

## FATTY ACID PROFILE AND OXIDATION TESTS OF FAT EXTRACTED FROM YOGURT USING ROSE HIP SEED OIL

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### ABSTRACT

**Background.** Yogurt is a dairy product with a high nutritional value. However, like all milk products, it contains milk fat and is rich in saturated fatty acids. It would be desirable to enrich dairy products in polyunsaturated fatty acids to increase dietary intake amongst consumers and improve their health. Also, some LAB bacteria are able to produce CLA and CLnA isomers from linoleic and linolenic acids. The aim of this study was to investigate the chemical properties and fatty acid profile of yogurt with the addition of 3.5% of rose hip seed oil.

**Materials and methods.** Yogurt was made from skimmed milk and yogurt starter culture YC-180 Ch. Hansen (Denmark), with the addition of 3.5% of rose hip seed oil. The peroxide value, acid value, iodine value, TBA rate and fatty acid profile were determined in fat extracted from the yogurt after 1 and 14 days of storage and in fresh rose hip seed oil. The fatty acid profile was determined using gas chromatographic methods with mass spectrometric detectors.

**Results.** Fat extracted from the yogurts had lower levels of peroxides than the fresh oil. It was more acidic and the iodine value was higher than in the fresh oil. Rose hip seed oil enriched the product with polyunsaturated fatty acids. After 14 days of storage, linoleic and linolenic acid levels had increased. Moreover, the content of myristic and palmitic acids had decreased.

**Conclusion.** The rose hip seed oil added to the yogurt was less susceptible to oxidation. The content of unsaturated fatty acids in the yogurt increased with the addition of the oil, making yogurt with rose hip seed oil an excellent source of  $\Omega$ -3 and  $\Omega$ -6 fatty acids. Conjugated linoleic (CLA) and linolenic (CLnA) acids were not detected. However, yogurt manufactured with appropriate adjunct cultures and with the correct oil addition could be a natural source of CLA and CLnA in the human diet.

**Keywords:** yogurt, rose hip seed oil, fatty acid, CLA

### INTRODUCTION

Yogurt is a dairy product made by the symbiosis of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbueckii* ssp. *bulgaricus*. Yogurt was probably first consumed by humans in the Middle East or India. Nowadays, the United States of America and Europe are the main producers of this fermented milk product (Tamime and Robinson, 2007). Yogurt has

a high nutritional value: it contains large amounts of easily digestible protein, vitamins and milk fat. Associated with probiotics, it has a positive impact on the human body (Lourens-Hattingh and Viljoen, 2001). High levels of yogurt consumption amongst consumers presents an excellent opportunity to introduce new and healthy products into people's diets (Serafeimidou

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et al., 2013; Trejo et al., 2014). However, the milk fat present in dairy products contains high levels saturated fatty acids. Some scientific publications suggest that too much saturated fat in the diet can lead to cardiovascular disease (Estrada et al., 2011; Hu et al., 2001). It would be desirable to enrich dairy products with polyunsaturated fatty acids, which are not synthesized in the human body, and the intake of which from food is often very poor. These acids have many health-promoting properties (Delgado-Lista et al., 2012; Kapoor and Huang, 2006; Ranieri et al., 2009). High levels of interest in the consumption of omega 3 fatty acids have made it possible to produce food fortified with EPA and DHA from marine sources (Kolanowski and Weißbrodt, 2007; Rognlien et al., 2012). Because of the fishy flavor which this gives, a good alternative to this is the addition of oils from vegetable sources (Dal Bello et al., 2015; Estrada et al., 2011). The rose hip seed oil is a little-known but rich source of linoleic acid (44.4–55.7%) and  $\alpha$ -linolenic acid (18.6–31.4%) in the preferred ratio of 2:1 recommended by dietitians, and it contains only three saturated fatty acids. This oil is mainly known as valuable ingredient in the regeneration of skin and damaged tissues, while there is no data about food fortification using this oil (Buchwald et al., 2007). Moreover, it has been shown that some lactic acid bacteria are capable of producing conjugated forms of linoleic (CLA) and linolenic acid (CLnA) (Bzducha-Wróbel and Obiedziński, 2009; Florence et al., 2012; Jiang, 1998; Van Nieuwenhove et al., 2007). CLA and CLnA are positional and geometric isomers of these acids naturally present in the meat and milk of ruminants and in vegetable oils. These isomers have a number of health-promoting properties, especially anti-carcinogenic, anti-arteriosclerotic, anti-inflammatory properties. The main subject of the present research on the effects of CLA and CLnA on the human body is to reduce body fat, increase muscle mass and improve lipid metabolism. Supplementation of these isomers may be useful in the treatment and prevention of obesity (Estrada et al., 2011; Hennessy et al., 2012; Sieber et al., 2004). Conjugated forms of linoleic is synthesized by the dehydrogenation of linoleic and linolenic acid in the rumen by bacterial enzymes.  $\alpha$ -linolenic, linoleic and oleic acids hydrolyzed in rumen are converted into cis and trans isomers by biohydrogenation and isomerization. Conjugated

