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Modification of fats by enzymatic interesterification

M. Bekbolatova[1], B. Sakenova[1], I. Temirova[1], D. Shaimerdenova[1], G. Sarbassova[1], A. Iztayev[2], G. Ospankulova[1,3]

Abstract. We describe the development of a method for determining the qualitative properties of mixtures of certain animal fats and soybean oil using Lipozyme TL IM, a lipase from the fungus Thermomyces lanuginosus. We first determined the interesterification activities of Lipozyme RM IM and Lipozyme TL IM and found that the latter was optimal for interesterification analysis of combined fat when present as 5 % (wt/wt) of a sample. After interesterification, the content by weight of three saturated triglycerides decreased by 14.5 % and that of unsaturated triglycerides increased by 12.6 %. In contrast, the content of monounsaturated triglycerides did not change. We also investigated the organoleptic, physical, and chemical properties of the combined fats, including the melting and pour points and mass fraction of triglycerides as a function of temperature. The content of trans fatty acids of the combined fat ranged from 6.5 % to 7.3 %.

Key words. Enzymatic interesterification, Lipozyme, organoleptic.

1. Introduction

Modern diets vary in their composition of natural fats and include a wide range of vegetable oils (traditional liquid vegetable and palm oils), milk fat, saturated animal fats, and fats from fish and marine mammals [1–3]. These fats have nutritional advantages and disadvantages. Researchers continually attempt to develop balanced fat products with a nutritionally optimum ratio of fatty acids that are highly resistant to oxidation. These efforts have led to the production of combined fat products, including margarine, shortening, spreads, and fat blends, using hydrogenation reactions to generate trans fatty acids. However, recent research reveals that synthetic and trans fatty acids derived from natural sources have the same biological effects. This has led to international efforts to develop alternative methods

[1]Kazakh Research Institute of Processing of Agricultural Products, 010000, Astana, Kazakhstan
[2]Almaty Technological University, 050000, Almaty, Kazakhstan
[3]Corresponding author; e-mail: bulashevag@mail.ru

to produce margarine, shortening, and other fats for special purposes [4–6]. One method to accomplish this, while ensuring adequate nutritional value, involves the enzymatic interesterification of fats. Interesterification technology makes it possible to generate new types of fat-containing foods with defined properties and provides the ability to regulate food fat content in a rational and enforceable manner.

Vegetable oil is used as one component of the combined fat and is obtained from the zoned varieties of soybean seeds in Kazakhstan. The design of fatty foods takes into account two major aspects: creation of a balance between nutrition and its biological source and technology that is capable of changing the proportion of fat to produce a set of products with appropriate rheological properties and composition that can be applied for specific purposes.

2. Materials and methods

2.1. Fats, fatty acids, and enzymes

Fats were prepared from sheep, goats, and camels that had undergone preliminary processing for meat. The immobilized enzymes Lipozyme RM IM and TL IM were obtained from Novozymes.

2.2. Determination of animal fat quality

The organoleptic and physicochemical properties of sheep, goat, and camel fat were determined using standard methods and refractive. The solid fat content in animal fat was determined using a Proton 20M nuclear magnetic resonance analyzer, according to a published method. Fatty acid composition was determined using a gas chromatograph (Agilent Technology).

2.3. Determination of vegetable oil quality

Organoleptic and physicochemical properties of refined oils that were analyzed are as follows: smell, taste, color, and transparency; moisture and volatile compounds; determination of moisture and volatile matter content); acid number; unsaponifiables; mass fraction of phosphorus compounds.

2.4. Enzymatic interesterification

Enzymatic interesterification of animal fats and soybean oil was performed with slight modifications according to a published method. Because freshly immobilized enzyme preparations contain 5 % water, they were subjected to dehydrationprior to analysis. A three-necked flask with a jacket served as a reactor, and the desired reaction temperature (60 °C) was maintained using a thermostat connected to a hose. The reactor was also equipped with a magnetic stirrer rotating at 300 rpm. During interesterification, the lid was left open to allow the remaining moisture in the enzymes to evaporate. The acid number for each mixture was determined at

baseline and at 2, 4, 8, 10, 12, 14, 16, 18, and 20 h intervals (Fig. 1).

