

RESEARCH CONCERNING PHYSICOCHEMICAL CHANGES AND FRESHNESS INDICATORS OF MILK FAT DURING STORAGE

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Physicochemical 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 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. Changes in freshness parameters and alterative processes installation, when butter becomes improper for consumption were also studied, inducing fatty acid content, acidity, peroxide value (PV), iodine value (IV) and the presence of epyhidrinic aldehyde. There was an increase of titrable acidity during storage; butter hydrolysis was installed after 15 days under refrigeration and after one month under freezing conditions. Hydrolysis processes are installed more quickly in terms of refrigeration and freezing than oxidative processes, being intensified by a higher water content in product and by hydrolytic enzymes presence. Results showed that butter was resistant to oxidation, advanced oxidation being installed after 6 months in chilled butter and after 9 months in frozen butter.

Keywords: cow butter, hydrolysis, oxidation, refrigeration, freezing.

1. Introduction

Milk fat is one of the most complex fats found in nature (Amer, Kupranycz, and Baker, 1985). This complexity stems from the extreme diversity of its fatty acids (FA) (e.g., chain length, degree of unsaturation and branching) and more than 400 of these have been identified recently (Jensen and Newburg, 1995). 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 (Gus, 2003).

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. The first factor which requires hydrolysis is water content of fat, the other factor being hydrolytic specific enzymes (Ciobanu, D., and Ciobanu, R., 2001; Banu et al., 2002).

Lipid oxidation includes fatty acid oxidation and generates compounds that affect food quality and even nutrition and food safety (Gertz, Klostermann, and Kochhar, 2000; Tofana Maria, 2006). Oxidative rancidity or autooxidation 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 (Moller and Wallin, 1998) that may be involved in aging process (Liu et al., 1998), hypertension (Russo et al., 1998) and cancer growth (Navarro et al., 1999).

Research motivation is the determination of physicochemical indicators of fresh cow butter, and the moment when changes occur in the organoleptic and physicochemical parameters of butter stored under refrigeration and freezing, following hydrolysis and oxidation, making it unsuitable for human consumption.

2. Materials and methods

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).

Fatty acid composition was determined using gas chromatography (GC-FID) Shimadzu GC-17 A 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 (SR EN ISO 3727-2, 2002).

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.

Determination of acidity is the basic criterion for assessing the intensity of hydrolysis. The method consists in neutralizing fat acidity with potassium hydroxide 0.1 N, using phenophtaleine, as an indicator. Acidity was expressed in oleic acid grams to 100 grams sample (SR EN ISO 661, 1996).

Iodine value was determined using Hanus method (STAS 145/19-90). Approximately, 0.5 g sample (dissolved in 15 ml CCl₄) 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 I₂ in the presence of 20 ml of KI (100 g/l) and 100 ml distilled water. Free I₂ was measured by titration with 24.9 g/l Na₂S₂O₃·5H₂O using starch (1.0 g/100 ml) as an indicator. IV was calculated as g I₂/100 g sample.

Peroxide value 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 (Fe³⁺) derived from the oxidation of ferrous ions (Fe²⁺) by hydroperoxides, in the presence of ammonium thiocyanate (NH₄SCN). Thiocyanate ions (SCN⁻) react with Fe³⁺ 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. Fe³⁺ expressed in µg) was constructed and peroxide value was expressed as meq O₂/kg sample (ISO 3976, 2006).

By Kreiss 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 (SR ISO 6884, 1993).

3. Results and discussions

Physicochemical examination of chilled butter

The content of saturated fatty acids in milk fat was higher (68.35%) than monounsaturated fatty acids (39.25%) and polyunsaturated fatty acids (2.4%), the major fatty acids present in milk fat were butyric, palmitic, capric and oleic acids (Figure 1). Palmitic acid was determined in the largest

proportion (26.85%), these results are in agreement with previous studies on different types of milk cow butter (Glew, Okolo, Chuang and Vanderjagt, 1999; Samet-Bali, Ayadi and Attia, 2008). Fatty acid composition was determined at the beginning of the experiment to determine butter authenticity and to detect its counterfeiting with fat of vegetable origin.

