

EMULSIFICATION OF VEGETABLE OILS WITH NONIONIC SURFACTANTS AND POLYPEPTIDES

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Some correlation between the emulsifying activity index (*EAI*) and the polymorphic states of the triacylglycerides (*TAG*) submitted to emulsification are presented. The results obtained by turbidimetric study of interfacial surface (*IS*) at the emulsification, using the model systems of polymorphic forms of palmitooleo-stearine (*POS*) separated from cocoa butter are reported. Cocoa butter was fractionated in temperature gradient from 15, 20 and 25% (w/v) acetone solutions. The fractions of sn-1-palmito-2-oleo-3-stearine (palmitooleo-stearine), which predominate in this fat (34 - 36%) were separated between 0 and 25°C and purified by repeated refractionations when the α , β and β' polymorphic forms were obtained. By emulsification in model systems with emulsifying agent and protection colloid for volumic fractions ranging between 0.05 and 0.4 it was established that the *IS* determined by turbidimetry decrease from β to β' to α forms in all the cases. BRIJ-35 was used as emulsifying agent and carboxymethylcellulose (CMC-Na) 1 % (w/v) and/or casein peptone ($M_w = 25$ kDa) 1 % (w/v) as stabilizing agent.

INTRODUCTION

Cocoa butter as well as Borneo butter and cattle fat has one of the narrowest distributions of saturated (palmitic 34% and stearic 51%) and unsaturated (oleic 12.5% and linoleic 1.5%) fatty acids residues. In the steric structure of the triacylglycerides, according to the stereospecific numbering, the rests of unsaturated acids distribute mainly in sn-2 position (over 80%) and those of saturated acids in sn-1 and sn-3 positions.

This particular distribution facilitates the separation of α , β and β' polymorphic forms in an extremely pure state using fractionation techniques with methanol or acetone solutions in temperature gradient from -40 to +25°C.^{1,2}

The polymorphic forms have different thermomechanic properties because of the differences in their microcrystalline structure, aspect that can be found not only in the solidification process of the melt, but also during the processes of emulsification^{1,3} and stabilization with emulsifying agent and protection colloid (especially polypeptides).⁴

Arishima *et al.*⁵ separated and studied the polymorphism of some saturated-unsaturated-

saturated (sat-unsat-sat) TAG with different ratios between the lengths of the carbon chains (p, q and r). Among these, sn-1-palmito-oleo-3-stearine has a priority place because it can be found in many fats currently used in food industry, including cocoa butter (*POS* is the main component - over 34%).

The studies concerning the emulsification of fats, such as those regarding the preparation of concentrated emulsions are numerous and diverse.⁶⁻⁸

An useful and fast method used for the study of interfacial surfaces in emulsions was elaborated by Pearce *et al.*⁹ and developed later by Cameron *et al.*¹⁰

In this paper we present some correlation between the emulsifying activity index and the polymorphic states of the TAG submitted to emulsification. We report the results obtained by turbidimetric study of *IS* using the model systems of polymorphic forms of *POS* separated from cocoa butter.

RESULTS AND DISCUSSION

Table 1 gives the results obtained by fractionation of cocoa butter at five temperatures and three concentrations of *POS* respectively.

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Table 1
The results of the fractionation of POS separated from cocoa butter

No.	Conc. (% w/v)	Temp. (°C)	Fractions ^{*)}			Melting point (°C)	Polymorphic form
			W _i (g)	q _i (%)	∑ q _i (%)		
1		5	0.0126	2.02	2.02	36.2	α
2		10	0.0345	5.53	7.55	37.0	β'
3	15(I)	15	0.3395	54.48	62.03	36.8	β'
4		20	0.1352	21.69	83.72	40.5	β
5		25	0.1014	16.28	100.00	40.6	β
POS mass, q = 0.75 g; ∑ W _i = 0.6232, recuperation degree RD = 83.09%							
6		5	0.0268	3.68	3.68	36.3	α
7		10	0.0464	6.37	10.05	36.9	β'
8	20(II)	15	0.3490	47.89	57.94	37.0	β'
9		20	0.1963	26.94	84.88	40.7	β
10		25	0.1102	15.12	100.00	40.6	β
POS mass, q = 1.00 g; ∑ W _i = 0.7287, recuperation degree RD = 86.87%							
11		5	0.0413	3.51	3.51	36.3	α
12		10	0.0794	6.75	10.26	37.1	β'
13	25(III)	15	0.6413	54.56	64.82	36.9	β'
14		20	0.2221	18.89	83.71	40.6	β
15		25	0.1914	16.29	100.00	40.7	β
POS mass, q = 1.25 g; ∑ W _i = 1.1755, recuperation degree RD = 94.04%							

