Installation of Hydrolysis and Oxidation Processes in Animal Fats during Refrigeration and Freezing Storage

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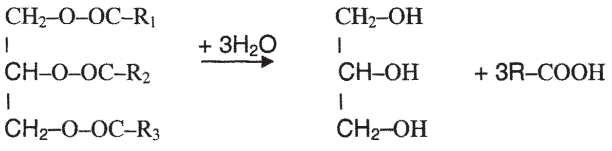
Physico-chemical characteristics and freshness indicators of cow butter during refrigeration (2 ... 4°C) and freezing (-15 ...- 18°C) storage were studied. Alteration (hydrolysis and oxidation) of food is responsible for the degradation of sensory quality, nutritional value and even the formation of toxic substances such as peroxides, which requires intimate knowledge of these processes and taking appropriate measures to avoid losses that can be registered. Research motivation was the determination of physico-chemical indicators in fresh milk fat, and the moment when occur changes in organoleptic and physico-chemical parametres of butter stored under refrigeration and freezing, making it unsuitable for human consumption. Changes in freshness parameters and the installation of alterative process when butter becomes improperly for consumption were studied inducing fatty acid content, acidity, peroxide index, iodine index and the presence of epyhidrinic aldehyde. The content of saturated fatty acids was higher (71.84%) than that of unsaturated fatty acids (27.09%), the main fatty acids present in butter were butyric, miristic, palmitic, oleic and stearic acids. There was an increase of titrabile acidity during storage, butter hydrolysis was installed after 15 days under refrigeration and after one month under freezing conditions. Results showed that butter is resistant to oxidation, epyhidrinic aldehyde was shown after 6 months of storage under refrigeration and after 11 months in freezing conditions.

Keywords: milk fat, physicochemical characteristics, refrigeration, freezing, freshness

Butter is considered one of the most popular concentrated milk products. Its nutritive value is high and is based on fat content. Digestibility of butter is 97% for fat and 94% for dry plasma, representing an important source of vitamin E [7].

Hydrolysis and oxidation occurring in animal fats during their storage have resulted in the depreciation of their quality and their exclusion from the diet.

Hydrolysis is the type of alteration which is finalized with the release of the two primary components: fatty acids and glycerine [1 , 4, 12].

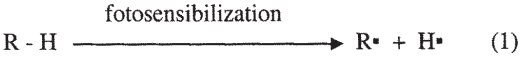


The first factor which requires hydrolysis is the water content of fat, the other factor being hydrolitic specific enzymes [12].

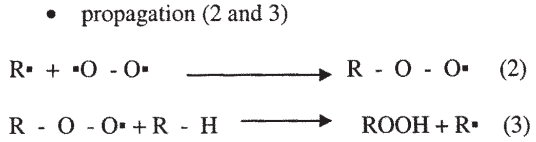
Lipid oxidation includes fatty acid oxidation and generates compounds that affect food quality, due to changes in color, flavor, texture and even nutrition and food safety [9, 14].

Autooxidation is the reaction of atmospheric oxygen and lipids, because unsaturated ties, after this process being irreversibly compromised the quality of fatty substances, not only in terms of organoleptic (taste and aroma) but also in terms of toxicology. Lipid oxidation involves radicals reactions which unfolds in three steps [14]:

- initiation (1)

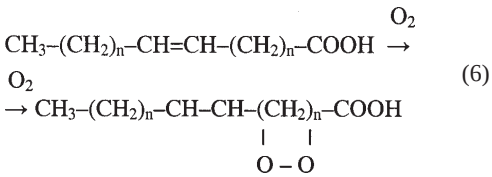


- propagation (2 and 3)



- interruption or end (4 and 5)

In the initial phase of oxidation, oxygen is fixed in the peroxidic form at double links of several molecules of unsaturated fatty acids [12, 14]:



In an advanced stage, the peroxidic bound is breaking, after which results a lot of chemical compounds of decomposition: aldehydes, ketones, alcohols, inferior acids, acids-alcohols, acids –aldehydes, acids-ketones, etc. [14]. Reached at this stage fat becomes unfit for consumption. 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 are installed organoleptic changes, easily discernible using the senses: yellow color, smell and taste of rancid [9, 14]. Peroxide index provides us information on the incipient oxidation, and Kreis reaction illustrates advanced oxidation.

