The Annals of the University *Dunarea de Jos* of Galati
Fascicle VI – Food Technology, New Series Year III (XXXII)
Paper presented at the International Symposium *Euro - aliment 2009*, 9th – 10th of October 2009, Galati – ROMANIA

DRY-FRACTIONING POSSIBILITIES OF ANIMAL LIPIDS

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Received 3 July 2009
Revised 12 October 2009

Animal fats and vegetable oils, conventional and unconventional, with various structural diversity and resources, which are known at present, cannot assure qualitative and quantitative necessity under economic efficiency. The present technological possibilities (fractioning, hydrogenation, interesterification), allows chemical and/or physico-chemical "modification", as a dependence of the object. Dry–fractioning, as a minimal processing technique also allows the sector valorisation of the different plant and/or animal lipid fractions, both primary and secondary. The authors of the present paper present the results of studying by applying dry-fractioning to pork lard as such and monitored association with cow grease with a view to obtain a range of reconstituted lipid assortments.
At the same time, this could also be a way of identifying possible food adulteration from the point of view of a given lipid composition.

Keywords: designer lipids, tailor-made lipids, fractionated lipids, lipids modified, fat-fractionation.

1. Introduction

The difficulties of oxidative, hydrological, crystallographic, senses and polymorphism stability imply supplementary restrictions that limit the lipid assortment of aliments in lead areas.

The actual techniques allow the modification (correction) of one or more characteristics of natural lipids so they become nutritionally and/or functionally up to the standards of a certain goal/product ("tailor-made lipids" or "designer lipids") (Costin, G. et al., 1999). Obviously, the products resulted after this operation can significantly differ from the initial sources.

The major objectives of oil and fat modification are (Podmore, J., 1987) [2]:

- obtaining some products with intermediary physic and chemical characteristics that are inexistent in the natural standard of natural fat (for example, harder or softer, with faster or slower melting, with changed colour, etc.);
- obtaining a similar product to the existent one but at a lower price;
- amelioration of oxidation resistance – (hydrogenation);
- optimization of palatability;
- the controlled modification of the lipid crystallization method;
- the extension of the product assortment nutritionally superior through the reduction of fatty saturated acids and of "trans-" isomers and the growth of the concentration of poly-unsaturated ones.

Fractioned crystallization of lipids from a mixture depends on the degree of un-saturation, respectively on the length of the hydro-carbonated chain.

Brown J. et al. (1955) made studies on wet crystallization of fatty acids at low temperatures (-20; -70^{o}C), underlining the simplicity of this separation technique. With the exception of the solvent, the other participants to the system are not involved in the oxidation process or other secondary processes that will affect fatty acids.

Kaufmann H. et al., (1957), Schlenk H. et al., (1957) sustain that through fractioned crystallization we can obtain fatty acids of high purity with possibility of extension on other lipids (cholesterol separation from products that can't be turned into soaps).

Feng S. et al., (2004) proposed a separation and evaluation method of fatty acids from milk, through centrifugation, different from the solvent extraction. They collected a sample of 20 ml raw milk cow that was introduced in conical recipients for the separation of the fats through the centrifugation method (12000 rot/min; 30 min; 40^{o}C). After centrifugation, from the superior layer they collected 1 g of fat and they transferred it in a micro tube of 1.5 ml. The sample is left in standby at room temperature (≈ 20^{o}C) for 20 minutes, while it melts, after that being introduced in a micro centrifuge where separation is realized at the following operating parameters: 13000 rot/min, 20 min, 20^{o}C. After separation we can see three layers: 1) superior (lipids); 2) middle formed by proteins and other substances unsolvable in water; 3) inferior – water. After this, from the superior layer they made a sample that was transformed to determine the composition of fatty acids. The procedure can be compared with the separation method of Hara A. et al. (1978) proposed for the extraction of lipids from the animal fat tissue from a mixture of hexane and isopropanol.

Luna, P. et al., (2005) developed a fast lipid separation method from milk with the purpose of determining the composition of fatty acids through the utilization of two successive centrifugations at 4^{o}C.

2. Materials and methods

2.1. Materials

- *pig lard*, obtained (figure 1), characterised (table 1), from domestic subjects rose traditionally in Romania's Western Plain (slaughtering year – December 2006).

- *cow grease* (the Italovini Slaughterhouse SRL in Giulvăz, Timiş County, Romania) obtained through heat-processing of bovine lipids.

