter taste, and the yellow color became more intense. It can be established a maximum value for TBARS of about 4 mg MDA/kg, value to which poultry fat presented a relative freshness, and over this value was installed a rancid taste and smell. Between MDA and PV there was a positive correlation up to 120 days of storage (R=0.875), an inverse correlation up to 150 days (R=-0.872), and between MDA and storage time there was observed a positive correlation (R=0.986).

Based on the obtained results it can be concluded that poultry fat stored under freezing was fresh up to 2 months (Figure 3), up to 4 months the freshness was relatively due to the slightly acidic taste and smell (Figure 4) and the high value of PV, and in the 5th month the oxidative spoilage was installed also highlighted by the color, taste and smell defects (Figure 5).

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 [28].

Table 2. Physico-chemical indices values of frozen poultry fat

Fat tipe	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	TBARS test (MDA mg/kg)	Kreis reaction
PF0	1.1±0.07	74.8±0.07	1.24±0.006	negative
PFf30	2.3±0.14*	74.1±0.14*	1.68±0.008*	negative
PFf60	3.7±0.16**	73.7±0.13*	2.37±0.010**	negative
PFf90	7.9±0.14***	72.2±0.11**	2.85±0.014**	negative
PFf120	8.6±0.07***	68.6±0.07***	3.76±0.007***	negative
PFf150	7.7±0.09***	66.4±0.15***	4.82±0.012***	positive

Values are expressed as the mean ± standard deviation of three determinations, significant differences: NS (P>5%); *(1%<P≤5%); **(0.1%<P≤1%); ***(P≤0.1%); PF0 –fresh poultry fat, PFf30 –poultry fat to 30 days of freezing, PFf60 – poultry fat to 60 days of freezing, PFf90 – poultry fat to 90 days of freezing, PFf120 – poultry fat to 120 days of freezing, PFf150 – poultry fat to 150 days of freezing

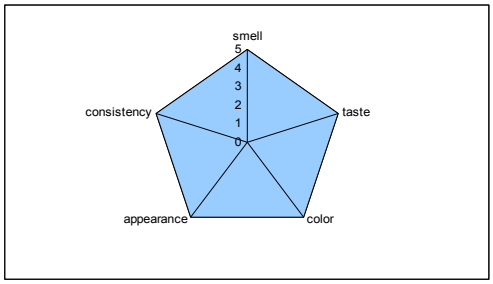

Figure 3. Sensory analysis of fresh poultry fat

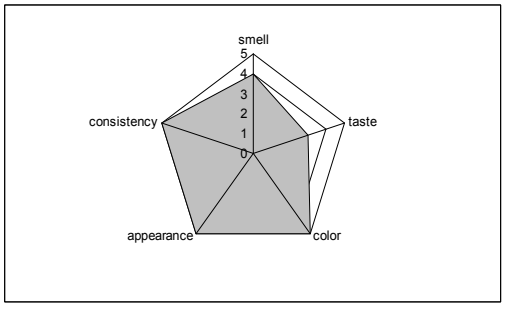

Figure 4. Sensory analysis of relatively fresh poultry fat

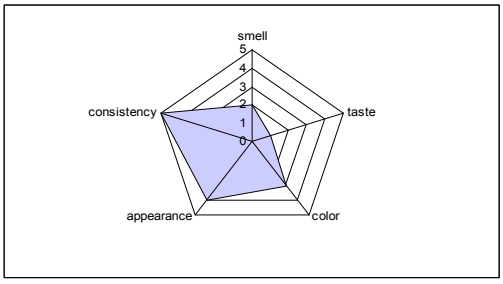

Figure 5. Sensory analysis of altered poultry fat

4.Conclusions

Storage temperature had a very significant effect (P≤0.001) and storage time a significant effect (P≤0.05) on the installation of advanced processes of hydrolysis and oxidation, the acceptability tolerance of fat stored under freezing was almost twice for that of fat stored under refrigeration. The time of changes occurring in hydrolysis and oxidation processes of poultry fat has particular importance in assessing the quality and its preservation.In the case of poultry fat, hydrolysis processes were installed earlier than oxidative ones, for both storage temperatures, oxidation was prevented by limiting the contact with

atmospheric oxygen and luminous intensity also by 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 and/or animal subjects (if exists) respect the specific regulations and standards.