Research motivation is the determination of physico-chemical indicators in fresh milk fat, and the moment when occur changes in the organoleptic and physico-chemical

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parametres of butter stored under refrigeration and freezing, making it unsuitable for human consumption.

Experimental part

Samples

Butter with a content of 80% fat and 16% water was collected immediately after obtaining in a processing milk unit and stored under refrigeration (2 ... 4°C) and freezing (-15 ...- 18°C), following the installation of altering processes (hydrolysis and oxidation).

Physicochemical examination

Fatty acid composition was determined using gas chromatography (GC-FID) Shimadzu GC-17 A (Tokyo, Japan), coupled with flame ionisation detector. Gas chromatography column is Alltech AT-Wax, 0.25 mm I.D., 0.25 μm thick stationary phase (polyethylene), used helium as carrier gas at a pressure of 147 kPa, temperature of the injector and detector was set to 260°C, the oven program was the following: 70°C for 2 min., then the temperature was raised up to 150°C with a gradient of 10°C/min., a level of 3 min. and the temperature was raised up to 235°C with a gradient of 4°C/min. The method consists in transforming fatty acids in methyl esters in the sample under analysis, followed by separation of components on a chromatography column, their identification by comparison with standard chromatograms and quantitative determination of fatty acids. By comparing the distances of each peak from analyzed sample chromatogram with peaks distances from standard chromatograms, we identify each fatty acid present in the analyzed sample. Results were expressed as w/w (%) total fatty acids [6, 11, 16] .

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 phenophtaleine, as an indicator. Acidity was expressed in oleic acid grams to 100 g of fat [16, 17].

Peroxide index was determined using UV - VIS T60U spectrophotometer (England): operating temperature 5 – 45°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 peroxide value, 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 of fat.

Iodine index was determined using Hanus method [16]. There have been weighing on analytical balance, 0.5 g fat, melted in advance, were added 10 mL chloroform under continuous agitation for complete dissolution and 25 ml solution Hanus. After homogenization, was plugged with cork and let in the darkness 30-60 min. Then were added 20 mL solution of potassium iodide 15%, fresh prepared, and 100 mL distilled water, that were well washed stopper and neck vessel, not to remain traces of iodine on them. In parallel, has been a witnessed sample, in the same conditions, but without fat and titrated with sodium thiosulphate 0.1 N. Around the end of titration (straw-yellow color), was add 1 mL starch solution 1% and continued titration, drop by drop, until the sudden disappearance of the color blue. Iodine index was calculated as the amount of iodine in g, in addition to 100 g fat.

By Kreis reaction we identify aldehydes results in advanced stages of fat oxidation. Epihidrinic 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 epihidrinic aldehyde, and so with the oxidation process [16].

Results and discussion

Fatty acid composition was determined at the beginning of the experiment and after 4 months of storage under refrigeration when alterative processes were installed, to determine if there are changes in saturated, mono-unsaturated and polyunsaturated fatty acid content.

Figure 1 illustrates sample chromatogram for fresh milk fat in which fatty acids are registered in the form of peaks separated from each other by increasing the length chain, and at the same length chain by increasing the unsaturated degree.

The content of saturated fatty acids of butter was higher (71.84%) than that of unsaturated fatty acids (27.09%), major fatty acids presented were butyric, miristic, palmitic, stearic and oleic acids. Palmitic acid was determined in the largest proportion (32.04%), these results are in agreement with previous studies on different types of milk cow butter. According to the researchers the content of palmitic acid in the „Fulani butter" was 30.2%, and Sagdic et al. presented a content of palmitic acid in the „Turkish butter" of 33.72% [5, 13].

