

Evaluation of oxidation and hydrolysis in milk fat during freezing storage

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Abstract

Changes in freshness parameters and the installation of alterative processes when butter becomes improperly for consumption were studied inducing fatty acid content, peroxide value (PV), iodine value (IV), acid value (AV), thiobarbituric acid reactive substances (TBARS) and the presence of epyhidric aldehyde.

The content of saturated fatty acids was higher (71.84%) than that of unsaturated fatty acids (27.09%), the main fatty acids presented in butter were butyric, miristic, palmitic, oleic and stearic acids.

Iodine index for butter stored under freezing conditions decreased significantly to the 8th month ($P \leq 0.01$), followed by a very significant decrease to the 9th month ($P \leq 0.001$), then the decrease was relatively slow until the 12th month, between the storage time and IV there was determined an inverse correlation ($R = -0.972$).

The advanced hydrolysis process was installed after about 30 days, acidity exceeded 2% (g oleic acid) ($P \leq 0.01$), the maximum permitted limit for fresh butter, between acidity values and storage time there was determined a perfect correlation ($R = 0.993$). The induction period for butter stored under freezing conditions was about 8 months, propagation period was about 3 months, and the period of decline began in the 12th month when were formed the secondary compounds of oxidation, after 11 months the oxidative status of butter sample passed from primary to secondary state

Keywords: milk fat, peroxide value, iodine value, oxidation, freezing

1. Introduction

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 and represents an important source of E vitamin [1].

Nowadays there are registered many metabolic imbalances, awarded on the one hand to reduction of physical effort, sedentariness, and on the other

to the growth of nerve demand and daily stress, to environmental pollution, food pollution implicitly.

Excessive consumption of fat food, especially saturated fat led to the emergence of health problems, increasing of blood pressure and cholesterol levels, increasing the number of patients with cardiac and circulatory diseases [2,3].

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

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 [4].

Unsaturated lipids are less dangerous and contain significant amounts of liposoluble vitamins useful to the body, which have antioxidant function in fat food and body, preventing many diseases due to oxidative stress. Of unsaturated fatty acids, very important are linoleic, linolenic and arachidonic acids called essential fatty acids, that can not be synthesized by the body, they should be brought by food intake [2,5]. Lipids are solvents for liposoluble vitamins, providing their introduction in the body by gastrointestinal tract. Some fats are themselves an important source of vitamins, butter and fish fat [3].

Autooxidation is the reaction of atmospheric oxygen and lipids, at unsaturated links, 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. In the initial phase of oxidation, oxygen is fixed in the peroxidic form at double links of several molecules of unsaturated fatty acids [2,6]. 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. [3]. 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 ranced. Peroxide index provides us information on the incipient oxidation, and *Kreis* reaction illustrates advanced oxidation [7].

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 [8,9]. During storage or food processing, lipid autoxidation is the main reaction for organoleptic (color, flavor, change of texture) and nutritional (loss of essential fatty acids, nutritional losses) deterioration [10].

The purpose of this study was to follow the stability under freezing storage (-15...- 18°C) of milk fat, by quality parameters monitoring to determine its validity.

2. Materials and Methods

2.1. Samples

Butter with a 80% fat content was collected at production from a milk processing unit, samples of 50 g were packed in foil and stored under freezing (-15...- 18°C), the research aim was to study the physicochemical and organoleptic changes, and the installation time of alterative processes (hydrolysis and oxidation).

2.2. Chemical analysis

2.2.1. *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 [11].

2.2.2. *Iodine value (IV)*. Iodine value was determined using Hanus method. Approximately, 0.5 g sample (dissolved in 15 mL CCl₄) 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 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 [11].

2.2.3. *Peroxide value (PV)*. Peroxide value was determined using UV-VIS spectrophotometer. 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). 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 [12].

2.2.4. 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 [12].

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 [11].

2.2.6. Fatty acids composition. Fatty acids content was determined using Hp-Hewlett Packard 5890 GC, chromatography column with high resolution, flame ionisation detector, by transformation in methyl esters of fatty acids in the sample under analysis, followed by components separation on a chromatography column, their identification by comparison with standard cromatograms and quantitative determination of fatty acids [11].

