Effects of storage on lipid oxidation in milk and egg mixed powder

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Summary
The aim of this study was to determine the effect of the duration of vacuum storage on oxidation of cholesterol and fatty acids, as well as on sensory properties of dried milk and egg mixed powder in comparison with whole milk powder and egg powder. Determination of the contents of fat and water, peroxide value and sensory evaluation were done according to American Oil Chemists' Society and ISO official methods. The application of vacuum packaging did not protect powders against progressing changes in lipids. Composition of the powders determined their oxidation stability and formation of oxycholesterols, hydroperoxides, the value of the browning index, as well as sensory properties. After production and during storage of mixed powders, the evaluated indeces had values between those recorded for milk powder and egg powder. On the basis of these findings, it can be concluded that the analysed milk-egg powder retained its good sensory attributes up to the 12th month of storage in spite of physical and chemical changes taking place.

Keywords
milk powder; egg mixed powder; storage; lipid oxidation; oxycholesterols

The production of dried foods has been increasing in recent years. These products require dissolution of a dry concentrate and only short subsequent culinary processing, such as cooking or baking. Production of dried semi-products is an increasingly important sector for the development of the milk and egg markets.

Production of milk in countries of European Union in 2011 was 156 million tonnes. A part of it (19.3 million tonnes) were processed to 2.1 million tonnes of milk powder [1]. The egg production in EU reached 6.4 million tonnes in 2008 [2]. The egg processing sector currently accounts for 28–30% of total egg production and is growing by 1.0% to 1.5% annually as a result of the growing trend towards the use of processed and convenience food, and the trend of increasing proportion of meals taken outside the home [3].

Dried eggs and whole milk powder are widely used in food preparation because of their higher microbiological safety in comparison with raw products. Functional properties of milk and eggs complement each other in terms of foaming, emulsification, stability and gelling ability. Egg yolk may also be applied as a colorant. Homogenous dried mixtures of milk and whole eggs or egg yolks may be used as a semi-product for preparation of cake mixes, omelet mixes, ice-creams, desserts, sausages and other food products, for preparation of which the above mentioned raw materials are used. Separate production of milk, egg or egg yolk powders and subsequent combining them requires from the producers a greater number of technological operations and creates additional problems due to the difficulty in obtaining a homogenous mixture with varying proportions of the components required in the final product.

Dry powdered food products, depending on their quality and storage conditions, are characterized by a long shelf life, ranging from several months to more than a year. However, even in such a strongly dehydrated food, adverse changes may occur, including e.g. oxidation of lipids or Maillard reactions. Literature data indicate that, in products containing considerable amounts of lipids, such as whole milk powder or egg powder, subjected to heating at high temperatures, undesirable changes caused by lipid oxidation may

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occur in the course of long-term storage [4, 5]. Several mechanisms of fatty acid oxidation yielding different products are known: autooxidation, photo-oxidation, lipooxygenase action and oxidation of sterols and fat-soluble vitamins (A, D, E and K) [6]. Lipid autooxidation is a complex of chemical reactions that causes loss of nutritive value and increase in the contents of products that can be harmful for health of consumers. This process is the main cause of food deterioration during processing and storage. Autooxidation is free-radical chain reaction initiated when fatty acids react with oxygen-forming peroxides. At the advanced lipid oxidation process, different toxic substances may be formed. Cholesterol oxidation process probably undergoes the same free-radical mechanism as the oxidation of fatty acids. More than one hundred cholesterol oxidation products (oxycholesterols, COP) were identified in food products. The best known oxycholesterols are epimers of 7α- and 7β-hydroxycholesterol (7α- and 7β-OHC), α- and β-epoxycholesterol (α- and β-EpC), as well as 7-ketocholesterol (7-keto), 20α-hydroxycholesterol (20α-OHC), 25-hydroxycholesterol (25-OHC) and cholestanetriol (triolC). Some of them demonstrated several deleterious effects to health such as cytotoxic, mutagenic, carcinogenic and atherogenic effects. COP are formed during the production and storage of food products of animal origin. All these compounds were detected in egg powder and whole milk powder. The content of COP in egg powders may vary from 8 mg·kg^{-1} to 311 mg·kg^{-1} and in milk powder from traces to 6.8 mg·kg^{-1} [7].

