JAP&T
TPA-Timişoara

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

Journal of Agroalimentary Processes and Technologies 2011, 17(1), 30-35

Journal of Agroalimentary Processes and Technologies

Installation of hydrolysis and oxidation processes in alimentary animal fats under different storage conditions

Flavia Pop[1]*

[1] *North University of Baia Mare, Chemistry Biology Department, 76A Victoriei Str., 430122, Baia Mare, Romania*

Received: 02 December 2010; Accepted: 30 January 2011

Abstract

Physicochemical characteristics and freshness indicators of pork lard 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. Based on the obtained results it can be said that pork lard stored under refrigeration is fresh until the 2nd month, in the 3rd month is relatively fresh showing slightly acidic taste and smell and a high value of PV, and from the 4th month, the advanced oxidation is installed due to the formation of secondary compounds that alter the sensory properties in rancid taste and odor, and yellow color. Storage temperature had a very significant effect ($P \leq 0.001$), and storage time had a significant effect ($P \leq 0.05$) on the installation of advanced processes of hydrolysis and oxidation, the shelf life under freezing was double than that under refrigeration.

Keywords: pork lard, hydrolysis, oxidation, refrigeration, freezing

1. Introduction

Animal products industry faced with the situation of unwanted organoleptic and physico-chemical changes of alimentary fats, reason for that it is of great interest the knowledge of physico-chemical parameters evolution and their correlation with the state of freshness and shelf life in both refrigerated and frozen storage, for accurate knowledge of their validity and to ensure consumer protection.

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. The effects of hydrolytic reactions can be minimized by cold storage, good transportation, careful packaging and sterilization.

However, oxidative rancidity or autoxidation cannot be stopped by lowering the temperature of storage since it is a chemical reaction with low activation energy.

Research into the problems concerning oxidative deterioration has been pursued for many years but it has been given a boost by the recognition that such oxidations can cause damage to cell membranes and DNA, that may be involved in aging process, hypertension and cancer growth [1-4]. Autoxidation can be reduced by vacuum packaging, packing under an inert gas to exclude oxygen (modified atmosphere) and refrigeration/freezing. Lipid oxidation proceeds through a typical self-propagating free radical mechanism where oxygen attack occurs mainly at the allylic positions adjacent to double bonds [5].

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

The photosensitised route is an alternative oxidative pathway that involves the direct reaction of excited singlet oxygen ($^1O_2$) to unsaturated lipids in the presence of sensitisers [6-8].

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 alkoxyl and alkyls 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 [9-13]. Lipid oxidation is induced by oxy- and/or lipid free radical generation and results in the generation of toxic compounds such as the malondialdehyde and cholesterol oxidation products [14, 15].

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. In this stage of oxidation, organoleptic changes are installed, easily discernible using the senses: yellow color, smell and taste of rancidity [16]. 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 [17-20] or on the shelf life of foods like oats, cream cheese and butter [21].

The objective of this study was to investigate physicochemical and stability properties of pork lard stored under refrigeration (2...4^{o}C) and freezing (-15...-18^{o}C) in order to establish its shelf life.

2. Materials and methods

*2.1. Samples.*Pork lard was obtained by fresh bacon and pork fat melting, packed in unvacuumate plastic bags and stored under refrigeration (2...4^{o}C) and freezing (-15...- 18^{o}C), following 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 T60U spectrophotometer (Bibby Scientific, London, UK): 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 500nm. 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 [22].

*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 (100g/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 (1.0g/100mL) as an indicator. IV was calculated as g I_2/100g sample [23].

*2.2.3. TBARS determination.*TBARS determination was carried out as follows: TBA Reagent (0.02M 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 T60U model spectrophotometer. The optical density value was converted to the moles of malondialdehyde per gram of oil sample by using a standard curve. A standard curve was prepared by making appropriate dilutions of the 1 x 10^{-3} M 1,1,3,3-tetraethoxypropane standard solution, to give amounts ranging from $1x10^{-8}$ to $7x10^{-8}$ mol of malondialdehyde in 1mL. These dilutions were reacted with TBA reagent and the optical densities were measured at the wavelength of 538 nm in the spectrophotometer.

Samples with an optical density higher than 0.5 were diluted to a known degree to get accurate values [22].

*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 [24].

*2.2.5. Kreis reaction.*By Kreis reaction we identify aldehydes resulted in advanced stages of fat oxidation. 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 [24].