forms of linoleic is produced from vaccenic acid in the mammary gland. Because the natural concentrations of CLA isomers in milk products are relatively low, to improve their health benefits, GRAS (Generally Recognized As Safe) bacteria with linoleate isomerase (a strain-dependent enzyme responsible for CLA conversion) activity could be used as a microbial culture to develop functional fermented dairy products with increased levels of CLA. In addition, the natural origin of these acids is important (Gorissen et al., 2015). The addition of vegetable oil to the fermented dairy product not only enriches the product in EFAs (Essential Fatty Acids), but also has the potential to increase the amount of CLA and CLnA.

## MATERIAL AND METHODS

### Materials

Materials consisted of raw cow's milk from a private farm in Kaczyna (Poland), DVS yogurt starter culture YC-180 Ch. Hansen (Denmark) which contained cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus delbrueckii* ssp. *lactis*, *Streptococcus thermophilus* and cold-pressed rose hip seed oil produced by Oleofarm Ltd (Poland).

Raw milk was transported under refrigerated conditions in September 2014 to the Department of Animal Product Technology laboratory, University of Agriculture in Krakow and it was heated to the temperature of 40°C, skimmed in a Belomo SM55 centrifuge (Belarus) and to 0.1% of milk fat content. The milk was pasteurized at 85°C for 15 minutes, cooled down to 60°C and rose hip seed oil was added, in an amount equivalent to 3.5% of the total volume. This oil was used because of the favorable ratio of linoleic acid and linolenic acid and the fact that it had never before been used as a food additive. Milk with added rose hip seed oil became twice homogenized in a homogenizer Armfield Ltd. Ringwood (England) under a pressure of 7 MPa at 60°C and cooled down to 44°C. The DVS starter culture was the added, according to the recommendations provided by the producer. The milk was poured into 200 jars and incubated in an incubator CLW 115 ECO Pol-Eco Aparatura (Poland) at 44°C to achieve pH 4.6 and stored in laboratory refrigerator at 4 ± 1°C for 14 days. To determine chemical properties, 10 g of yogurt was shaken with 100 ml of diethyl ether

in a glass vessel to dissolve the fat into an ether phase. Then the ether phase was collected and another portion of ether was added. This procedure was repeated 3 times. The ether was evaporated in 40°C to obtain a fat fraction.

### Basic composition of milk

The dry matter content, non-fat dry matter content, protein, fat, lactose and density were tested using the FOSS Milkoscan FT-1200 (Denmark). The pH of the milk was measured using a CP-411 Elmetron pH-meter (Poland). The fat content in normalized milk was checked by the Gerber method (AOAC, 2006).

### Oxidation tests

Chemical parameters were determined in the yogurt with 3.5% rose hip seed oil after 1 and 14 days of storage and in fresh rose hip seed oil. In these products, the peroxide value (PN-ISO 3960:1998), acid value (PN-ISO 660:1996), iodine value (PN-ISO 3961:1998) and the TBA rate (Hekmat and McMahon, 1997) were determined. All samples were analyzed at least in duplicate. The experiment was performed in three independent runs. The results were expressed as means  $\pm$  standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) using the Statistica 10.0 software. Duncan's multiple range tests were used to evaluate significant differences between the means at  $p < 0.05$ .

### Fatty acid composition

The fatty acid profile was determined in fresh oil and fat extracted from yogurt stored for 1 and 14 days. According to the IDF method (IDF standard 182:1999), 2 cm<sup>3</sup> 0.5 mol·L<sup>-1</sup> KOH in methyl alcohol solution was added to the 20 mg fat samples. The samples were incubated for 15 minutes at 60°C. In order to obtain fatty acid methyl esters, 2 cm<sup>3</sup> 14% boron trifluoride

in methyl alcohol solution was added to the samples. The samples were incubated for 15 minutes at 60°C. After cooling, 2 cm<sup>3</sup> of hexane and 2 cm<sup>3</sup> of saturated sodium chloride was added to the samples. The solution was then shaken for 10 minutes and left to separate into phases. The upper layer (hexane) was transferred to a separate vessel. Sodium sulfate was added to the vessel and analyzed.