References

1. 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
2. Paz, I.; Molero, M., Study of the thermal stability of edible vegetable oils in different environments, *Afinidad* **2001**, *58*, 190–196
3. 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**, *10*5, 661–667
4. Nawar, W.W., Biochemical processes: *Lipid instability*. In: *Food storage stability*, Boca Raton, Washington, 2001, pp. 89–103
5. Frankel, E.N., Lipid oxidation, *Progress in Lipid Research* **1997**, *19*, 1–22
6. 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
7. 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
8. Hamilton, R.J., *The chemistry of rancidity in foods*. In: *Food storage stability*, Boca Raton, Washington, 2001, pp. 113–135
9. Karatapanis, A.E.; Badeka, A.V.; Riganakos, K.A.; Savvaidis, I.N.; Kontominas, M.G., Changes in flavour volatiles of whole pasteurized milk as affected by packaging material and storage time, *International Dairy Journal* **2006**, *16*(7), 750–761
10. Chaiyasit, W.; McClements, D.J.; Decker, E., The relationship between the physicochemical properties of antioxidants and their ability to inhibit lipid oxidation in bulk oil and oil-in-water emulsions, *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4982–4988
11. Cosgrove, J.P.; Church, D.F.; Pryor, W.A., The kinetics of the autoxidation of polyunsaturated fatty acids, *Lipids* **1987**, *22*, 299–304
12. Davies, K.L.A.; Golberg, A.L., Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes, *Journal of Biological Chemistry* **1987**, *262*, 8220−8226
13. Faraji, H.; Lindsay, R.C., Characterization of the antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions, *Journal of Agricultural and Food Chemistry* **2004**, *52*, 7164–7171
14. Hara, A.T.; Kimura, N.; Saito, Y.; Miyashiit, K., Effects of emulsifiers and fatty acyl components on the oxidative stability of polyunsaturated triacylglycerols in emulsion system, 94th AOCS Annual Meeting & Expo, Kansas **2003**, USA.
15. Fillion, L.; Henry, C.J., Nutrient losses and gains during frying: a review, *International Journal of Food Science and Nutrition* **1998**, *49*, 157–165
16. Kolakowska, A., Lipid oxidation in food systems. In Z. E. Sikorski, A. Kolakowska (Eds.), *Chemical and functional properties of food lipids* **2002** (pp. 133–160). FL, USA: CRC Press
17. Krause, A.J.; Miracle, R.E; Sanders, T.H.; Dean, L.L.; Drake, M.A., The effect of refrigerated and frozen storage on butter flavor and texture, *Food Chemistry* **2007**, *89*, 57-65
18. Lagarda, M.J.; Manez, J.C.; Pilar Manglano, Rosaura Farré, Lipid hydroperoxides determination in milk based infant formulae by gas chromatography, *European Journal of Lipid Science and Technology* **2003**, *105*, 339-345
19. Nawar, W.W., Biochemical processes: Lipid instability. In I. A. Taub & R. P. Singh (Eds.), *Food storage stability*, pp 89–103, **1998**, Boca Raton, Boston, London, New York, Washington, DC: CRC Press
20. 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
21. Liu, Y.; Mu, X.M.; Yan, M.; Cui, S.; Hongxia, X.; Xiao, L., Antiaging effects of polysaccharide from spirulina platensis, *Zhongguo Yaolixue Tongbao* **1998**, *14*(4), 362–364
22. Russo, C.; Oliviero, O.; Girelli, D.; Faccini, G.; Zenari, M.L.; Lombardi, S., Antioxidant status and lipid peroxidation in patients with essential hypertension, *Journal of Hypertension* **1999**, *16*(9), 1267–1271
23. Navarro, J.; Obrador, E.; Carreter, J.; Petschen, I.; Avino, J.; Perez, P., Changes in glutathione status and the antioxidant system in blood and cancer cells associate with tumor growth in vivo, *Free Radical Biology Medicine* **2000**, *26*(4), 410–418
24. Nam, K.C.; Du, M.; Ahn, D.U., Cholesterol oxidation products in irradiated raw meat with different packaging and storage time, *Meat Science* **2008**, *58*, 431–435
25. Ahn, D.U.; Nam, K.C.; Du, M., Effect of irradiation and packaging conditions after cooking on the formation of cholesterol and lipid oxidation products in meats during storage, *Meat Science* **2007**, *57*, 413–418
26. *** International Standard ISO 3976, 2006
27. ***Romanian Standard SR EN 14082, Bucharest, 2003
28. Pop, F. ; Laslo, C., Research concerning physicochemical changes and freshness indicators of milk fat during storage, *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology, New Series Year III* **2009**, *32*, 91-96