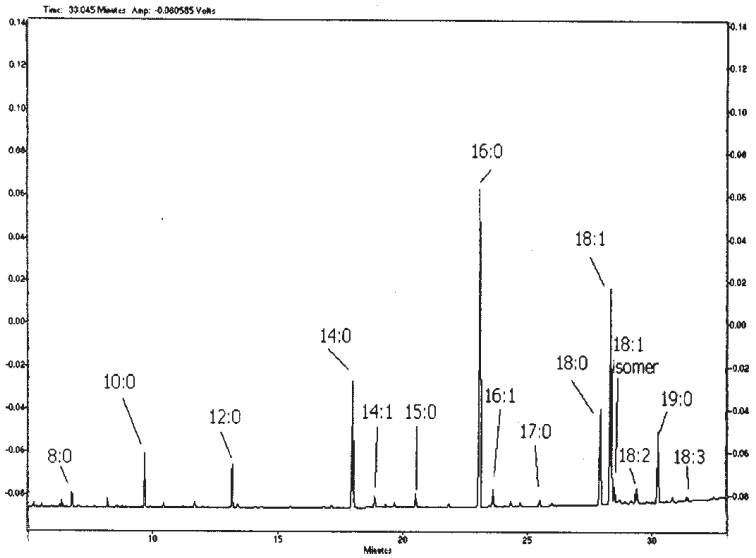

Fig. 1. Chromatogram of fresh cow milk fat

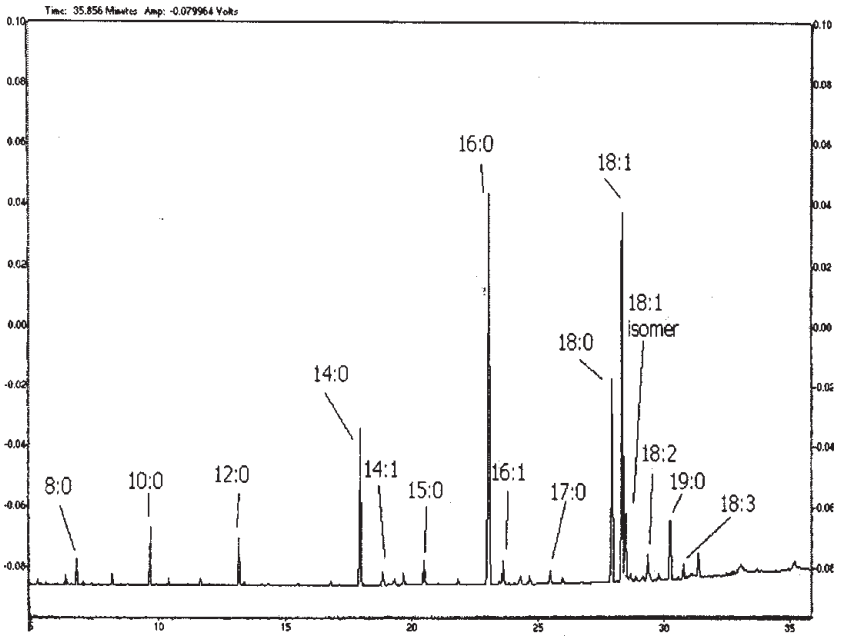

Fig.2 Chromatogram of milk fat to 4 months under refrigeration storage

In milk fat sample to 4 months refrigeration (fig. 2), fatty acid composition presented some differences from the fresh sample. In general, saturated fatty acids content increased to 70.41%, monounsaturated fatty acids content decreased to 28.23% and polyunsaturated fatty acids content decreased to 1.35%. It was concluded that the increase of saturated fatty acids content is due to hydrolysis leading to the release of acids from triglycerides structure, and the decrease of monounsaturated and polyunsaturated fatty acids is due to unsaturated fatty acids oxidation.

To watch the acid hydrolysis were determined following values of titrable acidity of butter stored under refrigeration (2 ... 4°C), determinations were executed at intervals of 5 days. The total acidity varied as follows: 1% (g oleic acid) for fresh butter, 1.1% after 5 days of refrigeration storage, 1.3% after 10 days of refrigeration storage, 1.7% after 15 days of refrigeration storage, and 2.1% after 20 days of refrigeration storage. The results are mean values of three determinations showing that for 16% water content butter, hydrolysis is triggered early and developed rapidly, after 5 days of refirgeration has registered a moderate increase of acidity, and this enhance during storage. It was found that advanced hydrolysis process appears after 15 days of refrigeration, acidity exceeds 2% (g oleic acid), the maximum permitted value, because there were released saturated fatty acids which are volatile, there are changes in color (yellow), taste (sour, rancid) and odour (butyric) and butter becomes improper for consumption.

