Influence of Temperature-Induced Phase Transitions on Fat Emulsions[1]

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ABSTRACT

The polymorphic behavior of a commercial monoglyceride emulsifier together with a liquid vegetable oil and water has been investigated by means of microscopic examination and differential scanning calorimetry. The results show that the stability regions of the liquid crystalline phases formed are highly dependent on the temperature and related both to the solubility of the monoglyceride emulsifier in the oil phase and to its polymorphic behavior. The formation and stability of emulsions are discussed in connection with the results obtained.

INTRODUCTION

The properties of fat emulsions depend to a high degree on the temperature during the pretreatment and mixing of the ingredients. It is our opinion that this is due to temperature dependent changes involving the crystalline and liquid crystalline phases which occur in fats and in lipid emulsifiers. Furthermore these changes show marked hysteresis phenomena and nonreversible behavior which can give rise to problems when processing emulsified products on a factory scale.

The phase behavior of aqueous systems of monoglycerides with chain length ranging from C12 to C22 has been studied by Lutton (1) who identified mesophases of the neat, viscous isotropic and middle structure. An extensive study at different temperatures of the phase behavior of a series of aqueous monoglyceride systems with chain length down to C6 was made by Larsson (2). The presence of seven liquid crystalline and micellar phases was shown. In particular a reversed middle phase was observed to be formed either by monoglycerides with long chain fatty acids or by combination of monoglycerides with diglycerides of the same fatty acid. This structure consists of hexagonally arranged cylinders of water surrounded by lipids. In an investigation by Krog and Larsson (3) of water mixtures of monoglycerides prepared from natural fats, the practical importance of the lamellar structured phase, called dispersion, in emulsion formation was emphasized. X-ray diffraction showed the occurrence of two crystalline forms, the metastable α-form and the stable β-form.

The determining influence of liquid crystalline phases on stability (4) and viscosity of emulsions (5) has been shown at our institute in a series of investigations on phase equilibria at 20 C in three-component systems.

In view of the importance of emulsions in food technology we have investigated the polymorphic behavior and the degree of reversibility of phase transitions in a system of a commercial monoglyceride in combination with water and liquid oil.

THREE-COMPONENT SYSTEM

DISTILLED WATER (W)	IND. DISTILLED MONOGLYCERIDE (DMG) 94%	COMMERC. TRIGLYCERIDE (SBO)
	Fatty acid comp:	ref. liq.
	C_{16} 7%	soybean oil
	C_{18} 85%	
	C_{20}+C_{22} 6%	
	others 2%	
	mp 72-74 C	
–pH-4–		

FIG. 1. Specifications and terms for the components used in the investigation.

EXPERIMENTAL PROCEDURES

The monoglyceride (DMG) was a commercially available product produced by molecular distillation of a mixture of mono-, di- and triglycerides obtained from interesterification with glycerol of a fully hardened soy bean oil. The monoester content was 94%, of which glyceryl monostearate was the main component together with minor amounts of other fatty acid monoglycerides (Fig. 1).

The triglycerides were derived from industrially refined liquid soy bean oil (SBO). The water was distilled twice.

All samples were observed grossly by polarizing microscope, melting point estimations and by differential scanning calorimetry (DSC).

The monoglyceride sample was mixed with water or soy bean oil in concentration intervals of 5%. Mixtures of 5 g were prepared in ampoules and heated to obtain isotropic

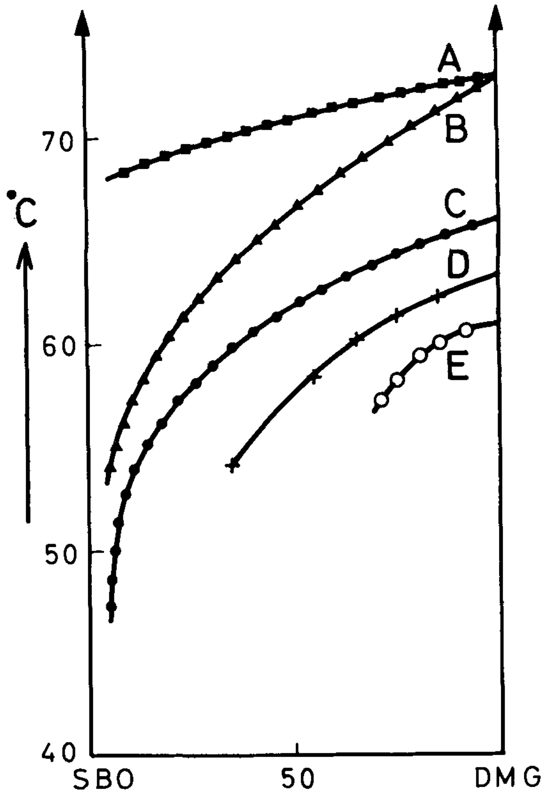

FIG. 2. SBO-DMG binary phase diagram. Line A: the melting curve for DMG crystals when slowly crystallized in SBO solution. Line B: the melting curve for the β-crystals when the solution has been rapidly cooled below line C and stored at room temperature for 18 hr. Line C: the solidification temperatures of the α-form for the solid solution of SBO-DMG obtained by rapid cooling. Line D and E: the solidification temperatures of the α-form of the solid solutions when 5 and 10% water, respectively, is solubilized.