*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. Descriptive test is looming over one product using a linear scale, as reported elsewhere [16]. Taster is presented with a single sample, and is required to assess the intensity of preselected attributes, this test was applied to the pork lard 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 = moderat 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 pork lard. For fresh pork lard the determined acidity was 0.29±0.007% (g oleic acid) and during refrigerated storage presented an upward trend, in the 35th day reaching 0.61±0.007% value (P≤0.001) and exceeded the maximum permissible limit of 1% (g oleic acid) for fresh lard in the 40th day of storage (Figure 1). At this time were found no essential changes for color, only the presence of a slightly sour taste and smell, because by hydrolysis, superior saturated fatty acids are released which are not volatile and do not affect the sensory properties of lard, between acidity values and storage time there was a strong positive correlation (r=0.994). The variation of physico-chemical parameters for refrigerated pork lard is presented in Table 1.

Peroxide index value for fresh lard was determined to be 0.9±0.07 meq O_2/kg. During the first 2 months of storage at 2...4^oC, PV increased significantly up to 2.8±0.14 meq O_2/kg (P≤0.01), followed in the 3rd month, by a very significant increase up to the value of 5.9±0.15 meq O_2/kg (P≤0.001), in the 4th month the value decreased due to the decomposition of hydroperoxides in secondary compounds. It can be said that from the 4th month, the oxidative status of the sample passes from primary to secondary state.

Iodine index value (IV), determined for fresh pork lard was 66.8±0.07 g I_2/100 g, this value gradually decreased during storage, a very significant decrease (P≤0.001) was registered in the 3rd month of storage, between IV and storage time there was an inverse correlation (r=- 0.913). Between PV and storage time there was a linear relationship up to the 3rd month of storage, the correlation coefficient (r) was 0.975, and from the 4th months of the refrigeration there was an inverse correlation (r=-0.946). Malondialdehyde was detected in the early storage period with a value of 0.40±0.007 mg/kg, its value increased during storage, a very significantly increase (P≤0.001) was registered in the 4th month of storage at 2...4^oC.

Based on the obtained results it can be established a maximum value of TBARS test about of ≈ 1.9 mg MDA/kg, amount by which pork lard have a relative freshness, and above this value a rancid taste and smell is installed. Also in the 4th month, the presence of epyhidrinic aldehydes was identified, and regardless of the intensity of the reaction (positive or weak positive), pork lard should be excluded from the food channel.

Based on the obtained results it can be said that pork lard stored under refrigeration is fresh until the 2nd month, in the 3rd month is relatively fresh showing slightly acidic taste and smell and a high value of PV, and from the 4th month, the advanced oxidation is installed due to the formation of secondary compounds that alter the sensory properties in rancid taste and odor, and yellow color.

Table 1. Physico-chemical indices values of refrigerated pork lard

Fat tipe	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	TBARS test (MDA mg/kg)	Kreis reaction
UP0	0.9±0.07	66.8±0.07	0.40±0.007	negative
UPr1	1.5±0.07/*	65.3±0.15/**	0.63±0.008/*	negative
UPr2	2.8±0.14/**	63.5±0.14/**	1.26±0.009/**	negative
UPr3	5.9±0.15/***	57.1±0.07/***	1.82±0.014/**	negative
UPr4	4.7±0.08/***	56.3±0.08/***	2.42±0.007/***	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%)

UP0 –fresh pork lard, UPr1 –pork lard to 1 month refrigeration, UPr2 – pork lard to 2 months refrigeration, UPr3- pork lard to 3 months refrigeration, UPr4 – pork lard to 4 months refrigeration