$$*) q(\%) = (W_i / \sum W_i) \cdot 100$$

The values in table 1 show that the α form separates from POS at 5°C, while β' and β POS crystallize as two separated fractions at 10 and 15°C or 20 and 25°C respectively so they have almost equal melting points (ΔMp < 0.2°C). This suggests that the polymorphic β and β' forms can be recuperated by cooling the solutions with a chosen dQ/dt rate in order to cover the ΔT = 5 - 10°C range in 10 - 12 hours (that was made in several experiments). The advantage of the method consists in the possibility of crystallization mainly of the most thermodynamically unstable fractions (α >> β' > β).

The cooling rate and the concentration of acetone solutions are important for the separation of pure fractions (which are supplementary crystallized). Even though the recuperation degree of the polymorphic forms from POS grows from 83.09% to 94.04% by increasing the concentration from 15% to 25%, it is recommendable to work at a 15% concentration which assures more accurate separations.^{2, 5, 11} At this level of concentration POP (palmitooleo-palmitine) and SOS (stearooleo-stearine) were also crystallized. The types of polymorphic forms and the melting points for all the fractions are given in table 2, compared with the results from the literature.^{12 - 14}

Table 2
Melting points of some polymorphic forms of the TAG separated from cocoa butter

TAG	Polymorphic form	Melting point (°C)	
		Literature	Experimental
POP	α	33.3 - 33.5	33.5
	β'	35.3 - 35.5	35.7
	β	41.6	41.8
POS	α	36.0 - 36.3	36.3
	β'	36.7 - 37.7	36.9
	β	40.9	40.6
SOS	α	38.2 - 38.9	38.7
	β'	39.3 - 39.8	39.2
	β	43.5	43.2

Correlating the results from table 2 with those obtained for molecular weights using the saponification, iod and hydroxyl indices we established that the polymorphic forms of the separated TAG correspond to the forms we attributed to, and are extremely pure. Considering the fact that the differences between POP, POS and SOS appear in the number of methylen groups from the lateral positions (sn 1- and 3-) (two supplementary groups for stearine comparatively with palmitine), it would be normal that their melting points depend linear on the number of carbon atoms. This dependence is linear only for α and β' forms while for β it is nonlinear.

The differences in the thermal and thermomechanical properties are due not only to the length of the alkyl chains, but also to the packing degree of the chains (p, q, and r) according to the concept of chain conformation.

Analyzing the results from Table 2 one can see that the simultaneous crystallization of the polymorphic forms of POP and POS (for SOS the difference ΔT is big enough for a distinct separation) is possible. This impediment can be eliminated by adjusting the cooling rate according to the Differential Scanning Calorimetry curves, or

by using mixtures of solvents (for ex. acetone-tetrahydrofuran, methanol-acetone) as the literature mentions. The δ form of POS mentioned in the literature⁵ couldn't be isolated in pure state. According to Friberg¹³, the polymorphism of mono-, di- and triacylglycerides (MAG, DAG and TAG) is of major importance in the realisation of stable emulsions in which monoacylglycerides (MAG) and diacylglycerides (DAG) could be used as emulsifying agents and the TAG are the emulsioned components. It was confirmed later¹⁴ that in aqueous media the MAG forms micelles, the DAG forms lamellar associations and the TAG distributes at the interface.

There appeared two different situations:

Emulsions prepared at 50°C, in which the polymorphic forms of POS remake, by melting, the microcrystalline structure after cooling at 20°C;

Emulsions prepared by mechanical dispersion of the TAG at 20°C in model system.

This situation leads to significative differences between the values of the direct and corrected interfacial surfaces (IS and IS_c) as well as between the direct and corrected emulsifying activity index (EAI and EAI_c). The results from Table 3 for POS are relevant from this point of view.