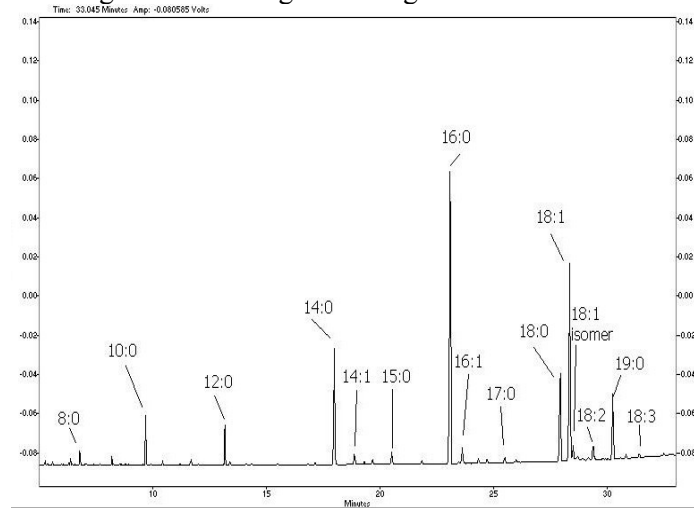


Figure 1. Fatty acids composition of cow butter

Following values of titratable acidity for butter stored under refrigeration (2 ... 4°C) were determined, determinations being made every 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 was triggered early and developed rapidly, in 5 days post refrigeration registering a moderate increase of acidity enhanced during storage. It was found that advanced hydrolysis process appeared after 15 days under refrigeration, acidity exceeded 2% (g oleic acid), the maximum permitted value, because saturated fatty acids which are volatile were released. There were changes in color (yellow), taste (sour, rancidity), odour (butyric), and butter become improper for consumption. The following values for iodine index were determined: for fresh butter 34 g I₂/100 g sample, butter to 1 month refrigeration 33.6; butter to 2 months refrigeration 33; butter to 3 months refrigeration 32.3; butter to 4 months refrigeration 27.9; butter to 5 months refrigeration 27.2 and butter to 6 months refrigeration 26.4. In the first 3 months iodine index values fell slightly, in month 4 the decrease was more pronounced, in line with the propagation phase of lipid oxidation that formed the largest quantity of hydroperoxides, then the decrease presented a slow slope as indicated in Figure 2.

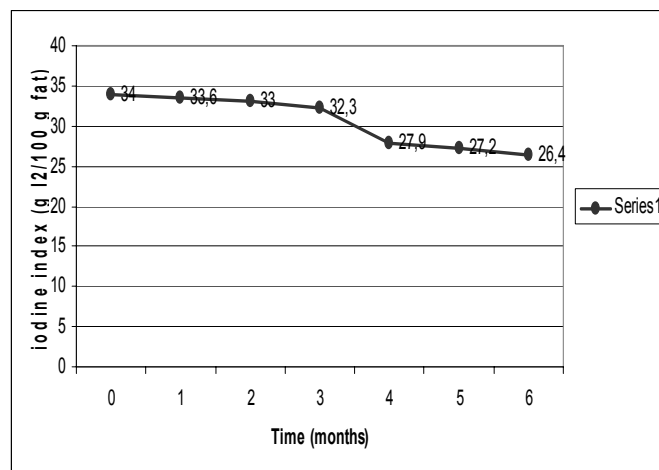


Figure 2. Iodine index variation of chilled butter

During the refrigeration storage there was a fall of iodine index values, because the beginning of oxidation processes decreased the degree of unsaturation due to unsaturated fatty acids oxidation (Vito, Ferioli, Riciputi, Iafelice, Marconi, & Caboni, 2008).

For fresh butter the peroxide value was determined to be 0.4 meq O₂/kg and followed an upward slope. In the first 4 months of storage under refrigeration, there was a slow increase of the peroxide index corresponding to the initiation phase of oxidation (Naz et al., 2005), followed by a sharp increase corresponding to the propagation phase in which the largest amounts of hydro-peroxides, as primary compounds of oxidation, were formed, their values reaching 3.4 meq O₂/kg in the 6th month, the growth being relatively constant upwards from 3.9 meq O₂/kg, as the balance between peroxides and secondary compounds was followed by a decrease of the peroxide value as a result of the hydroperoxides split into secondary compounds. It is at this moment when Kreiss reaction is positive, indicating epoxyhydrinic aldehyde presence (Figure 3).