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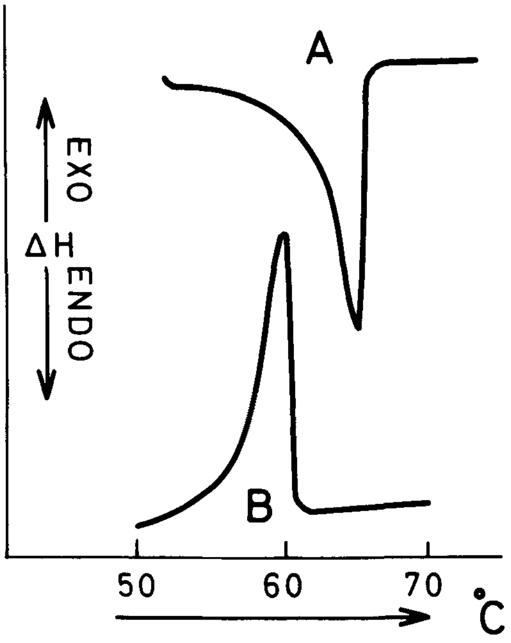

FIG. 3. DSC curves of a SBO-DMG mixture 40:60. Curve A: heating curve after shock chilling then standing for 18 hr at room temperature. The endothermic peak shows the melting point of the β-form. Curve B: at subsequent cooling the exothermic peak shows the solidification point of the α-form.

solutions. For rapid cooling ampoules were placed in a water bath at room temperature and then in a thermostatically controlled bath at the required temperature previously determined to be in the vicinity of a phase transition point. For slow cooling the ampoules were placed in an insulated water bath at 90 C which was allowed to slowly cool down to the required temperature. The ampoules were centrifuged at this temperature and the homogeneous content or the separated layers were investigated by microscopy, melting point estimations and DSC measurements. Small samples of the mixtures were also directly tempered on slides on the microscopic stage, in the melting capillaries or in the small pans for the DSC.

Microscopic Examination

The samples were examined by a polarizing microscope, Leitz Orthoplan, fitted with a special heating-cooling stage, thermostated to an accuracy of ±1 C. To ensure good thermal contact with the heating-cooling stage, samples were placed on thin glass slides, 0.16 mm thick. The different liquid crystalline phases were optically identified. The temperatures at which phase transitions occurred were determined by approaching the transition points both from higher and lower temperatures. Each sample was studied in duplicate or triplicate. Photographs were taken in polarized light and with a "1st-order red" plate as compensator.

Capillary Melting Point

Besides the microscopic examination of melting and transition points, the instantaneous melting point test (6) was used to estimate the melting point of the phases when solidified in the unstable α-form. A slip-point test after a tempering period of 18 hr was used for the determinations of β-form melting points.

Differential Thermal Analysis

Perkin Elmer DSC was used. The samples were 2-4 mg and heated at a rate of 4 C/min until phase transformations were observed. The method was used to check the transition and melting points found by microscopic examination or capillary melting point estimation.

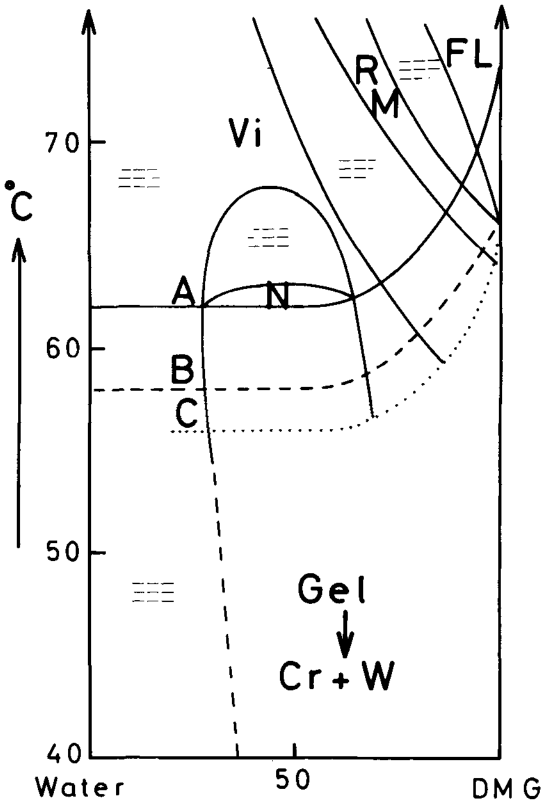

FIG. 4. Water-DMG binary phase diagram. Liquid crystalline phases: Vi = viscous isotropic; N = neat; RM = reversed middle; FL = fluid isotropic. Line A (unbroken): the melting points for crystals of the water-containing β-form. Line B (dashed): the solidification temperatures of the water-containing α-form, the gel. Line C (dotted): the solidification temperatures of water-containing α-form when 10% SBO is solubilized. The dashed areas represent two-phase regions.