The rate of cholesterol oxidation reaction and the type of products formed during this process are influenced by heat treatment, the content and composition of lipids, powder packaging systems and access to light during storage. Simultaneously with lipid changes, sensory properties of the products change as well. In view of the above, in this study an attempt was made to develop a new milk and egg powder mix and record the changes occurring during its storage. In particular, changes over the storage time were studied in a vacuum-packed product by following the oxidation of fatty acids and cholesterol, as well as sensory changes, in comparison with whole milk powder and egg powder.

MATERIALS AND METHODS

Materials

Mixed powder was prepared in our laboratory by combining liquid whole milk and liquid eggs at a proportion of a typical of pancake batter (1 dm^3 : 480 g). The control comprised whole milk powder and egg powder. The mixture was heated at 55 °C and homogenized (Rannie homogenizer, Rannie, Copenhagen, Denmark) at 18 MPa for 10 min. Then it was pasteurized by plate heat exchanger (Alfa Laval, Lund, Sweden) at 60 °C for 30 min. Temperature was controlled by thermostatic apparatus. The liquid mass was dried in a spray dryer (GEA Niro, Søborg, Denmark) with inlet and outlet temperatures of 170 °C and 75 °C, respectively. Before preparing the samples, temperatures and flow speed were measured using water instead of products. Well-matched parameters of drying conditions were used for the production of egg, milk and mixed powders. Final products were packaged in bags made from laminated, blue-coloured polyethylene terephthalate/polyethylene (PET/PE 1260/330) foil. The laminate had water vapour permeability of 12 g·m^{-2} per 24 h and oxygen permeability of 73 cm^3·m^{-2} per 24 h at 0.1 MPa. Samples were vacuum-packaged but the residual of oxygen was not checked in the samples at the start of storage and during the shelf life. Samples were stored with no access to light in a room at 20 ± 1 °C and relative humidity of max. 75% for a period of 24 months. The powders were produced in three replicates.

Reagents and standards

The internal standards 19-hydroxycholesterol and 7α-hydroxycholesterol (7α-OHC) were obtained from Steraloids (Newport, Rhode Island, USA). Solvents and 5α-cholestane, 7β-hydroxycholesterol (7β-OHC), α-epoxycholesterol (α-EpC), β-epoxycholesterol (β-EpC), 25-hydroxycholesterol (25-OHC), 7-ketocholesterol (7-keto), cholestanetriol (triolC) and anhydrous pyridine were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Silylation mixture of BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) was obtained from Fluka Chemie (Buchs, Switzerland). Sep-Pak amino cartridges were obtained from Waters (Milford, Massachusetts, USA).

For identification of fatty acids, analytical standards of 37-component fatty acid methyl esters FAME mix (Supelco, Bellefonte, Pennsylvania, USA) and polyunsaturated fatty acids PUFA No. II (Animal source; Supelco) were used. Purity of COP standards ranged from 90% to 98% (the purities were: 7keto > 90%, 7β-OHC and α-EpC > 95%, 7α-OHC, 25-OHC, β-EpC and triolC > 98%).

Methods

Water and lipid contents were determined after the production of the powder. Cholesterol, oxycholesterols, fatty acid composition and browning index were determined after the production of the powder and after 3, 6, 12 and 24 months of storage. Peroxide value was determined every three months during the stotage. Sensory evaluation was done after the production of the powder and after 6, 12 and 24 months of storage.

Lipid extraction and contents of water

Lipids were extracted by the method of FOLCH et al. [8]. The amount of total lipids was determined after evaporation of the solvent in the stream of nitrogen. Water content was determined by the standard gravimetric method, described by the International Dairy Federation [9], consisting in drying of the test sample (1–3 g) at 102 °C ± 2 °C under atmospheric pressure for 2 h. A test for constancy of mass was performed by additional drying steps of 1 h until the difference in mass did not exceed 0.5 mg.