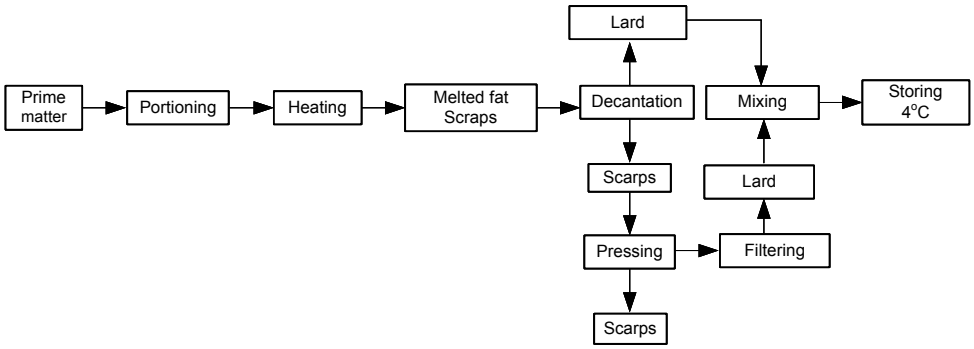


Figure 1. Operation chart in processing pig lard

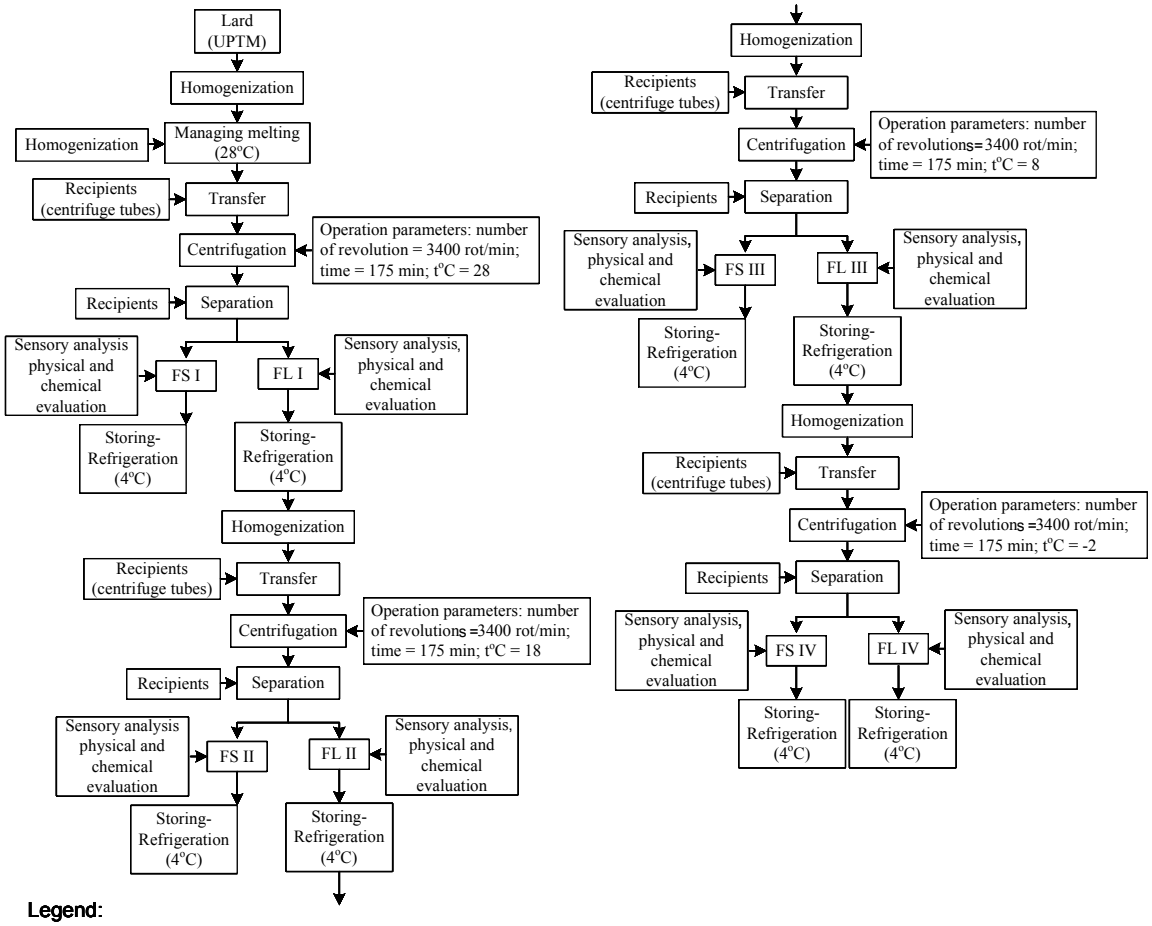
Table 1. Physical and chemical characteristics of lard (prime matter) accessed for dry-fractioning (average value)