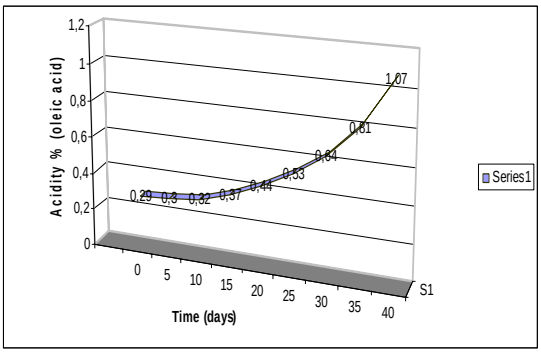

Figure 1. Acidity variation of pork lard during refrigeration storage

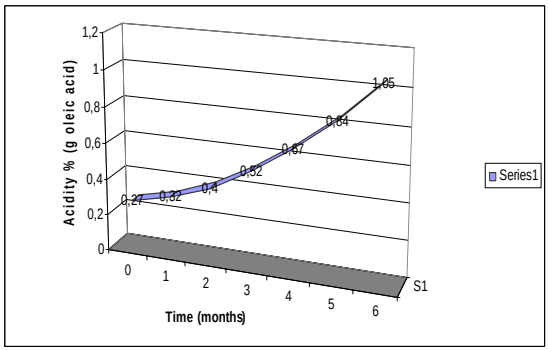

Figure 2. Acidity variation of pork lard during freezing storage

Table 2. Physico-chemical indices values of frozen pork lard

Fat tipe	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	TBARS test (MDA mg/kg)	Kreis reaction
UP0	0.9±0.07	66.8±0.07	0.40±0.007	negative
UPc1	1.4±0.14/*	65.9±0.14/*	0.52±0.007/*	negative
UPc2	2.1±0.13/*	64.6±0.15/**	0.76±0.009/*	negative
UPc3	2.9±0.14/**	62.8±0.14/**	0.84±0.014/**	negative
UPc4	5.4±0.07/***	57.7±0.16/***	1.23±0.013/**	negative
UPc5	5.9±0.14/***	57.2±0.07/***	1.54±0.014/**	negative
UPc6	6.3±0.15/***	56.4±0.08/***	1.96±0.008/***	negative
UPc7	5.6±0.07/***	55.5±0.14/***	3.25±0.007/***	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%)

UP0 –fresh pork lard, UPc1 –pork lard to 1 month freezing, UPc2 –pork lard to 2 months freezing, UPc3 – pork lard to 3 months freezing, UPc4 – pork lard to 4 months freezing, UPc5 – pork lard to 5 months freezing, UPc6 – pork lard to 6 months freezing, UPc7 – pork lard to 7 months freezing

3.2. Physicochemical examination of frozen pork lard

During feezing storage, acidity increased gradually in the 6th month reaching the value of 1.05±0.007% (g oleic acid) (P≤0.001), exceeding the maximum permissible limit of 1% for fresh pork lard (Figure 2). From this time, a slightly sour taste and smell was installed but the color, consistency and appearance remained unchanged. The variation of physico-chemical parameters for frozen pork lard is presented in Table 2.

PV recorded an increase during storage at -15…-18^{o}C, observing a very significant increase in the 4th month when reached the value of 5.4±0.07 meq O_2/kg (P≤0.001) due to the formation of a large amount of peroxides, in the 5th and 6th months, value increased relatively slowly regarding the 4th month, in the 7th month there was a fall of the value to 5.6±0.07 meq O_2/kg corresponding to the interruption phase and division in secondary compounds.

IV decreases during freezing storage, a very significant decrease (P≤0.001) was observed in the 4th month of storage, there was an inverse correlation (r=-0.867) with PV, because of the unsaturated bounds split in the structure of fatty acids, between IV and the storage time there was an inverse correlation (r=-0.924). Malondialdehyde content increased gradually, registering the highest value in the 7th month of storage, respectively 3.25±0.007 mg/kg (P≤0.001), 8.12 times from the first month, also in this month was identified epyhidrinic aldehyde presence and other secondary compounds of oxidation which are volatile and gives pungent odor and bitter taste to pork lard, affecting the color which becomes yellow. Between MDA content and storage time there was a positive linear correlation, the correlation coefficient was r=0.975.

Based on the obtained results it can be said that pork lard stored under freezing is fresh until the 5th month (Figure 3), in the 6th month is relatively fresh showing slightly acidic taste and smell (Figure 4) and a high value of PV, and from the 7th month, the advanced oxidation is installed, underlined by color, taste, smell and appearance defects (Figure 5).