Determination of cholesterol

Cholesterol content was determined by gas chromatography, after saponification with 1 mol·l^{-1} KOH in methanol. Cholesterol was extracted with diethyl ether, solvent evaporated under vacuum and residue derivatized by silylation (AOCS Official Methods Ch 6-91) [10]. Silylated derivatives were separated on Agilent model 6890 SII gas chromatograph (Agilent, Santa Clara, California, USA) equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 mm; J&W Scientific, Folsom, California, USA). Helium was used as a carrier gas at a flow rate of 1.6 ml·min^{-1}. Analysis was performed isothermally at 290 °C, and injector and detector temperatures were set at 310 °C. Samples were injected using a split ratio of 1:25. For quantification, 5α-cholestane was applied as the internal standard, while identification was based on the retention data for compounds previously identified in our laboratory by mass spectrometry and based on published data [11].

Determination of cholesterol oxidation products

Cholesterol oxidation products (COP) were determined according to the method described by PRZYGOŃSKI et al. [12]. Isolated lipids were saponified with 10% sodium methylate in methyl-*tert*-butyl ether (4:6, v/v), cholesterol and its oxidation products were extracted with chloroform, extracts fractionated on a Sep–Pak NH2 column (Waters), solvent evaporated under vacuum and the residue was silylated. Silylated oxycholesterols were separated and quantified on an Agilent model 6890 gas chromatograph using a DB-5MS capillary column specified above. Separation was run using programmed temperature at a rate of 25 °C·min^{-1} from 60 °C to 270 °C, followed by 2.5 °C·min^{-1} until 290 °C. Helium was used as a carrier gas at a flow rate of 1 ml·min^{-1}. Injector and detector were held at 300 °C, split ratio was set at 1:40. The amount of COP was calculated using internal standard 19-hydroxycholesterol. Oxycholesterols were identified based on the retention data for compounds previously identified in our laboratory by mass spectrometry and based on published data [11].

Fatty acid composition

The fatty acid composition was analysed by following AOCS Official Method Ce 1h-05 [13]. The fatty acid methyl esters were separated on Agilent model 6890 SII gas chromatograph equipped with a SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm; Supelco). Hydrogen was used as a carrier gas at a flow rate of 1.5 ml·min^{-1}. Column temperature was programmed from 70 °C to 160 °C at 25 °C·min^{-1}, held for 30 min, then further programmed to 210 °C at 3 °C·min^{-1}. Initial and final temperatures were held for 5 min and 30 min, respectively. Detector temperature was set at 250 °C. Fatty acids were identified by comparison of the retention times with those of authentic standards and the results were reported as a weight percentage of the lipid.

Peroxide value

For the assessment of oxidative status of stored products, peroxide value was determined according to AOCS methods Cd 8-53 [14]. The results were expressed in milliequivalents of active oxygen per kilogram of lipid. Three replicates from each production part were analysed colorimetrically.

Browning index

Colour was measured using a Hitachi U-3000 apparatus (Hitachi, Tokyo, Japan) equipped with an integrating sphere at a wavelength range of 380–780 nm. Colour was determined using the C illuminant together with the Commission Internationale del'Eclariage ($L^*a^*b^*$) scale. The temperature of the assessed sample ranged from 22 °C to 24 °C. The Browning index (BI_{L^*}) was calculated using the formula of Hirschler [15]:

$$BI_{L^*} = 100 - L^* \quad (1)$$

Sensory analysis

The products were evaluated by a 10-person trained panel for sensory characteristic using

Tab. 1. Descriptive evaluation scale for food concentrates.

Score	Descriptive note
1.00–1.50	Bad
1.51–2.50	Unsatisfactory
2.51–3.50	Satisfactory
3.51–4.50	Good
4.51–5.00	Very good

a five-point scoring scale [16]. Attributes of colour, aroma, consistency and taste were evaluated using scores in accordance with the description specified in the standard. The scores were multiplied by weighting indeces for a given attribute (colour 0.1, aroma 0.3, consistency 0.2, taste 0.4) and the total was ascribed its equivalent in the descriptive scale (Tab. 1). Each sample was tested three times, $n = 90$ (10 persons, 3 replicates from each production part, three times tested).