Because the DMG was a mixture of glycerol monoesters of several fatty acids of different chain length, the temperature range for melting and phase transitions was about 2-3 C.

RESULTS

DMG-SBO

On addition of SBO both the melting and solidification temperatures of the DMG are reduced. The reduction is small for additions of less than 50% SBO, but increases rapidly at higher amounts (Fig. 2). In accordance with the polymorphic behavior of pure glycerides, the DMG-SBO solutions solidified in two modifications, the low melting unstable α-form and the higher melting stable β-form. When rapidly cooled the α-modification is formed, and this transforms readily in solid state to the β-modification (Fig. 3). Slow cooling of DMG-SBO solutions causes a crystallization in the β-form of the fairly insoluble DMG. When water is added further depression of the crystallization temperature of the α-form occurs, and this depression is independent of the initial amount of SBO. Up to 10% water can be solubilized when the ratio to DMG in SBO is high.

DMG-Water

The general form of this phase diagram (Fig. 4) is similar to earlier published results (1-3). Besides the neat, viscous and fluid isotropic phases previously reported in commercial DMG-samples and described as monostearins, we also found a reversed middle phase. This phase can likely be accounted for either by the presence of fatty acid chains longer than eighteen carbon atoms or by the presence of

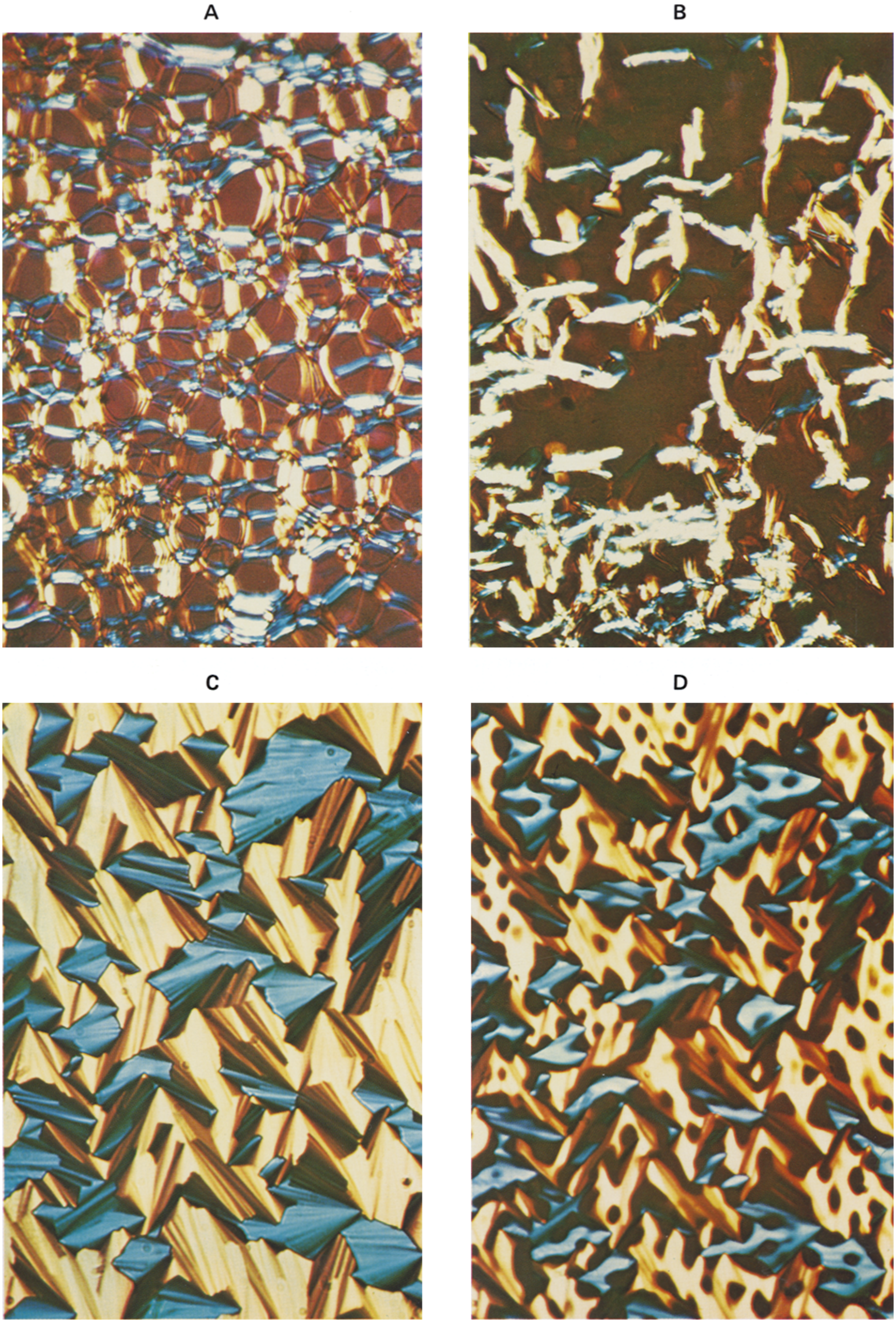

FIG. 5. Microphotographs of water DMG mixtures. Crossed Nicols and 1st order red plate. The length of each photograph corresponds to 0.33 mm. Series A-B shows irreversible transformation of neat viscous isotropic phase. Series C-D shows reversible transformation of reversed middle viscous isotropic phase. A: a mixture of water-DMG 50:50 forms a neat phase when heated to 60-63 C. Further heating results in transformation to the viscous isotropic phase. B: on subsequent cooling no neat phase appears but crystals of the α-modification are formed at 58 C. C: a mixture of water and DMG 20:80 forms a reversed middle phase at 70-72 C. D: when cooled to 68 C the viscous isotropic phase appears as dark areas; when heated again the image C is restored.

small amounts of monoisomers or di- and triglycerides in the DMG. Although mixed glycerides have a tendency to behave as a single component in accordance with the main compound, the small ratio of long chain fatty acids or impurities directed the phase behavior at high DMG concentrations. Low concentration of water caused marked and parallel depression of the maximum temperature region for the existence of the water-containing α- and β-crystalline forms. Beyond 40% addition, water had no further effect upon these temperatures.