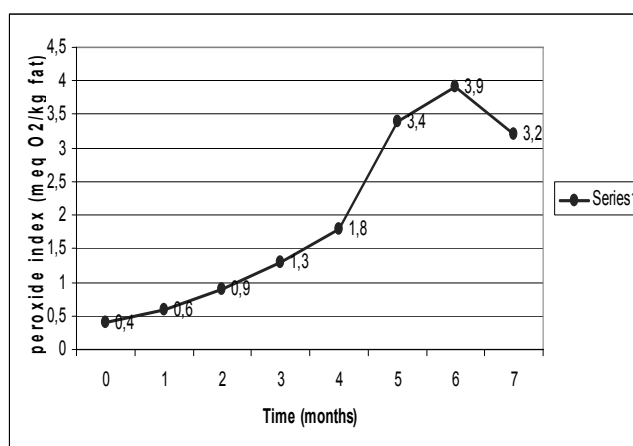


Figure 3. Peroxide index variation of chilled butter

Physicochemical examination of frozen butter

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, the following values of iodine index for butter stored under freezing were determined: for fresh butter sample, 34 g I₂/100 g; for butter sample, one month post-freezing, 33.7 g I₂/100 g; for butter sample, two months post-freezing, 33.2 g I₂/100 g; for butter sample, 3 months post-freezing, 32.5 g I₂/100 g; for butter sample, 4 months post-freezing, 31.8 g I₂/100 g; for butter sample, 5 months post-freezing, 30.9 g I₂/100 g; for butter sample, 6 months post-freezing, 28.7 g I₂/100 g; for butter sample, 7 months post-freezing, 24.4 g I₂/100 g; for butter sample, 8 months post-freezing, 23.6 g I₂/100 g; for butter sample, 8 months post-freezing, 22.3 g I₂/100 g. The values are expressed as the average of three determinations. The results showed a fall of iodine index values, as the beginning of oxidation processes caused the decrease of the degree of unsaturation accounted for by unsaturated fatty acids oxidation (Figure 4).

Figure 5 illustrates that in the first six months of storage under freezing, there was a slow increase of the peroxide value, corresponding to the initiation phase of oxidation, followed by a sharp increase corresponding, in its turn, to propagation phase in which the largest amounts of hydroperoxides were formed as primary compounds of oxidation (Naz et al., 2005), their value reaching 3.5 meq O₂/kg. In the next 3 months, the growth was relatively constant, on account of the balance between peroxides and their secondary compounds. After nine months, the peroxide value decreased as a result of the split of hydroperoxides in secondary compounds. It is at this moment when Kreiss reaction is positive indicating epoxyhydrinic aldehyde presence.

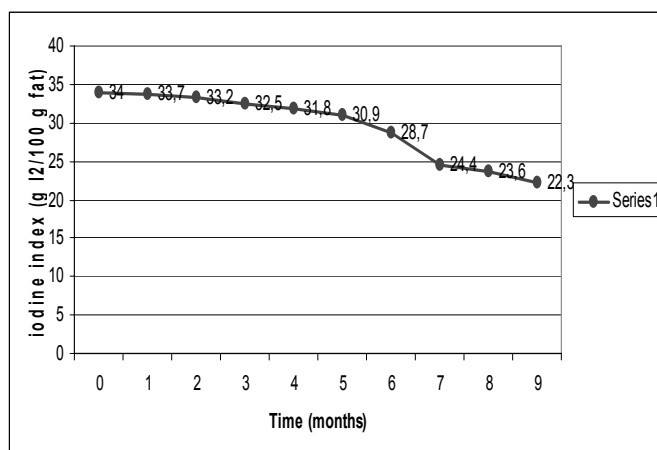


Figure 4. Iodine index variation of frozen butter

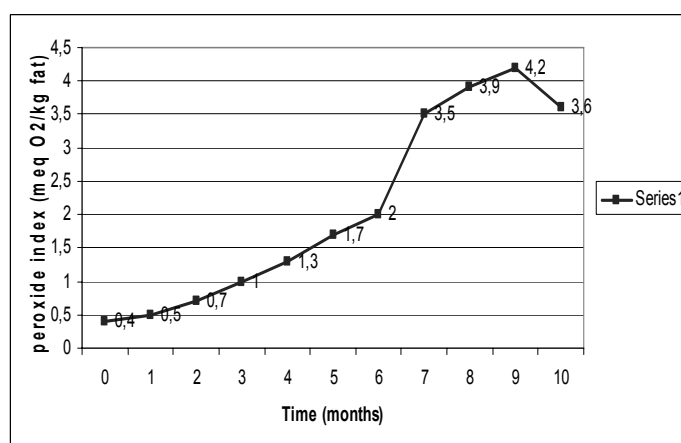


Figure 5. Peroxide index variation of frozen butter

4. Conclusions

The timing of changes occurring in hydrolysis and oxidation processes of cow butter has particular importance in assessing the quality and its preservation. In frozen butter altering processes take place more slowly than in that stored under refrigeration. In case of both, refrigeration and freezing storage, the hydrolysis process installed more quickly than the oxidative processes, being intensified by the high water content of the product and by lipases presence.

It should be noted that acid hydrolysis was installed quickly in butter due to the high water content (16%), which favors glycerides hydrolysis translated by the increase of the titratable acidity until it exceeds 2%. The butter was resistant to oxidation due to low unsaturated fatty acids content; the advanced oxidation was installed after 6 months in case of chilled butter and after 9 months in case of frozen butter.

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