Statistical calculations

Results given in the tables are means from 9 measurements (3 replicates from each production part). Data collected experimentally were analysed statistically using the Fisher test in the Statistica 6.0 software package (StatSoft, Tulsa, Oklahoma, USA)

RESULTS AND DISCUSSION

Lipid and water contents of dried powders

Lipid content of the tested milk powder was 26.4%, egg powder 42.9% and mixed powder 34.8%. The content of water was 2.4%, 4.0% and 3.2%, respectively. According to American Dairy Products Institute, dry whole milk powder should contain not less than 26% but less than 40% milk fat, and not more than 5% moisture [17]. Fitzpatrick et al. [18] and Liang [19] tested commercial whole milk powders and found the lipid contents to range from 26.0% to 28.2%. The American Egg Board as well as United Nations Economic Commission for Europe declared that good quality dehydrated egg powder should have the lipid content in the range from 39.0% to 41.5%, and a maximum of 5% of moisture [20, 21]. Whole dried eggs analysed by Ayadi et al. had lipid content of 33.6% [22], but those produced and stored by Caboni et al. [23] contained 45.8% of lipids and 4.7% of moisture. Obara et al. [24] found the lipid content of spray-dried whole egg powder to be 43.5% and water content to be 2.8%.

Milk and egg powders are useful for product developers in the formulation of a variety of bakery products. They provide flavour and functionality in bakery products such as biscuits, breads, cakes, cookies and muffins. These semi-products with addition of wheat flour, salt, sugar, raising agents, β-carotene contain from 22% to 26% of lipids.

Cholesterol content

The type of produced whole milk, egg and mixed powder had a statistically significant effect on cholesterol content, but there was no significant difference in the cholesterol contents during storage (Tab. 2). Concentration of cholesterol in bovine milk was from 104 $mg \cdot l^{-1}$ to 117.8 $mg \cdot l^{-1}$, which is equivalent to the content of 3.25–3.75 $g \cdot kg^{-1}$ of lipids [25, 26]. Cholesterol content in whole milk powder ranged from 0.73 $g \cdot kg^{-1}$ to 0.93 $g \cdot kg^{-1}$ of the product, while in baby formulas it was from 0.24 $g \cdot kg^{-1}$ to 0.75 $g \cdot kg^{-1}$ of the product [27]. Cholesterol content in whole spray-dried egg powder ranged from 12.2 $g \cdot kg^{-1}$ to 17.3 $g \cdot kg^{-1}$ of the products [24, 28, 29]. Obara [30] reported that the differences in cholesterol contents in egg powder during storage were only slight, although statistically significant. Mazalli and Bragagnolo [29] did not determine any changes of cholesterol content during storage of egg powder for 12 months.

Whole milk powder, as well as egg powder, are products rich in lipids and cholesterol. Their production involves a spray-drying process at high temperatures, which accelerates reactions between oxygen and lipids, resulting in the formation of cholesterol oxidation products and degradation of fatty acids.

Cholesterol oxidation products

The effect of storage time on the increase in total contents of oxycholesterols was found for all types of powder. The greatest increase in oxysterol contents (13.5-fold) after 24-month storage was recorded in the mixed powder, in egg powder it was 9-fold, while in milk powder it was 5.5-fold (Tab. 2). COP might be influenced by different values of residual oxygen in the samples, but data on this were not available.

After 24-months of storage, the percentage contribution of oxycholesterols in the total amount of cholesterol was the biggest in the milk powder, then in egg powder and the lowest was in mixed powders. Total content of COP after this storage period determined in mixed powder was 19.14 $mg \cdot kg^{-1}$, which was smaller than the value calculated as a sum of oxycholesterols from milk

powder (46%), from egg powder (54%) or from mixed powder (27.43 mg·kg^{-1}). The rate of cholesterol oxidation in mixed powder during storage depends on the oxidation of milk and egg powders. Both compounds of mix powder, milk and egg, were antioxidatively influencing each other.

In whole milk powders immediately after production, NOUROOZ-ZADEH and APPELQVIST [4] recorded total contents of eight oxycholesterols amounting from 0 mg·kg^{-1} to 0.672 mg·kg^{-1}. After 12 months of storage of milk powder packaged in paper bags at a temperature of 20°C, the total content of oxycholesterols ranged from 2.328 mg·kg^{-1} to 4.008 mg·kg^{-1}. CALVO et al. [31] determined 7-keto and 25-OHC in milk powder stored in an open can for 24 months and observed an increase from 0.68 mg·kg^{-1} to 1.86 mg·kg^{-1}.