2.2.7. 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

In the case of butter was determined a content of 72.87% saturated fatty acids (SFA), greater than the content of monounsaturated fatty acids (MUFA) (24.37%) and polyunsaturated fatty acids (PUFA) (2.72%), that reflects the small value of refractive index and iodine index, the moderate value of melting point, as well as the high value of saponification index.

The fatty acid content for fresh and oxidized milk fat is presented in Table1.

For fresh butter was determined a SFA:MUFA:PUFA=26.79:8.95:1 report, and essential fatty acids:non-essential fatty acids = 1:8.95.

Variations in fatty acid content with a more significant decrease for unsaturated fatty acids were found in the case of milk powder storage at ambient temperature and at 15°C [10].

For butter stored under freezing conditions it was found that the advanced hydrolysis process installed after about 30 days, acidity exceeded 2% (g oleic acid) ($P \leq 0.01$), the maximum permitted limit for fresh butter, between acidity values and storage time there was a perfect correlation ($R=0.993$). There are changes of taste (sour) and odor (butyric, sudorific), due to the release of lower saturated fatty acids from triglycerides structure, and butter became unsitabile for consumption.

In the first 8 months of storage under freezing conditions there was a significant increase of peroxide index from 0.4 ± 0.07 meq O_2/kg to 2.9 ± 0.14 meq O_2/kg ($P \leq 0.01$), which corresponded to the initiation stage of oxidation, followed by a very significant increase in the 9th month ($P \leq 0.001$), this increase corresponded to the propagation phase of oxidation when are formed the largest amount of peroxides, in the 10th and 11th months the increase was relatively constant, assuming that was due to the balance formed between the peroxides and decomposition products, and in the 12th month peroxide index value decreased due to hydroperoxides division in secondary compounds (Figure 1), in this month was also identified the presence of epyhidrinic aldehyde, indicating the installation of advanced oxidation process. It can be concluded that the induction period for butter stored under freezing conditions was about 8 months, propagation period was about 3 months, and the period of decline began in the 12th month when were formed the secondary compounds of oxidation, after 11 months the oxidative status of butter sample changed from primary to secondary state. Between PV and storage time there was determined a linear relationship ($R=0.972$) until 11th months of storage, and after this month there was an inverse correlation ($R=-0.937$).

Iodine index for butter stored under freezing conditions decreased significantly to the 8th month ($P \leq 0.01$), followed by a very significant decrease to the 9th month ($P \leq 0.001$), then the decrease was relatively slow until the 12th month (Figure 2), between the storage time and IV there was determined an inverse correlation ($R = -0.972$). Vito et al. [14] also reported a decrease in IV levels on the stability of products enriched with n-3 polyunsaturated acids.

Malondialdehyde (MDA) content increased during storage in freezing conditions, in the 12th month was registered a very significant increase ($P \leq 0.001$) (Figure 3), 12.12 times more than in the first month of storage. The increased values of MDA content were recorded in the interruption stage of oxidation, there was an inverse correlation with PV which decreased in this stage ($R = -0.689$).

Based on the obtained results it can be concluded that for frozen butter the advanced hydrolysis process was installed after 30 days and the advanced oxidation process was installed after 11 months. 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 [15].

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 almost double from that under refrigeration conditions.

For butter, hydrolysis processes were installed much earlier than oxidative processes, because of the high water content, the presence of hydrolytic enzymes and microbial agents, oxidation was prevented by limiting the contact with atmospheric oxygen and light intensity through the packaging method.

Based on the obtained results it can be concluded that for butter with 80% fat and 16% water content, the shelf life under freezing storage was about 30 days, even if the oxidation process was not yet installed, butter should be excluded from the food circuit because of rancid taste and odor produced by the lipolysis when were released lower saturated acids which are volatile.

In another study [15] was noted that advanced oxidation process in milk fat was installed after 7 months under refrigerated storage and after 12 months of storage under freezing.