Table 3

Optical and superficial characteristics of the α -POS model emulsions

φ	DO ₅₀₀	$\tau^{*)}$	w(g)	EAI	EAI _c **)	IS	IS _c **)
0.4	1.632	375.85	0.01	0.752	0.485	501.13	323.30
	0.297	68.40		0.137	0.088	91.33	58.92
0.2	0.989	227.8	0.005	0.182	0.118	227.77	146.90
	0.275	63.33		0.051	0.033	63.75	41.13
0.1	0.401	92.35	0.0025	0.296	0.191	82.09	052.91
	0.251	57.81		0.018	0.012	50.00	32.26
0.05	0.165	37.99	0.00125	0.486	0.314	31.99	20.64
	0.230	52.97		0.008	0.005	43.05	27.77

*) the turbidity τ was calculated according to the relation: $\tau = 0.023 \cdot DO_{500}$

***) the corrected emulsifying activity index

EAI was calculated using the relation:

$$EAI = \frac{IS}{w}$$

where w is the mass of emulsifying agent, and

$$IS = \frac{2\tau \cdot w}{[c(1-\varphi)]}$$

φ being the volume fraction ($\varphi = w/V_e$), where V_e is the volume of emulsion, τ is the turbidity and

c is the concentration (g/mL). EAI_c and IS_c correspond to the results of Cameron at al.¹⁰ Results similar to those from table 3 were obtained for β' -POS and β -POS. While OD₅₀₀ and IS at 50°C decrease with the volume fraction of TAG, EAI has its minimum at $\varphi = 0.2$, then increases at $\varphi = 0.05$ having almost the same value as for $\varphi = 0.4$. This can only be explained using the redistribution of the alkyl chains from the TAG in the interfacial structures in extended

lamellar form, when the concentration of the dispersed phase decreases.

At higher concentrations of dispersed phase (TAG) mainly known "microcrystalline" associations (cubic, hexagonal or multilamellar forms) are obtained.⁴ At 20°C the growing of *EAI* and *IS* is normal. They will probably depend on the frequency used for disintegration (we used only a 3000 rpm frequency).

For both temperatures (20°C and 50°C) the growth of *IS* are almost linear for the range of volume fractions we analyzed (for $\varphi > 0.4$ important deviations appear in the correlation of the turbidity coefficient τ with the concentration of TAG).

The dependences of *IS* (m²) on φ for β and β' -POS for 15% solution are represented in Figure 1.