Eggs dried at 170/117°C and 225/140°C contained oxycholesterols at levels of 56.35 mg·kg^{-1} and 86.94 mg·kg^{-1}, respectively [32]. During storage of the vacuum-packed egg powder at room temperature, total contents of oxycholesterols increased from 30.36 mg·kg^{-1} to 48.98 mg·kg^{-1} after 5 months and to 84.21 mg·kg^{-1} after 10 months. In the egg powder produced and analysed in this study, the content of oxycholesterols increased from 5.161 mg·kg^{-1} to 47.179 mg·kg^{-1} after the 24-month storage. In our study, both immediately after production and in the last month of storage, total content of oxycholesterols in the egg powder was markedly lower than in the study by GUARDIOLA et al. [32]. However, it needs to be stressed that in our study, after 12-month storage of egg powder, the content of oxycholesterols increased 3-fold, while in the study by GUARDIOLA et al. [32] it was 2.7–fold. It may not be excluded that, after the next 2 months, a 3–fold increase in oxysterol levels would also be recorded.

The same oxycholesterols were identified in egg mixes by PIE et al. [5]. The raw materials for their studies included commercial mixes with a 2%,

Tab. 2. The effect of powder type and its storage time on the contents of cholesterol, oxycholesterols, contribution of oxycholesterols in cholesterol content and on the percentage contribution of saturated, mono- and polyunsaturated fatty acids.

Type of powder	Storage time [months]	Cholesterol [g·kg^{-1}]	Total COP [mg·kg^{-1}]	Contribution of COP in cholesterol contents [%]	Saturated fatty acids [%]	Monounsaturated fatty acids [%]	Polyunsaturated fatty acids [%]
Whole milk powder	0	0.473 ± 0.025^{a}	0.77 ± 0.10^{a}	0.2	73.2 ± 2.3^{e}	24.3 ± 0.9^{a}	2.5 ± 0.1^{a}
	3	0.472 ± 0.027^{a}	1.29 ± 0.11^{ab}	0.3	73.2 ± 3.2^{e}	24.3 ± 0.8^{a}	2.5 ± 0.1^{a}
	6	0.451 ± 0.023^{a}	1.94 ± 0.15^{bc}	0.4	73.1 ± 3.0^{e}	24.4 ± 0.7^{a}	2.5 ± 0.1^{a}
	12	0.445 ± 0.022^{a}	3.71 ± 0.31^{de}	0.8	73.4 ± 3.3^{e}	24.2 ± 0.6^{a}	2.4 ± 0.1^{a}
	24	0.433 ± 0.020^{a}	4.25 ± 0.38^{ef}	1.0	74.5 ± 3.0^{e}	24.2 ± 1.0^{a}	2.3 ± 0.1^{a}
Egg powder	0	13.786 ± 1.023^{b}	5.16 ± 0.47^{g}	0.0	32.3 ± 1.4^{a}	52.7 ± 1.6^{c}	15.0 ± 0.5^{e}
	3	13.737 ± 1.105^{b}	5.02 ± 0.45^{g}	0.0	32.4 ± 1.5^{a}	52.8 ± 1.4^{c}	14.8 ± 0.4^{e}
	6	13.724 ± 1.121^{b}	13.41 ± 1.10^{i}	0.1	33.9 ± 1.0^{b}	52.0 ± 1.5^{c}	14.1 ± 0.5^{de}
	12	13.643 ± 1.108^{b}	16.14 ± 1.32^{j}	0.1	33.2 ± 1.2^{b}	53.3 ± 1.8^{c}	13.5 ± 0.4^{d}
	24	13.624 ± 1.251^{b}	47.18 ± 3.61^{l}	0.3	34.1 ± 1.2^{c}	54.9 ± 1.9^{d}	11.0 ± 0.5^{c}
Mix powder	0	4.411 ± 0.321^{c}	1.41 ± 0.11^{ab}	0.0	54.3 ± 1.5^{d}	38.3 ± 1.5^{b}	7.4 ± 0.2^{b}
	3	4.378 ± 0.311^{c}	2.82 ± 0,25^{cd}	0.1	54.3 ± 1.4^{d}	38.4 ± 1.5^{b}	7.3 ± 0.2^{b}
	6	4.367 ± 0.315^{c}	2.89 ± 0.25^{d}	0.1	54.2 ± 1.2^{d}	38.5 ± 1.7^{b}	7.3 ± 0.2^{b}
	12	4.344 ± 0.308^{c}	9.69 ± 0.82^{h}	0.2	54.1 ± 2.0^{d}	38.8 ± 1.8^{b}	7.1 ± 0.2^{b}
	24	4.344 ± 0.305^{c}	19.14 ± 1.37^{k}	0.4	54.5 ± 2.0^{d}	38.7 ± 1.4^{b}	6.8 ± 0.2^{b}