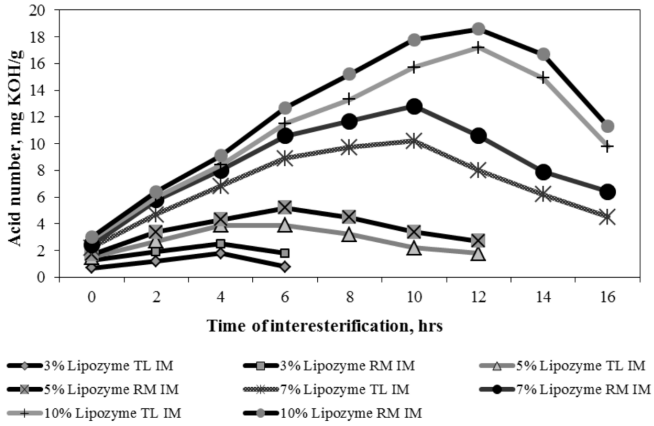

Fig. 1. Change in acid number of fat mixtures during enzymatic interesterification

As shown in Fig. 1, the acid number increased at the beginning of interesterification, increased at 12 h with higher enzyme concentrations (7 % and 10 %), and then decreased. A similar pattern was observed with fat mixtures containing lower enzyme concentrations and shorter interesterification times. This could have been caused by parallel hydrolysis occurring during interesterification i.e., due to the residual water present in the enzyme preparations. As the reaction time increased, more moisture evaporated and interesterification dominated, resulting in a decrease in acid number.

2.5. Analysis of triglyceride mixtures before and after interesterification

Triglyceride content in mixtures of animal fat and vegetable oil before and after interesterification was analyzed using high performance liquid chromatography (HPLC) using an Agilent 2100 according to published procedures GOST 30623-98 "Vegetable oils and margarine. The method of detecting fraud".

2.6. Determination of the quality of animal fat and vegetable oil mixtures

The physical and chemical properties of interesterified mixtures were detected according to a published method. Fusion temperatures of the combined mixtures Thermal systems of Mettler Toledo FR 900, the hardening temperature, and the mass fraction of triglycerides in combined fats and solid fat content (ISO 8292:2008 Animal and vegetable fats and oils - Determination of solid fat content - Pulsed nuclear magnetic resonance method, 2 The indirect method) 15 were determined according to the published methods. The content of trans isomers was determined using combined infrared spectrometry using an Infralum (Russia) as described previously.

3. Results and discussion

The optimum result was achieved using 5 % Lipozyme TL IM. Thus, acid number was the lowest, and the enzyme is considered more suitable for industrial use where free fatty acid content in fat mixtures is regulated. Moreover, 5 % Lipozyme TL IM significantly decreased interesterification time. Adding 3% Lipozyme TL IM or Lipozyme RM IM had no effect on fat mixtures because this quantity may have been insufficient for complete enzymatic interesterification. Thus, full liberation of free fat acids was not observed. Neither the 7 % nor 10 % preparations of Lipozyme TL IM and Lipozyme RM IM affected free fatty acid composition compared with lower amounts.

The organoleptic, physical, and chemical characteristics of animal fat are presented in Table 1. The camel fat moisture content was higher (10.2 %–13.6 %) than goat or sheep fats, while the acid number in goat fat was lower (59 %–68 %) than camel or sheep fats. The refractive index of fats ranged from 1.447 to 1.452. For goat fat, the drop point temperature on Mettler ranged from 3 % to 11 %, the *trans* isomer content was 6 %–35 %, and the Kaminsky hardness measure was 18 %–40 % higher than in sheep and camel fats. All three animal fats had specific aromas and tastes and were transparent when melted. The relationship between solid fat contents and melting curves is depicted in Fig. 2.