Number		Quality indicator		Measure unit	Value
1	a) physical	density		[g/ml]	0.9070
		colour		-	white
		viscosity[1)] (t = 40 ^{o}C)		[cP]	51.84114
		aspect		-	fluid, granulated and unitary
2	b) chemical	acidity value		[mg KOH/g]	2.2
		esterification value		[mg KOH/g]	196
		saponification value		[mg KOH/g]	198.2
		unsaponifiable		(%)	1.453
		iodine value	Hanus	[g I_2/100 g]	82.73
			Wijs	[g I_2/100 g]	79.82
			Margosches	[g I_2/100 g]	73.92

[1)] Hoppler method.

2.1. Apparatus:

- centrifuge Sorvall RT6000 Refrigerated Bench Top Centrifuge, with the following technical characteristics (maximum number of revolutions = 6000 rot/min; maximum capacity 4 x 250 ml; work temperature -20 ÷ +40°C);
- gas chromatograph equipped with: glass column 1 m long; Chromosorb stationary phase W 80-100 mesh, impregnated with 10% silicon oil SE-30; N_2 gas carrier, flow capacity 10 ml/min; temperature gradient 5°C/min; flame ion detector;
- filtering installation with void adequately refrigerated;
- Penski – Martens apparatus;
- Boetius microscope (to determine melting/solidification interval).



Legend:
UpTm = lard (initial sample); FS I = solid fraction from the first centrifugation; FL I = liquid fraction from the first centrifugation; FS II = solid fraction from the second centrifugation; FL II = liquid fraction from the second centrifugation; FS III = solid fraction from the third centrifugation; FL III = liquid fraction from the third centrifugation; FS IV = solid fraction from the forth centrifugation; FL IV = liquid fraction from the fourth **centrifugation;**

Figure 2. Operation chart of dry-fractioning of pig lard

3. Results and discussions

Preliminary separation trials of the heterogenic system led to the observation that the operation parameters have a determined influence on the efficacy of dry-fractioning. So, the direct separation of anterior un-homogenised lard at 10°C and centrifugation for 5 minutes with 1600 rot/min did not lead to significant results. Through homogenisation in advance, followed by centrifugation at 10°C, 10 minutes with 3400 rot/min three phases are formed: superior fluid (oily) yellow, partially opaque; middle viscose, strongly opaque; inferior solid with very low fluidity.

By putting together these observations with the quality parameters (indexes) of initial lard (table 1) we can admit, with sufficient precision, that the extreme phases belong: the superior one – in majority to the unsaturated lipid fractions, respectively the inferior one – in majority to the saturated lipid fractions, and the intermendiary layer to the mixture of the two extremes, not yet separated.

The interpretation was confirmed by the growth of the centrifugation duration in similar conditions from 10 to 20 minutes when there are still three phases, but the intermediary layer is proportionally reduced with its opaqueness.

From the compared evaluation of all the preliminary experimental data, regarding the normal decisive influence of the operating parameters we adopted the operation chart (figure 2). The obtained fractions were evaluated physically and chemically (table 2a and 2b).

The colour index (aspect) evaluated on both scales has an increasing tendency (FL I, FL II, FL III, FL IV) respectively, decreasing (FS I, FS II, FS III, FS IV) which is explainable through the yellow brown colour, more accentuated for the unsaturated lipid fraction (oleic, linoleic, linolenic) with the advanced degree of purity and the waxy aspect, and the white colour of the saturated lipid fraction (stearic, palmitic). The explanation was confirmed also through the chromatic charts in the gas phase of the two types of products (FL; FS).

The density of the two separated lipid fractions grows in the FL series and in the FS.

Compared to the initial reference product (UPTM) we can see that the inferior limit value is framed in both phases above the noted parameter ($0.9094/_{FL}$; $0.9119/_{FS}$ for density respectively, of $0.9040/_{FL}$; $0.9064/_{FS}$) for the relative density. The situation can be explained through the presence in variable quantity of the free volatile acid feature in the initial product which in the initial separation process (fractioning) is subsequently distributed between the two major phases because of its solubility. The Reichert – Meissl and Polenke indexes through their evolution in both phases confirm the formulated hypothesis and gratify the explanation. We can also see that, insoluble in water, volatile acids are distributed preferentially in the fluid phase (FL) in comparison with their water insoluble homologous that is found in the separated solid phase (FS).