Contents of cholesterol, total oxycholesterols and fatty acids are expressed as mean ± standard deviation (n = 9). Different letters in superscript within column denote statistically significant differences at p = 0.05. COP – oxycholesterols.

2.5%, 3.0% and 5.7% share of egg powder. Total content of oxycholesterols in these mixes increased with an increase in the share of egg powder and ranged from 2.059 mg·kg^{-1} to 6.608 mg·kg^{-1}.

The powders have a large surface area and contact with residual oxygen as well as the low water content make them susceptible to oxidation reactions. The quality of the raw materials and drying conditions determine the oxidative status of these products. Milk powder, which contains lower levels of cholesterol in comparison with egg powder, showed higher ratio of oxycholesterols before and after storage (0.16 : 0.04 and 0.98 : 0.35, respectively, Tab. 2). The presence of unsaturated fatty acids in lipids extracted from egg powder can protect cholesterol against oxidation by autooxidation.

Tab. 3. The effect of powder type and its storage time on the peroxide value.

Type of powder	Storage time [months]	Peroxide value [meq·kg^{-1}]
Whole milk powder	0	0.23 ± 0.01^{a}
	3	0.30 ± 0.01^{ab}
	6	0.38 ± 0.02^{bc}
	9	1.10 ± 0.05^{e}
	12	1.95 ± 0.07^{h}
	15	2.23 ± 0.10^{i}
	18	2.38 ± 0.12^{j}
	21	2.43 ± 0.12^{j}
	24	3.71 ± 0.18^{o}
Egg powder	0	0.25 ± 0.01^{a}
	3	0.42 ± 0.05^{cd}
	6	1.20 ± 0.04^{f}
	9	2.18 ± 0.11^{i}
	12	2.44 ± 0.15^{j}
	15	2.73 ± 0.14^{l}
	18	2.59 ± 0.12^{k}
	21	2.93 ± 0.11^{m}
	24	3.89 ± 0.18^{p}
Mix powder	0	0.36 ± 0.02^{bc}
	3	0.51 ± 0.02^{d}
	6	1.61 ± 0.08^{g}
	9	2.78 ± 0.13^{l}
	12	3.01 ± 0.15^{m}
	15	3.53 ± 0.14^{n}
	18	3.70 ± 0.18^{o}
	21	3.99 ±0.19^{r}
	24	4.34 ± 0.20^{s}

Peroxide values are expressed in milliequivalents of O_2 per kilogram of lipids (n = 9). Different letters in superscript within column denote statistically significant differences at p = 0.05.

Fatty acid composition

During storage of powders, statistically significant differences were observed in the percentage contribution of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the lipids of egg powder. These changes indicated the occurrence of oxidation reactions of fatty acids (Tab. 2). The fatty acid composition in milk and egg powders depends on many factors, like the quality of the raw material, technological and storage conditions. Golay et al. [33] found in whole milk powder 70% of SFA, 23% of MUFA, 3% PUFA and 4% other fatty acids.