Fatty acid composition was determined using gas chromatographic methods with mass spectrometric detectors. A gas chromatograph with a QP5050A Shimadzu mass spectrometer, equipped with a 100 m capillary column, 0.2 mm i.d. and 0.2 mm film thickness was used for the analysis. The carrier gas was helium. Chromatographic separation was carried out according to the following temperature program: 60°C maintained for 5 minutes, followed by an increase of 5°C per minute until a temperature of 220°C was reached, and this was then maintained for 23 minutes. The total time of incubation was 60 minutes. The analysis was performed using electron-impact ionization (EI). The percentage value of the fatty acid methyl esters was calculated using the formula:  $A_i / \sum A_i \cdot 100$ , where  $A_i$  is the signal of the  $i$ -th ester and  $\sum A_i$  is the amount of all analytical identified signals.

## RESULTS AND DISCUSSION

The basic composition of milk with 3.5% added rose hip seed oil, prepared for yogurt production, is shown in Table 1. All physico-chemical parameters are typical for cow's milk and similar to those obtained by Jasińska et al. (2011), Król et al. (2011) and Bonczar et al. (2009).

Fats and oils are very unstable. The changes that occur in the structure of fats are known as rancidity. These changes mainly involve hydrolysis and oxidation, which decrease the quality of fat, leading to

**Table 1.** The composition and properties of milk for yoghurt production (mean values from three series  $\pm$  standard deviations)

	Dry matter, %	Protein, %	Fat, %	Non-fat dry matter, %	Lactose, %	Density, g/cm <sup>3</sup>	pH
Milk with 3.5% of rose hip seed oil addition	12.31 $\pm$ 0.01	3.80 $\pm$ 0.01	3.5 $\pm$ 0.20	8.81 $\pm$ 0.18	4.70 $\pm$ 0.06	1.0330 $\pm$ 0.0000	6.6 $\pm$ 0.2

**Table 2.** The chemical properties of the fresh rose hip seed oil used to modify the fat fraction in yogurt and fat extracted from yogurt stored for 1 day and 14 days

Properties	Fresh rose hip seed oil	Fat extracted from yogurt stored for	
		1 day	14 days
Peroxide value (LOO), mEqO <sub>2</sub> /kg	11.79 ±0.21 <sup>A</sup>	4.36 ±0.93 <sup>B</sup>	4.18 ±1.50 <sup>B</sup>
Acid value, mg KOH/kg	0.54 ±0.10 <sup>A</sup>	3.78 ±0.83 <sup>B</sup>	3.71 ±1.00 <sup>B</sup>
Iodine value, g I/100 g	24.47 ±2.00 <sup>A</sup>	38.32 ±0.30 <sup>B</sup>	37.43 ±0.38 <sup>B</sup>
Thiobarbituric acid (TBA) rate [E <sub>1cm</sub> <sup>1%</sup> /λ = 533 nm]	1.90 ±0.10 <sup>A</sup>	0.97 ±0.10 <sup>Ba</sup>	1.18 ±0.12 <sup>Bb</sup>

<sup>A,B</sup>Mean values followed by different letters in the same line are significantly different ( $p \leq 0.01$ ).

<sup>a,b</sup>Mean values followed by different letters in the same line are significantly different ( $p \leq 0.05$ ).

the formation of an unpleasant flavor and the loss of nutritional value. The increase in free fatty acids and increasing acidity of the fat was due to hydrolytic degradation caused by water, light, air and enzymes. The oxidation of fat caused by the action of oxygen, light and catalysts led to the formation of peroxides and free and bound aldehydes. Oxidation products are extremely biologically active. They damage internal and external cell structures and reduce the activity of enzymes. They are both cytotoxic and atherogenic. Rancidity is dependent on fatty acid composition, the presence of antioxidants and storage conditions (Velasco and Dobarganes, 2002).