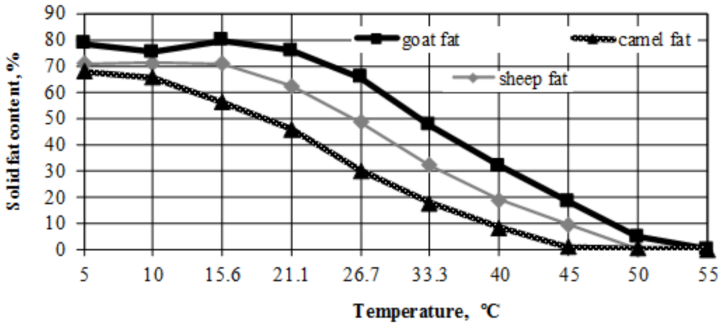

Fig. 2. Relationship between solid fat contents and melting curves

The solid fat content in goat fat was 10 %–13 % higher than that in sheep and camel fats. The camel and sheep fats completely melted at 45–50 °C in contrast to 55 °C for goat fat. Figure 3 shows the fatty acid content of goat, sheep, and camel fat. Fatty acid compositions of animal fats are shown in Fig. 3. Camel fat had the highest content of lauric acid (6 %–35 %), capric acid (16 %–29 %), and margaric acid (24 %–73 %). Sheep fat had the highest content of palmitic acid (10 %–15 %), myristic acid (17 %–35 %), and linoleic acid (21 %–41 %). Goat fat had the highest content of stearic acid (21 %–25 %). In contrast, it had the lowest content of oleic acid (5 %–25 %), linoleic acid (19 %–80 %), and arachidonic acid (21 %–61 %).

Table 1. Organoleptic, physical, and chemical characteristics of animal fats

Name of indicators	Animal fats		
	Sheep	Goat	Camel
Moisture content, %	0.19 ± 0.006	0.2 ± 0.006	0.22 ± −0.0012
Acid number (mg KOH/g)	2.8±0.006	0.9±0.06	2.2±0.06
Peroxide value (mg of iodine)	0.02±0.006	0.025±0.003	0.028±0
The number of refraction	47±0	48±0.12	46±0.12
Refractive index	1.452±0.15	1.450±0.03	1.447±0
Mettler drop point on Mettler (°C)	49.1±0.15	50.5±0.2	44.5±0.2
The content of *trans* (%)	13.2±0	14.16±0.04	9.19±0.01
Hardness by Kaminsky at 15 °C (g/cm)	581±0.58	715±1.15	435±0
Transparency	Transparent in the molten state		
Smell and taste	Specific, with characteristic odor		
Color	Pale yellow color	Yellow	White

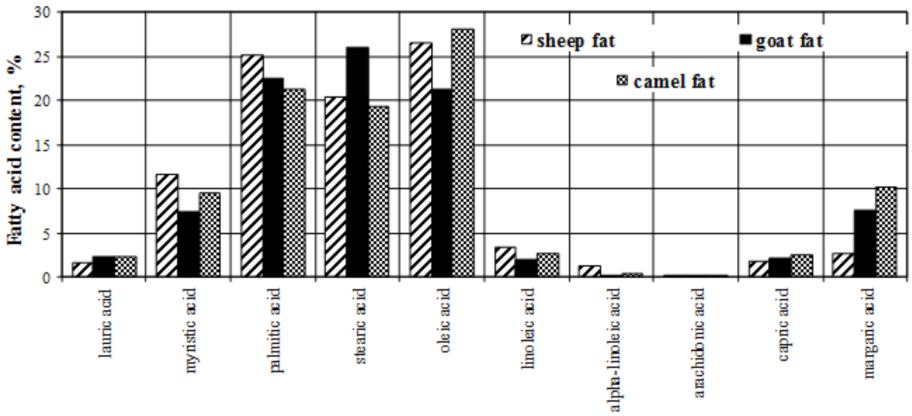

Fig. 3. The fatty acid content of goat, sheep, and camel fat

3.1. Analysis of triglyceride composition before and after transesterification

Animal fats containing higher levels of saturated fatty acids melt at higher temperatures. Therefore, the melting point of the mixture of goat fat and soybean oil was higher than that of a mixture of camel and lamb fat because of the higher content of saturated fatty acids. The same was true for the pour point (Table 2). In general, the process of transesterification had a positive impact on the melting point of combined fats.