Table 1. Fatty acid composition for fresh and oxidised milk fat

Fatty acid name	Abbreviation	Fresh milk fat	Oxidised milk fat
		%	%
Butyric	4:0	18.59	traces
Caprylic	8:0	1.60	1.74
Capric	10:0	1.91	1.99
Lauric	12:0	0.67	0.88
Myristic	14:0	7.98	8.08
Myristoleic	14:1	0.62	0.60
Pentadecanoic	15:0	1.26	1.32
Palmitic	16:0	24.09	24.30
Palmitoleic	16:1	1.25	1.23
Margaric	17:0	0.74	0.80
Stearic	18:0	11.73	11.84
Oleic	18:1	22.76	22.65
Vaccenic	18:1 isomer	4.04	4.01
Linoleic	18:2	1.92	1.82
Alfalinolenic	18:3	0.80	0.70

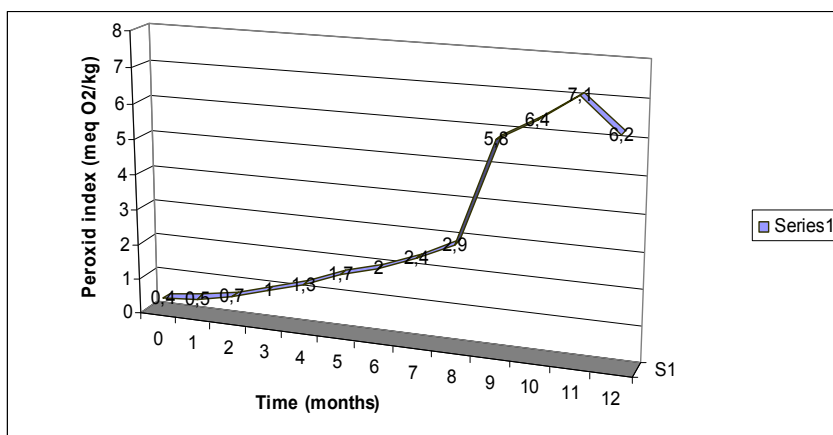


Figure 1. Peroxide index variation of butter during frozen storage

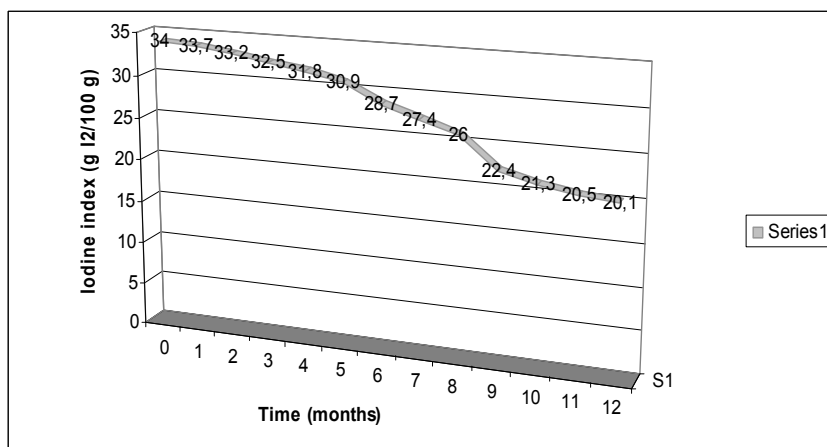


Figure 2. Iodine index variation of butter during frozen storage

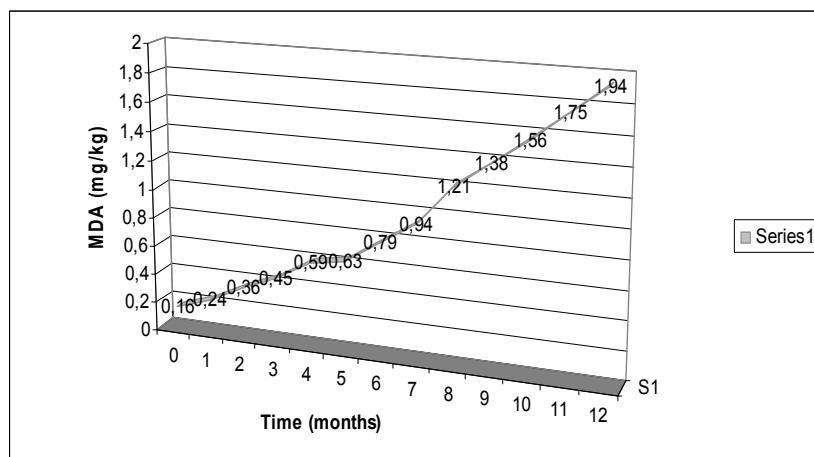


Figure 3. MDA content variation of butter during frozen storage

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For butter, hydrolysis processes were installed much earlier than oxidative processes, because of the high water content, the presence of hydrolytic enzymes and microbial agents, oxidation was prevented by limiting the contact with atmospheric oxygen and light intensity through the packaging method.