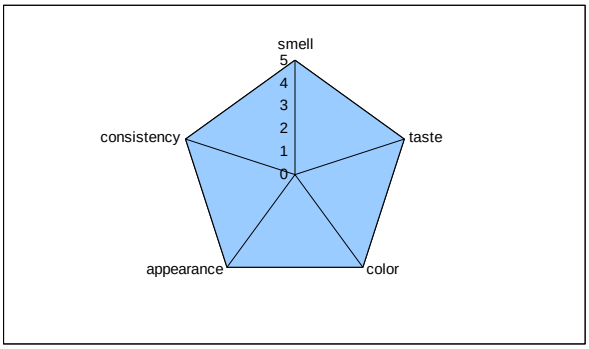

Figure 3. Sensory analysis of fresh pork lard

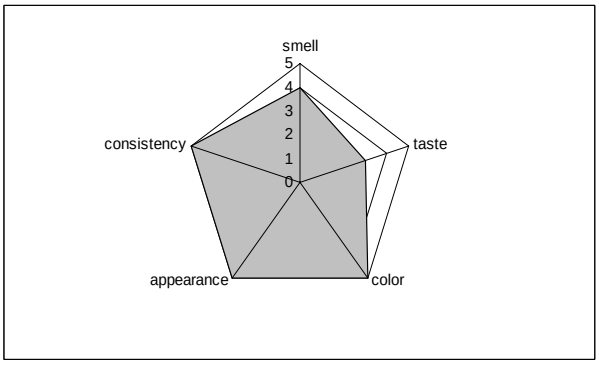

Figure 4. Sensory analysis of relatively fresh pork lard

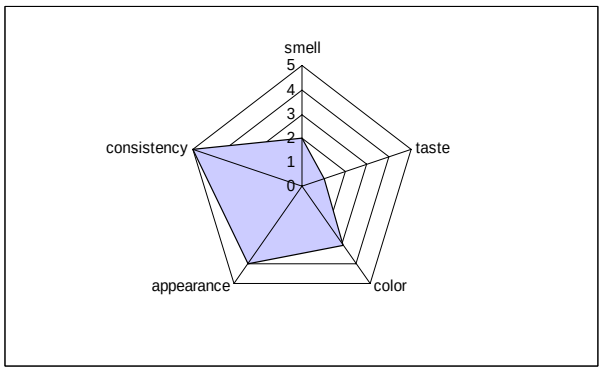

Figure 5. Sensory analysis of altered pork lard

4. Conclusions

Storage temperature had a very significant effect (P≤0.001), and storage time had a significant effect (P≤0.05) on the installation of advanced processes of hydrolysis and oxidation, the shelf life under freezing was double for that under refrigeration.

In the case of pork lard, hydrolysis process installed earlier than oxidative process, both under refrigeration and freezing, oxidation being prevented by limiting the contact with atmospheric oxygen and light intensity because pork lard was packed in unvacuumate plastic bags.

References

1. 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, doi:10.1016/S1383-5742(97)00041-0
2. 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
3. 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
4. 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*(3-4), 410–418, doi:10.1016/S0891-5849(98)00213-5
5. Nawar, W.W., Biochemical processes: *Lipid instability*. In: *Food storage stability*, Boca Raton, Washington, 2001, pp. 89–103
6. Frankel, E.N., Lipid oxidation, *Progress in Lipid Research* 1980, 19, 1–22
7. Hamilton, R.J., *The chemistry of rancidity in foods*. In: *Food storage stability*, Boca Raton, Washington, 2001, pp. 113–135
8. 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*(4), 121–125, doi: 10.1007/BF02640400
9. Contarini, G.; Pavolo, M., Volatile fraction of milk: Comparison between purge and trap and solid phase microextraction techniques, *Journal of the American Oil Chemistry Society* **2004**, *50*(25), 7350–7355, doi: 10.1021/jf025713a
10. 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, doi:10.1016/j.idairyj.2005.06.008
11. Kim, Y.D.; Morr, C.V., Dynamic headspace analysis of light activated flavor in milk, *International Dairy Journal* **2000**, *6*(2), 185–193, doi:10.1016/0958-6946(95)00001-1
12. 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
13. 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, doi: 10.1017/S0022029902005782
14. Boselli, E.; Caboni, M.F.; Rodriguez-Estrada, M.T.; Gallina Toschi, T.; Daniel, M.; Lercker, G., Photoxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions, *Food Chemistry* **2005**, *91*(4), 705–713, doi:10.1016/j.foodchem.2004.06.043
15. Simion, C.; Albu H.; Alina Simion, *Calitatea si Controlul Alimentelor*, Ed. Printech, Bucharest, 2007, pp. 120-175
16. Chávez-Servín, J.L.; Castellote, A.I.; López-Sabater, M.C., Analysis of potential and free furfural compounds in milk-based formulae by highperformance liquid chromatography: Evolution during storage, *Journal of Chromatog. A*, **2005**, *1076*(1-2),133–140, doi:10.1016/j.chroma.2005.04.046
17. Nam, K.C.; Du, M.; Ahn, D.U., Cholesterol oxidation products in irradiated raw meat with different packaging and storage time, *Meat Science*, **2001**, *58*(4), 431–435, doi:10.1016/S0309-1740(01)00046-8
18. Stanescu, V.; Savu, C., *Controlul de laborator al produselor alimentare de origine animala*, Atelierele I.A.N.B., Bucharest, 1992, pp.35-89
19. 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* **2001**, *57*(4), 413–418, doi:10.1016/S0309-1740(00)00119-4
20. Du, M.; Nam, K.C.; Ahn, D.U., Cholesterol and lipid oxidation products in cooked meat as affected by raw-meat packaging and irradiation and by cooked-meat packaging and storage time, *Journal of Food Science* **2001**, *66*(9), 1396–1401, doi: 10.1111/j.1365-2621.2001.tb15221.x
21. Eie, T.; Larsen, H.; Sorheim, O.; Pettersen, M.K.; Hansen, A.A.; Wold, J.P.; Naterstad, K.; Mielnik, M., New technologies for extending shelf life, *Italian Journal of Food Science* **2007**, *19*(2), 127–152
22. *** International Standard ISO 3976, 2006
23. ***Romanian Standard SR EN-14082, Bucharest, 2003
24. *** Romanian Standard SR EN 661, Bucharest, 1998