Table 1. Melting points and solidification of combined fats

Ratio	Melting temperature, °C			Pour temperature, °C		
	sheep fat + soybean oil	goat fat + soybean oil	camel fat + soybean oil	sheep fat + soybean oil	goat fat + soybean oil	camel fat + soybean oil
90:10	38	41	36	33	36	31
80:20	35	38	32	29	31	28
70:30	32	35	29	27	29	26
60:40	28	29	25	18	22	16
50:50	22	25	20	15	19	15

The melting curve characterizes the mass fraction of triglycerides over a certain temperature range, which is the most important criterion of quality fat systems, and represents the amount of solid phase-fat crystals dispersed in a liquid fatty phase and determines the hardness of the product. The results are shown in Fig. 4.

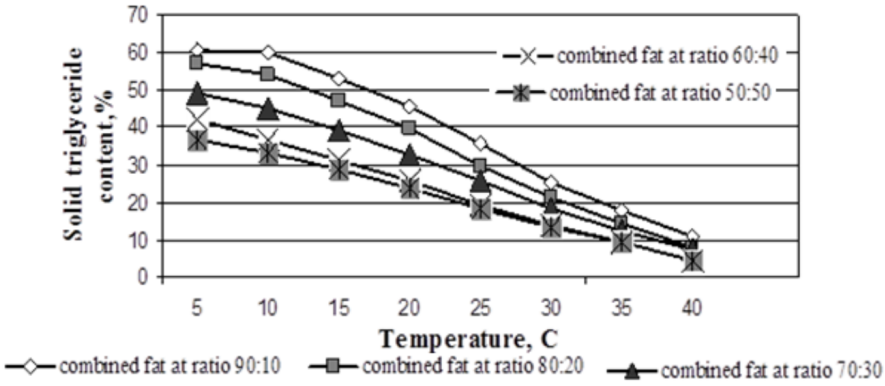

Fig. 4. The melting curve obtained by the combination of fat enzymatic transesterification

Normally, high quality confectionery fats have a higher content of firm triglycerides at room temperature (>50 %), which then sharply decreases, providing full fusion of the majority of products at temperatures from 35 °C to 39 °?. The combined fats at 80:20 and 70:30 can be used in flour confectionery because they impart similar properties to confectionery fats, although combined fats at 60:40 and 50:50 may be more suitable for baking and culinary purposes. Fat and vegetable combinations, such as 60:40 and 50:50, can also be used as a fat basis in the margarine industry because they meet special requirements such as plastic consistency and more specific temperatures of fusion. The fatty acid trans isomer content of combined fats was 6.5 %–7.3 %, which corresponds to the natural *trans* isomer content of animal fats (the fatty acid *trans* isomer content obtained by hydrogenation can exceed 40 %).

4. Conclusion

The results of the present study indicate that the animal fat and vegetable oil mixtures developed here comply with quality requirements and could be used by various sectors of the food industry, including confectionaries, bakeries, and for culinary purposes. This approach would help use animal fat efficiently without industrial utilization, which undoubtedly would contribute to the economy and an agricultural "no waste and healthy nation" policy as stated in the National Strategy-2020 address by the President of the country. Such mixtures with specific trans fatty acid and triglyceride content may also contribute toward the decrease in mortality rate from cardiovascular or other chronic diseases.

References

[1] R. O'Brien: *Fats and oils. Production, composition and properties, application.* Professiya, Russian (2007), p. 752.

[2] E. M. Davydov, V. A. Petrovichev: *Practical experience of using enzyme technology.* Fat promyshlennost (2002), No. 3, 24–25.

[3] N. A. Ibrahim, Z. Guo, X. Xu: *Enzymatic interesterification of palm stearin and coconut oil by a dual lipase system.* Journal of the American Oil Chemists' Society *85* (2008), No. 1, 37–45.

[4] A. MacKenzie, A. Douglas: *Lipase catalysed modification of animal fats.* Thesis (PhD–Chemistry)–University of Auckland (2000), No. 00-474.

[5] Z. k. Kadyrov: *Improved technology interesterification of lipids using biocatalysts.* Dissertation for the degree of candidate of technical sciences, Tashkent (1984).

[6] L. V. Zaitseva: *The use of enzymatic interesterification for the modification of oils.* Prischevaya industry (2011), No. 5, 22–25.

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