Based on the obtained results it can be concluded that for butter with 80% fat and 16% water content, the shelf life under freezing storage was about 30 days, even if the oxidation process was not yet installed, butter should be excluded from the food circuit because of rancid taste and odor produced by the lipolysis when were released lower saturated acids which are volatile.

In another study [15] was noted that advanced oxidation process in milk fat was installed after 7 months under refrigerated storage and after 12 months of storage under freezing.

Lozano et al. [16] analyzed the flavor compounds that are formed at butter storage using GC-MS, assessing the influence of storage time and package material (wax parchment paper vs. foil) on the aroma components and sensory properties of butter stored under refrigeration and freezing. Aroma compounds detected in the highest concentrations were: butanoic acid, δ -octolactone, δ -decalactone, 1-octen-3-one, dimethyl trisulfure and diacetyl. The authors also found that the amount of styrene increased with storage time in refrigerated storage compared to frozen storage and the fact that the foil package performed better than wax parchment paper to prevent styrene

migration in butter, in minimizing lipid oxidation and the formation of acid compounds which contribute to the loss of fresh flavor, and they concluded that refrigerated storage caused greater loss of fresh flavor than frozen storage.

The purpose of microscopic examination was to follow changes at microscopic level that occur when advanced oxidation process was installed in animal fats. At microscopic examination of fresh butter, fat molecules were presented under the form of molecules with different sizes, scattered in the butter water phase (Figure 4), while for oxidized butter this dispersion was not visible, fat molecules showed a more compact image with small fat globules (Figure 5).

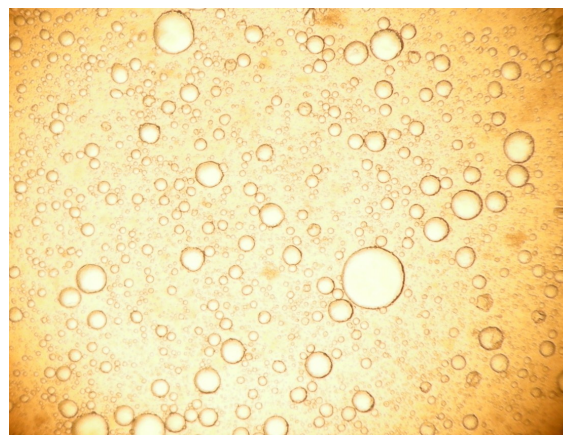


Figure 4. Microscope view for fresh milk fat

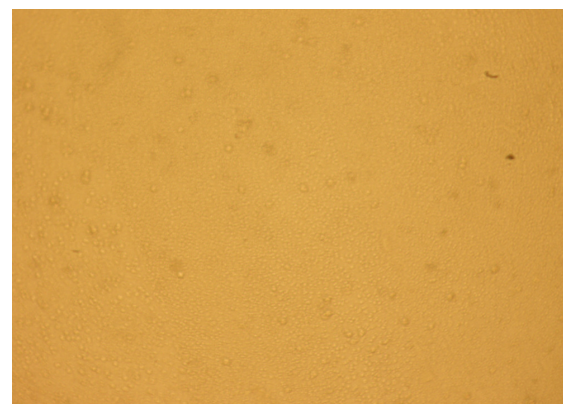


Figure 5. Microscope view for oxidised milk fat

4. Conclusions

The content of inferior saturated fatty acids, which are volatile: butyric, caprylic, capric, lauric, makes at the installation of hydrolysis process that butter to present butyric and sweaty smell, which can be confused with the installation of oxidation process, wrong appreciating that butter is oxidative altered, that is why it is necessary physicochemical analysis to determine the installation of hydrolysis process.

For butter, hydrolysis processes were installed much earlier than oxidative processes, because of the high water content, the presence of hydrolytic enzymes and microbial agents, oxidation was prevented by limiting the contact with atmospheric oxygen and light intensity through the packaging method

Based on the obtained results it can be concluded that for butter with 80% fat and 16% water content, the shelf life under frozen storage was about 30 days, even if the oxidation process was not yet installed, butter should be excluded from the food circuit because of rancid taste and odor produced by the lipolysis when were released lower saturated acids which are volatile, advanced oxidation process was installed after 11 months for frozen butter.

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.

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