High significant differences were found between the peroxide value, acid value, iodine value and TBA of the fresh oil and that observed in the fat extracted from yogurt (Table 2). The high peroxide value indicates peroxide decomposition caused by the action of oxygen on the unsaturated bonds of fatty acids catalyzed by light and various other catalysts, leading to the formation of peroxides. According to the standard of FAO/WHO, the acid value of cold pressed vegetable oil should not be higher than 4.0 mg KOH/g, and the peroxide value should not exceed a concentration of 15 mEqO<sub>2</sub>/kg (Alimentarius, 2013). The peroxide value of the fat extracted from the yogurt (after both 1 and 14 days of storage) was lower than that of the rose hip seed oil. The lower peroxide value of the fat extracted from yogurt may be indicative of a lower susceptibility to oxidation, due to the protective effects of the yogurt components, the activity of microflora which could convert peroxides during their life

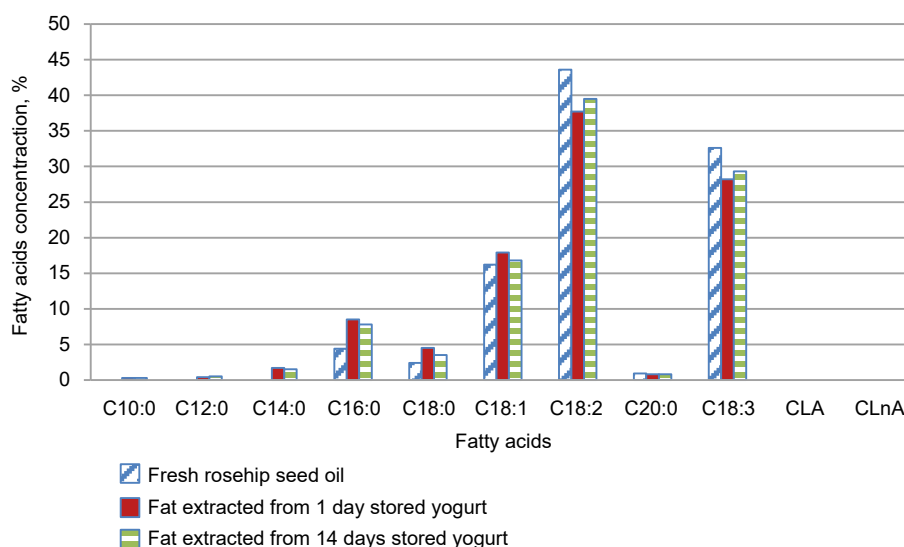
processes, or possibly the decomposition of the oil at the high temperature (60°C) to which it was exposed. For example, Dan Bello et al. (2015) reported a significant increase in the peroxide value of fresh and stored yogurt with added vegetable oils compared to pure oil. Let et al. (2007) compared the oxidative stabilities of fish oil-enriched milk, yoghurt and salad dressing and investigated the effects on oxidation of adding either fish oil or a fish oil-and-water emulsion to these products. They found that fish oil-enriched yoghurt generally had higher oxidative stability, as determined by the peroxide value and volatile oxidation products, than fish oil-enriched dressings, so yoghurt therefore seems to be a good delivery system for lipids containing n-3 polyunsaturated fatty acids. The acid value was higher in the fat extracted from stored yogurt (after both 1 and 14 days) compared to the fresh oil. The high acid value of the yogurt resulted from the fermentation process and the production of short chain fatty acids (lactic, acetic), and the presence of lipolytic enzymes and water, which contribute to an increase in the acid value of the fat. The iodine value, which is a measure of the amount of the unsaturated fatty acids, was higher in the fat extracted from yogurts than in the oil. This may be the result of the conversion of saturated fatty acids into unsaturated fatty acids by the yogurt bacteria. A high iodine number indicates a high degree of unsaturation, i.e. a large number of double and triple bonds. However, the greater the degree of unsaturation, the greater the susceptibility of the oil or fat to oxidative rancidity, but in the case of the tested yogurt this is not true (Firkins and Eastridge, 1994).

Anisidine value (TBA test) for the fresh oil was highly significantly different from that of the fat extracted from the fresh and stored yogurt. This value pointed to a high level of lipid peroxidation and aldehyde formation. A significant difference in the TBA rates of the fat extracted from fresh oil and fat extracted from yogurts was demonstrated. It can be assumed that the storage and addition of oil into yogurt caused a reduction of aldehydes compared to the fresh oil. This is also reflected in the reduction of the peroxide content in the fat extracted from the yogurt. It was observed by Estrada et al. (2011) that the TBA rate more than doubled during storage in strawberry yogurt with the addition of salmon oil.