Density growth in both series (FL; FS) can be contradictory with the evolution of the same values of the major individual unsaturated lipid components from the mixture [$C_{18}(1\Delta)$ 0,891; $C_{18}(2\Delta)$ 0,902; $C_{18}(3\Delta)$ 0,914]. If we correlate the evolution of viscosity (fluidity) of the same components with temperature, the explanation of the density of fractions FL from table 2 becomes obvious. The same thoughts are also valid for the similar values of the FS.

The refraction index evolves in the same way as the density index presented above. The explanation of the values in both series FL; FS is based on the same ideas.

The evolution of melting and solidifying intervals for the liquid and solid phase confirm that dry-fractioning made as in the proposed chart allows separation of unsaturated lipids through adequate repeated correlation of the refrigeration temperatures and the centrifugation speed.

In the technological alimentary practice, we encounter a great compositional variety of animal fats (including lard) that came from the same source, but resulted in different processing stages and/or keeping (storing).

The chromatographic profile of the initial fat but also of the separated fluid and solid lipid fractions can represent a quality indicator of the technological "process". If we consider as a reference "chromatographic fingerprint" of lard initially accessed in the paper (UPTM), accidentally or on-purpose adulteration with cow grease (mostly saturated) in the technological flow modifies "fingerprint".

The experimental results (table 3) confirm that the percentage of saturated fatty acids *[$C_{14}(0\Delta)$; $C_{16}(0\Delta)$]* is, practically, similar for pig lard and for cow grease, but the percentage for the unsaturated lipid fraction *[$C_{18}(1\Delta)$; $C_{18}(2\Delta)$]* for cow grease is lower than the pig lard. Their presence grows proportionally with the lard in the mixture of fat submitted to dry-fractioning. The report $C_{16}(0\Delta)/C_{18}(1\Delta)$ respectively $C_{16}(0\Delta)/C_{18}(2\Delta)$ (table 4) is maintained in the same trends in the separated phases liquid/solid.

Table 2a. Values of the physical and chemical indicators that correspond to the analyzed samples (average value)

Number	Lipid separated fraction	IA[1] [mg KOH/g]	IS[2] [mg KOH/g]	IE[3] [mg KOH/g]	CI[4] [g I_2/100 g]			IP[5] [ml $Na_2S_2O_3$ 0.01N/g]	Reichert-Meissl[6] index [ml NaOH]	Polenske[7] index [ml NaOH]	SNs[8] [%]	Colour index [10]	
					Hanus method	Wijs method	Margosches method *[9]*					Iodine scale [mg I/100 ml]	Potassium bi-chromate [mg $K_2Cr_2O_7$/ 100 ml]
1	FL I	0.3	198.7	198.4	63.83	63.2	63.83	1.6	0.96	0.11	3.932	4	5
2	FL II	0.3	197.2	196.9	64.1	64.52	62.8	1.8	0.86	0.22	3.115	5	7
3	FL III	0.2	197.2	196.98	61.6	63.2	62.3	1.52	0.86	0.22	4.162	5	6
4	FL IV	0.2	196.4	196.18	62.4	64.3	63.3	1.27	1.36	0.22	3.391	6	7
5	FS I	0.3	193	192.7	67.5	68.9	69.16	4.6	2.22	0.56	1.68	7	7
6	FS II	0.3	194.6	194.3	69.2	68.1	73.92	3.4	1.6	0.62	1.8	6	6
7	FS III	0.2	192.7	192.48	67.4	69.1	67.1	1.52	1.6	0.79	0.977	6	6
8	FS IV	0.2	193.6	193.38	66.9	69.4	65.5	2.41	1.74	0.52	1.676	5	5
9	UPTM	2.2	198.2	196	82.73	79.82	73.92	6.4	1.85	0.56	1.453	5	6

[1]IA – acidity index *[11]*; [2]IS –saponification index *[11]*; [3]IE – ester index *[11]*; [4]CI – iodine number (unsaturation index)– STAS 145/19-90 *[12]*; [5]IP – peroxide index *[11]*; [6] Reichert-Meissl index– the quantity of volatile acids soluble in water *[11]*; [7]Polenske index– the quantity of volatile acids insoluble in water *[11]*; [8]SNs – unsaponifiable substances – STAS 145/15-91 *[13]*.