At present, the ratios of SFA : MUFA : PUFA recommended from the nutritional point of view should be 1 : 1 : 2. This proportion may promote expression of adiponectin, improve metabolism of glucose and lipids, and increase insulin sensitivity [34]. In this respect, the mixed powder has a better ratio of SFA to MUFA than milk or egg powder. However, due to the low level of PUFA, it could be suggested to supplement the mixed powder with these acids. On the other hand, if this powder is used to make pancakes, ice-cream and other similar foods, vegetable oils that are used together with it may have a beneficial effect on the ratio of the fatty acids groups in the final food.

Peroxide value

Peroxide value (PV) is one of the most used analytical indices for the quality control of fatty foods [35]. Peroxides are the first indicators of autooxidation because they are the first molecules to be formed in the degradation process of unsaturated fatty acids [36]. Data on the effect of storage on the peroxide value of the powders are presented in Tab. 3. Fonseca et al. [37] obtained similar peroxide values for goat milk powder immediately after production (0.1–0.2 meq·kg^{-1}) and after 3 months of storage (0.4–0.7 meq·kg^{-1}). In another study [38], peroxide values of powders packaged in air atmosphere and stored for 1 year ranged from 1.0 meq·kg^{-1} to 2.0 meq·kg^{-1}. For comparison, the peroxide value of good quality lipids should be lower than 1 meq·kg^{-1} [39].

In the analysed mixed and egg powders, the peroxide value exceeded the level of 1.0 meq·kg^{-1} in the 3rd month of storage, while for the milk powder it was in the 6th month (Tab. 3). By sensory evaluation, the maximum acceptable level of the peroxide value in milk fat was determined to be

Tab. 4. The effect of powder type and storage time on the sensory evaluation.

Type of powder	Storage time [months]	Colour score	Aroma score	Consistency score	Taste score	Total score
Whole milk powder	0	0.50 ± 0.00^{a}	1.50 ± 0.03^{e}	1.00 ± 0.00^{c}	2.00 ± 0.00^{c}	5.00 ± 0.04^{d}
	6	0.50 ± 0.01^{a}	1.50 ± 0.03^{e}	1.00 ± 0.03^{c}	2.00 ± 0.04^{c}	5.00 ± 0.11^{d}
	12	0.50 ± 0.01^{a}	1.40 ± 0.14^{de}	0.80 ± 0.05^{b}	1.73 ± 0.19^{b}	4.43 ± 0.17^{cd}
	24	0.50 ± 0.02^{a}	0.90 ± 0.07^{a}	0.40 ± 0.13^{a}	1.20 ± 0.19^{a}	3.00 ± 0.30^{a}
Egg powder	0	0.50 ± 0.01^{a}	1.50 ± 0.00^{e}	1.00 ± 0.00^{c}	2.00 ± 0.04^{c}	5.00 ± 0.05^{d}
	6	0.50 ± 0.01^{a}	1.30 ± 0.14^{cde}	1.00 ± 0.02^{c}	1.87 ± 0.19^{bc}	4.67 ± 0.28^{d}
	12	0.50 ± 0.02^{a}	1.30 ± 0.14^{cde}	0.80 ± 0.06^{b}	1.73 ± 0.19^{b}	4.33 ± 0.23^{cd}
	24	0.50 ± 0.02^{a}	1.00 ± 0.14^{ab}	0.40 ± 0.12^{a}	1.33 ± 0.19^{a}	3.23 ± 0.22^{ab}
Mixed powder	0	0.50 ± 0.00^{a}	1.50 ± 0.02^{e}	1.00 ± 0.03^{c}	2.00 ± 0.00^{c}	5.00 ± 0.05^{d}
	6	0.50 ± 0.02^{a}	1.50 ± 0.03^{e}	1.00 ± 0.03^{c}	2.00 ± 0.04^{c}	5.00 ± 0.10^{d}
	12	0.50 ± 0.02^{a}	1.20 ± 0.08^{bcd}	0.80 ± 0.07^{b}	1.33 ± 0.22^{a}	3.83 ± 0.19^{bc}
	24	0.50 ± 0.02^{a}	0.90 ± 0.03^{a}	0.33 ± 0.10^{a}	1.20 ± 0.23^{a}	2.93 ± 0.24^{a}

Scores were obtained as multiplication by their weighting factors. Different letters in superscript within column denote statistically significant differences at $p = 0.05$ ($n = 90$).