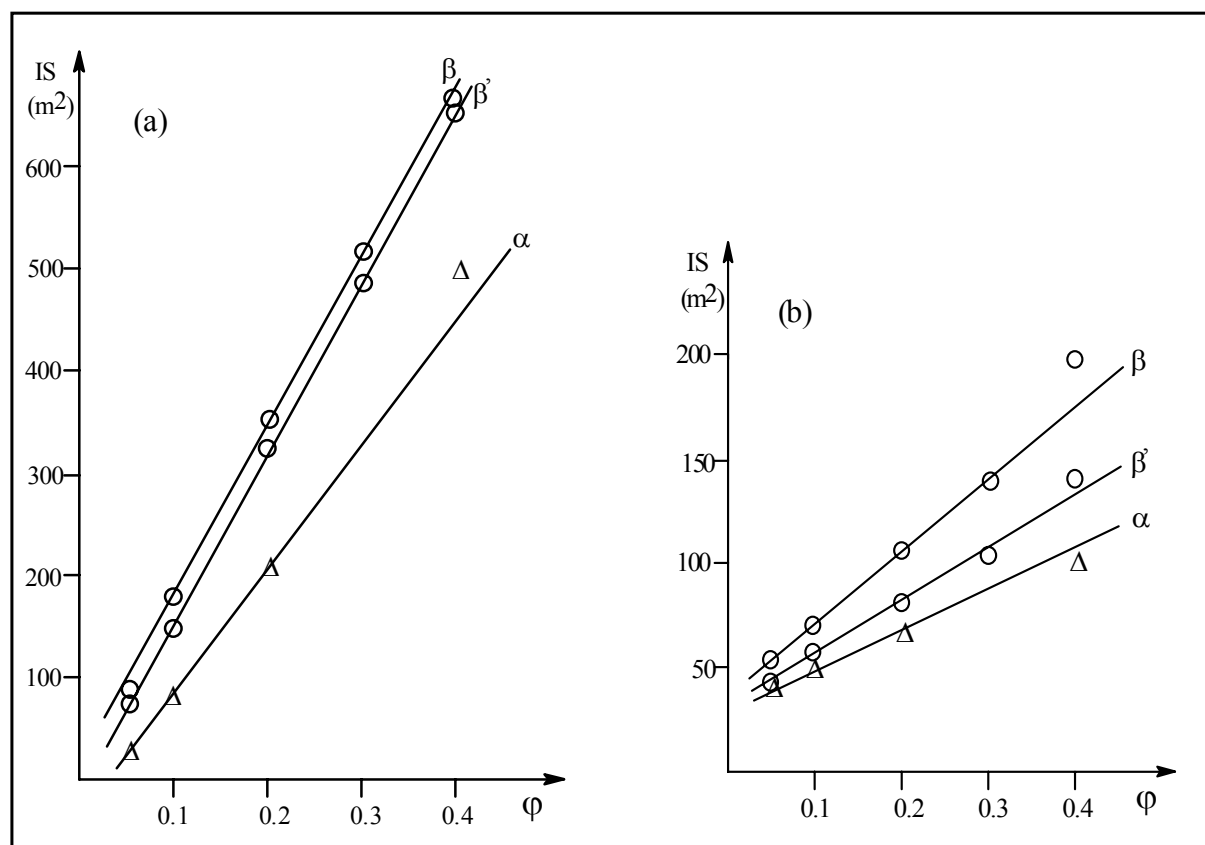


Fig. 1 – *IS* dependence on the volume fraction φ for the model emulsions prepared at 50 °C (a) and 20 °C (b) for each of the polymorphic forms for 15% acetone solutions.

For the emulsions prepared at 50°C the linear dependences are close each other for β and β' forms and *IS* values show the growth of the thermodynamic stability from α to β (Fig. 1.a).

The same aspect can be observed in Figure 1.b for emulsions obtained at 20°C, but here the differences are much more significant: the β form is more dispersible compared with β' , which is close to α , giving the higher interfacial surface for each volume fraction.

It is important to emphasize that similar orders for the linear dependences $IS = f(\varphi)$ were obtained for the fractions separated from the 20% and 25% solutions, but *IS* decreases with 5 - 10 m² when the cocoa butter concentration increases.

EXPERIMENTAL PART

The cocoa butter from S.C. "Dunareana" S.A. Roman which processes cocoa beans from Ivory Coast was used. Cocoa butter has a slipping point of 34.8°C, an acidity index of 0.46 mg KOH/g and the oxidation tests (peroxide index, carbonyl index and TBA) showed a good conservation. The reagents used for analyses were p.a., from Merck, Darmstadt Germany and S.C. Reactivil S.A. Bucharest. The TAG fractions from cocoa butter were separated from acetone 15, 20 and 25% (w/v) solutions (15 mL) thermostated ($\pm 0.1^\circ\text{C}$) for 12 hours at temperatures between 5 and 25°C. The solid part deposited was centrifuged, filtered and washed with 5 mL of cold acetone (-5°C). The filtrated part went to a next fractionation. The solid part was crystallized from acetone in the same conditions and was dried in vacuum at 200 mmHg.

The analytical identification of the fractions was made using the melting points determined with an Electrotherm

9100 Mettler Toledo apparatus. The emulsification of the polymorphic forms of POS and other fractions was made after the standard method of Pearce and Kinsella.⁹

The emulsions were prepared in model system containing TAG with volumic fractions ϕ between 0.05 and 0.4 at 20°C. As emulsifying agent BRIJ-35 1 % (w/v) was used and as stabilizing agent solutions of carboxymethylcellulose (CMC-Na) 1 % (w/v) and/or casein peptone (Mw = 25 kDa) with the same concentration. The determination of *EAI* was made using the turbidimetric modified method of Cameron et al.¹⁰, transforming the optical density (DO) at $\lambda = 500$ nm at a Secomam S 750 spectrophotometer (Secomam Sp.A., France) in turbidity coefficient.

CONCLUSIONS

The fractionation of saturated fatty acids residues in temperature gradient from acetone solutions at initial fat concentration of 15, 20 and 25% was done. The separation of the polymorphic forms takes place within a narrow temperature ranges using cooling rates lower than 2°C/h in a temperature range 20°C lower than the melting point of each component.

The stability of emulsions expressed by *IS* is higher when they are prepared at the melting point of each fraction, increasing up to 5.5 times compared with the values obtained for the mechanical dispersion at 20°C.

The order of increase of the stability of the emulsions prepared with POS polymorphic fractions corresponds with their thermodynamic stability. It is accepted that the interfaces of emulsions contain films or microcrystalline associations of TAG molecules in regulate structures which depends on the concentration

(volume fraction), temperature and the method of dispersion.

The method can be used as a reference in studying other complex fats currently used in the food industry to establish the optimal emulsification conditions.

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