Table 2b. Values of the physical and chemical indicators that correspond to the analyzed samples (average value)

Number	Lipid separated fraction	Ash *[11]* [%]	Insoluble in HCl *[11]* [%]	Penski – Martens Method[1] *[14]* [°C]		Melting interval[2] [°C]	Solidification interval[2] [°C]	Water [%]		Refraction index (n_D^{50})[3]	d_{40}^{40} [4] [g/ml]	d_{40}^{40} [4] [-]
				"Fog" point	Inflammable point			Drying closet *[11]*	Karl Fischer method *[15]*	Abbé method *[16]*	The picno-meter method *[16]*	
1	FL I	0.15	0.23	257	334	28	8	0,0812	0,782	1.452	0.9094	0.9040
2	FL II	0.13	0.35	261	335	18	4.5	0.0838	0.658	1.466	0.9142	0.9088
3	FL III	0.04	0.42	253	336	8	4	0.007	0.591	1.4681	0.9247	0.9112
4	FL IV	0.11	0.67	234	339	-2	4.2	0.013	0.607	1.4676	0.9168	0.9137
5	FS I	0.24	0.21	245	325	42	28.5	0.1658	0.965	1.4528	0.9119	0.9064
6	FS II	0.21	0.22	250	328	33	20.5	0.0619	0.908	1.454	0.9092	0.9038
7	FS III	0.23	0.25	254	329	35	10	0.013	1.034	1.4542	0.9240	0.9028
8	FS IV	0.17	0.36	253	334	31	2	0.087	0.970	1.4532	0.9277	0.9003
9	UPTM	0.39	0.33	255	331	37	22	0.9820	1.211	1.4528	0.9070	0.9016

[1]Penski – Martens method – STAS 145-67 *[14]*; [1] average value of the initial and final melting/solidification interval; [2] n_D^{50} – refraction index determined at 50 °C; [3] d_{40}^{40} - density, respectively relative density determined at 40°C.

Dry fractioning of these systems allow such monitored capitalization of the individual components and/or associated in mutual mass report determined by the chromatographic profile.

The values in Table 2a and 2b represent technological guide marks that can monitor the fractioning operation in the direction of obtaining (separating) the lipid assortment requested by the subsequent alimentary technological practice.

Results presented in this paper have never been supplied as such by literature.

Table 3. The dependency of the chromatographic profile1) of lard (UPTM) on the share of cow grease (SB) in mixture

Sample	$C_{14}(0)$	$C_{16}(0)$	$C_{16}(1)$	$C_{18}(0)$	$C_{18}(1)$	$C_{18}(2)$
UPTM (I)	5.978	30.451	1.032	24.015	36.950	1.614
SB (II)	1.952	27.014	2.543	11.873	48.031	8.593
98% II + 2% I	5.893	30.294	1.203	22.491	37.492	2.601
95% II + 5% I	5.890	30.273	1.549	21.052	38.003	3.031
92% II + 8% I	5.847	30.002	1.627	19.498	39.014	3.985
90% II + 10% I	5.808	30.451	1.758	17.032	39.897	5.492
88% II + 12% I	5.00	30.375	1.842	16.558	40.958	5.601
85% II + 15% I	4.813	30.298	1.905	13.082	42.275	7.508
82% II + 18% I	4.523	29.031	2.094	15.114	42.302	7.498
80% II + 20% I	4.019	28.392	2.242	14.752	42.593	7.893

[1)] has been used boron trifluoride in methanol (12-14 % w/v) as a transesterification catalyst of esterifying free fatty acids [17].

Table 4. The dependency of the report C16(0Δ)/C18(1Δ) respectively C16(0Δ)/C18(2Δ) in the pig lard mixture [UPTM (I)] with cow grease [SB (II)]

Sample	Chromatographic profile	
	$C_{16}(0\Delta)/ C_{18}(1\Delta)$	$C_{16}(0\Delta)/ C_{18}(2\Delta)$
98% II + 2% I	0.824	12.156
95% II + 5% I	0.795	8.439
92% II + 8% I	0.773	7.256
90% II + 10% I	0.757	5.118
88% II + 12% I	0.732	5.093
85% II + 15% I	0.718	4.826
82% II + 18% I	0.709	4.739
80% II + 20% I	0.708	4.626

4. Conclusions

The analyzed experimental results confirm the fact that dry-fractioning of animal fat (pig lard) processed with heat and/or not can represent a technological diversification perspective of physically and chemically modified lipids with utility in the functional processing of food.

Acknowledgments

The authors are indebted to the University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology – Timişoara for financial and technical support.

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