2.0 meq·kg^{-1} [40]. In the study of Şenel et al. [41], some flavour and aroma defects such as bitterness and rancidity were perceived in butter made from yoghurt when the peroxide value reached 3.22 meq·kg^{-1}.

Storage time of powders had a statistically significant effect on the peroxide value, and thus on the deterioration of the quality of lipids in all tested powders. Most probably due to the air contained between powder grains, oxidation processes were active in spite of the application of vacuum packaging of the powders. These results suggest that probably a better, though obviously more expensive, packaging method would be packaging in a gas atmosphere or in the atmosphere of neutral gases, e.g. nitrogen or carbon dioxide.

Browning index

The browning index (*BI*) was calculated as an indicator of the intensity of the brown colour. In milk, egg and mixed powders, *BI* after production was 6.87 ± 0.34, 13.73 ± 0.62, 10.9 ± 0.49, respectively. These values increased to 9.34 ± 0.44, 17.69 ± 0.88 and 13.17 ± 0.60, respectively, on the 12th month of storage and then decreased to 5.89 ± 0.30, 14.06 ± 0.70 and 9.56 ± 0.47, respectively, on the 24th month of storage. It was shown that the type of powder and storage time have an effect on *BI* values. Reactions causing darkening of powders occurred during storage. This may be explained by the course of Maillard reactions and the formation of several compounds described in detail by Fieldman [42]. Ruszkowska and Palich [43] observed that darkening of baby formulas during storage was most probably connected with crystallization of lactose. The study of Thomsen et al. [44] indicated that the processes of lactose crystallization, browning and formation of radical species are strongly coupled. As reported by Davies and Labuza [45], colour compounds are formed during the last stage of the Maillard reaction. Some of them are stable, while others are readily degradable. Melanoidin or brown products are responsible for colour changes, which can also be formed by sugar caramelization without participation of amino groups [46]. Another reason for darkening (reddening) of powders may be connected with the migration of free lipids and β-carotene to the surface of powder globules [47]. In general, *BI* increased (L^* parameter decreased) because of reactions causing darkening.

BI decreased (L^* increased) after 24 months because of lightening powder. Guardiola et al. [32] explained this phenomenon, on the basis of storage experiments of egg powder, by the loss of β-carotene during prolonged storage. This loss could be caused by oxidation reaction of this pigment. In a study by Caboni et al. [23], loss of vitamins A and E during 12-month storage of egg powder at a temperature of 20 °C amounted to 40% and 25%, respectively.

Sensory evaluation

With an extension of storage time, almost all the examined sensory attributes deteriorated, i.e. aroma, taste and consistency (Tab. 4). Colour was the only exception in this respect. In powders, the greatest changes during the storage were observed

in consistency. This could be caused by the application of vacuum packaging and the use of too high pressure when packaging samples. Taste and aroma also deteriorated during storage. These attributes had the greatest effect on the total score and descriptive evaluation of powders due to the highest weighting coefficients, amounting to 0.3 and 0.4, respectively. Both lipid oxidation products and products of the browning reaction might have influenced the scores and notes in the evaluation of these attributes. The effect of storage time on the total result of sensory evaluation of powders was shown starting from the 12th month of storage in the mixed powder and in egg powder, while it was as late as the 24th month for milk powder. No effect of powder type was found on the results of sensory evaluation. Both immediately after powder production and after 6 months of storage, all powders received very good notes. After 1 year, the note for all tested powders was "good", while after 24 months of storage it was "satisfactory".

CONCLUSIONS

Composition of mixed powder (obtained by combining whole milk and liquid egg at a proportion typical of pancake batter) determined their oxidation stability and formation of oxycholesterols, hydroperoxides, the value of the browning index, as well as the results of sensory examination. After production and during storage of mixed powders, the evaluation indeces had values between those recorded for milk powder and egg powder. An exception was found in the case of peroxide value, for which the highest values during storage were recorded for mixed powders. Application of vacuum packaging did not protect powders against rapidly progressing changes in lipids. Milk, egg and mixed powders retained their good sensory attributes up to 12 months of storage.

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