In assessing the degree of freshness and intensity of oxidation process for chilled butter were determined iodine index, peroxide index as an indicator of incipient oxidation [10, 15] and epihidrinic aldehyde which is an indicator of advanced oxidation [12], determinations were performed at intervals of one months until it was pointed Kreis positive feedback, when was determined the installation of advanced oxidation process.

There were determined the following values for iodine index, the results are mean values of three determinations: for fresh butter 34 g I_2 / 100 g butter, butter at 1 month refrigeration 33.6; butter at 2 months refrigeration 33; butter at 3 months refrigeration 32.3; butter at 4 months refrigeration 29.9; butter at 5 months refrigeration 25.2, butter at 6 months refrigeration 23.7 and butter at 7 months refrigeration 22.3. In the first 3 months iodine index values falls slightly, in month 5 the decrease was more pronounced, in line with the propagation phase of lipid oxidation that forms the largest quantity of hydroperoxides, then the decrease represents a slow slope as presented in figure 3. During storage there was a fall of iodine index values, because with the beginning of oxidation processes decreases the degree of unsaturation due to unsaturated fatty acids oxidation [15].

For fresh butter the peroxide index was determined to be 0.4 meq O_2/kg butter, followed an upward slope. In the first 4 months of storage under refrigeration there was a slow increase of the peroxide index, which corresponds to the initiation phase of oxidation [10, 15], followed by a sharp increase corresponding to propagation phase in which are formed the largest amount of hydroperoxides as primary compounds of oxidation, value reached at 3.4 meq O_2/kg, in month 6 the growth is relatively constant, more than 3.9 meq O_2/kg because the balance formed between peroxides and secondary compounds, after which the peroxide index decreases as a result of the split of hydroperoxides in secondary compounds, in this moment Kreis reaction is positive indicating epihidrinic aldehyde presence (fig.4).

To follow the acid hydrolysis of butter stored under freezing conditions (-15 ...- 18°C), the following values of titrable acidity were determined, determinations being made on a time interval of one month: fresh butter had 1% (g oleic acid) acidity, the one month post-freezing butter had 1.6%, and the two month post-freezing butter had 2.1%. The results showed that butter acidity can reach 2.1% (g oleic acid), 1 month post freezing, exceeding the maximum limit permitted, the advanced hydrolysis being installed, butter becoming unsuitable for consumption.

To watch the installation of oxidation process were determined the following values of iodine index for butter store under freezing conditions, the results are mean values of three determinations: for fresh butter 34 g I_2/100 g of fat; butter at 1 month freezing 33.7; butter at 2 months freezeing 33.2; butter at 3 months freezing 32.5; butter at 4 months freezing 31.8; butter at 5 months freezing 30.9; butter at 6 months freezing 28.7; butter at 7 months freezing 27.4; butter at 8 months freezing 26, butter at 9 months freezing 22.4, butter at 10 months freezing 21.3, butter at 11 months freezing 20.5 and butter at 12 months freezing 20.1. Results showed that during storage there is a fall of iodine index values, because with the beginning of oxidation processes