Analysis of the fatty acid composition of the fresh rose hip seed oil showed that the saturated fatty acids characteristic of animal fats such as capric, lauric and myristic acids were not present (Fig. 1). Fat extracted from fresh yogurt and stored yogurt contained only small amounts of capric, lauric and myristic acids. They might have originated from the small amounts of milk fat which remained in the yogurt after the centrifugation of the milk. In the fat extracted from the fresh yogurt as a result of the addition of oil, an increase in long chain saturated fatty acids such as palmitic acid and stearic acid was observed. The amount

of monounsaturated oleic acid was also higher in the fresh yogurt and stored yogurt. It can be assumed that the content of the fatty acids increased during fermentation due to the action of yogurt bacteria, and decreased due to storage of yogurt. The level of long chain polyunsaturated fatty acids (linoleic and linolenic) increased during storage. This fact is evidence of the ability of the active microflora in yogurt to synthesize these acids. The amount of linoleic and linolenic acids was higher in fresh oil, but this was the result of a higher concentration of these acids in the tested oil sample.

Conjugated forms of linoleic and CLnA were not detected in any analyzed samples. Therefore, Ogawa et al. (2005) believe that too high a concentration of certain fatty acids could inhibit microbial growth. The amount of arachidonic acid did not change, despite the decreasing pH and the time in storage. Estrada et al. (2011) studied the fatty acid profile of strawberry yogurt with added fish oil. They observed an increase in the amount of palmitic acid, stearic acid and oleic acid, a slight increase in the amounts of linoleic acid and a decrease in the amount of linolenic acid in the yogurt containing fish oil, compared to the microencapsulated salmon oil. Van Nieuwenhove et al. (2007) demonstrated an increase in the amount of palmitic,



**Fig. 1.** The concentration of fatty acids in fresh rose hip seed oil and fat extracted from fresh and stored yogurt

stearic, oleic, linoleic and linolenic acids in buffalo cheese manufactured with added sunflower oil and *Streptococcus thermophilus* CRL728, compared to the same cheese without oil. However, they observed an increase in the linolenic acid content and a decrease in the myristic acid content over the 15 days of storage. Zaręba (2009), in a study regarding changes in the fatty acid profile in soy milk rich in polyunsaturated fatty acids, such as linoleic acid and  $\alpha$ -linolenic acid, by the action of yogurt bacteria, found the highest linoleic acid content of all other acids presented in the yogurt. He observed a decrease in all measured fatty acids with a chain length of C16 to C22 after 2 and 4 weeks of refrigerated storage, regardless of the type of yogurt bacteria used. This author claims that the changes in fatty acid content in yogurts depends on their acidity, the amount and activity of lactic acid bacteria, the activity of bacterial enzymes, and on their ability to assimilate unsaturated fatty acids.

There are numerous scientific publications concerning the production of conjugated forms of linoleic acid and linolenic vegetable oil added to dairy products (Jiang et al., 1998; Lin, 2003; Van Nieuwenhove et al., 2007; Florence et al., 2012). For example, Lin (2003) studied the production of cis-9, trans-11-18:2 in on-fat yogurt by *Lactobacillus acidophilus* CCRC 14079 and yogurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) which contained 0.1% linoleic acid. The statistically significant increase in the CLA content (from 0.93  $\mu\text{g g}^{-1}$  to 2.95  $\mu\text{g g}^{-1}$ ) was observed only with the application of yogurt bacteria. However, in our study, the concentration of polyunsaturated fatty acids, especially linoleic and oleic, was too high for *S. thermophilus* growth, despite the fact that it is able to tolerate high linoleic acid concentrations in cultures producing CLA (Van Nieuwenhove et al., 2007).

## CONCLUSIONS

From the presented results of oxidation tests, it can be assumed that yogurt with rose hip seed oil is oxidatively stable over 14 days of storage. The amount of unsaturated compounds was higher in the yogurt samples compared to the fresh oil. This fact is very significant because it demonstrates the increase in health-promoting properties of the tested product, due to the lipolytic

activity of the starter culture. The addition of oil to the yogurt enriched the fatty acid profile with polyunsaturated fatty acids. No conjugated linoleic and linolenic acids forms were found. This could be the result of an oil content which was too high (3.5%) and inhibited bacterial growth. The results of this study may be useful to produce new yogurt and yogurt-like products. To determine the potential for commercialization of yogurt with added rose hip seed oil, further research is suggested to evaluate some physicochemical and sensory parameters, to determine the optimal dose of oil and to evaluate the growth of microorganisms.

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