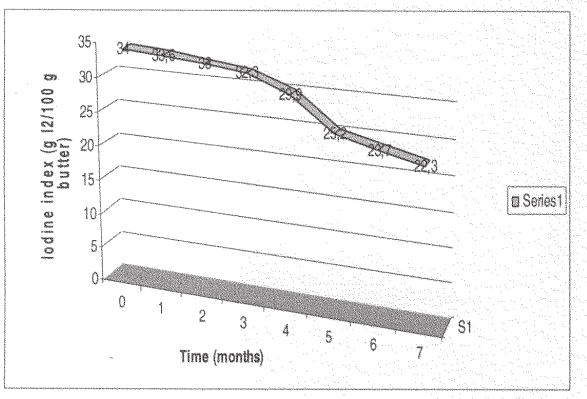

Fig. 3. Iodine index variation of butter stored under refrigeration

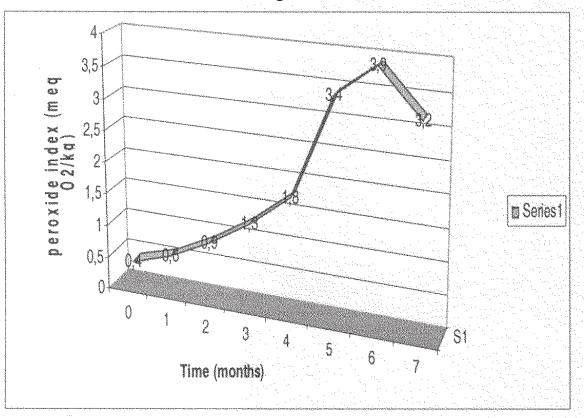

Fig. 4. Peroxide index variation of butter stored under refrigeration

decreases the degree of unsaturation due to unsaturated fatty acids oxidation (fig.5).

Figure 6 shows that in the first 7 months of storage under freezing there was a slow increase of the peroxide index, which corresponds to the initiation phase of oxidation, followed by a sharp increase corresponding to propagation phase [10, 15] in which are formed the largest amount of hydroperoxides as primary compounds of oxidation, value reached at 5.6 meq O_2/kg, in the next 2 months the growth is relatively constant because the balance formed between peroxides and secondary compounds, and after 11 months the peroxide index decreases as a result of the split of hydroperoxides in secondary compounds, in this moment Kreis reaction is positive indicating epihidrinic aldehyde presence.

Conclusions

The timing of changes occurring in hydrolysis and oxidation processes of cow milk fat has particular importance in assessing the quality and its validity.

In frozen butter altering processes take place more slowly than in that stored under refrigeration. Hydrolysis process is installed more quickly in terms of refrigeration and freezing than oxidative processes, being intensified by a higher water content in product and by hydrolitic enzymes presence. Results showed that butter is likely to acid hydrolysis due to the high water content (16%), which favors glycerides hydrolysis translated by increasing of titrable acidity until it exceeds 2%, and is resistant to oxidation due to low composition in unsaturated fatty acids, advanced oxidation is installing after 6 months in chilled butter and after 11 months if frozen butter.

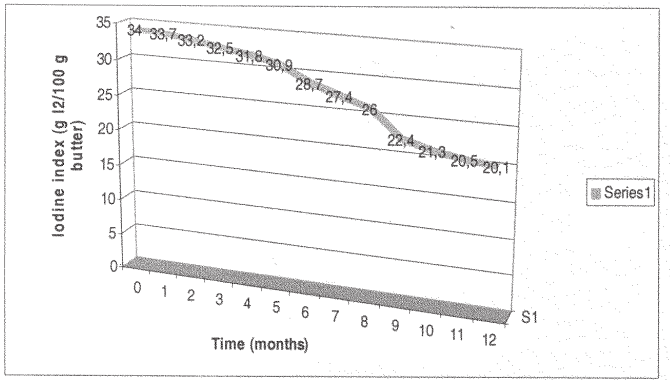

Fig. 5. Iodine index variation of butter stored under freezing

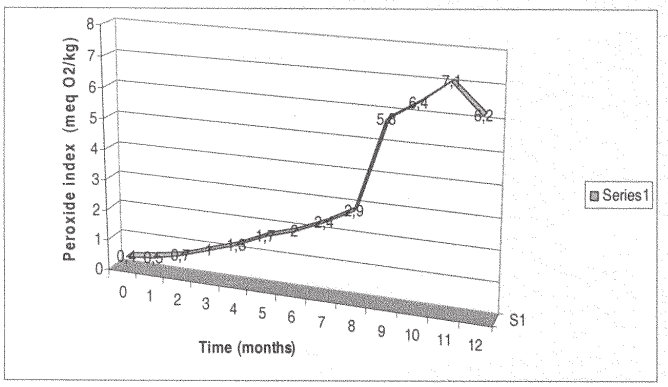

Fig. 6. Peroxide index variation of butter stored under freezing

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