When cooled a liquefied mixture solidified in the α-modification and formed a gel which in time transformed to β-form and water. Renewed heating gave rise to formation of one or more liquid crystalline phases when the temperature exceeded the melting point of the α- or β-form. The transformation rate for the α to β phase change is highly temperature dependent. The rate is highest at temperatures in the vicinity of the melting point of the α-form. In the region above this temperature the rate of β-crystals forming is increased if the temperature is raised from the α-form region.

Water contents between 40-65% give rise to a neat phase with a narrow temperature region of existence. At higher temperatures and at increased amounts of DMG the neat phase is transformed into a viscous isotropic phase. On further increase of either the DMG concentration or the temperature, a reversed middle phase is formed.

The phase change from the neat to the viscous isotropic state has been found to be irreversible. This is demonstrated by the absence of birefringence in the polarizing microscope. When the viscous isotropic phase is cooled the solution remains optically isotropic until the solidification point of the α-form is reached and crystals are formed in a pattern resembling neat phase structures (Fig. 5A and 5B). When the reversed middle phase was cooled the viscous isotropic phase was formed. When heated again the reversed middle phase was reformed. This transformation was completely reversible, contrary to the transition between the neat phase and the viscous isotropic phase. Figures 5C and 5D show the isotropic phase appearing as black areas in the color pattern of the reversed middle phase when the temperature is slowly decreased. An increase of temperature restored the pattern of the reversed middle phase. In both the neat phase and the reversed middle phase up to 15% SBO could be solubilized.

DISCUSSION

Owing to the difference in molecular interactions the effect of added water on the polymorphic behavior of DMG was different from that of added triglyceride. Water forms hydrogen bonds to the polar part of the monoglyceride, thereby reducing the directive forces between the glycerol residues. Consequently the crystallization of the α-form after addition of water is determined by forces of lower strength, viz., the dispersion forces of the hydrocarbon chains. On the other hand the main influence of triglyceride is on the packing conditions of the hydrocarbon chains. Small amounts of triglyceride have small effect on the melting point and will not influence the more solid matrix of the polar glycerol parts bound by hydrogen bonds. The effect of triglyceride will consequently become pronounced first at such high concentrations that the association equilibria of the monoglyceride begin to exert their influence. This is in agreement with the fact that water has an additive effect on the melting point reduction in the systems which contain triglycerides and monoglycerides (Fig. 2, lines D and E).

The maximum solubility of water in the α-form of DMG is found to be 12 mol aq/mol DMG which corresponds approximately to the amount of water required for the saturation of all available hydrogen bonding sites in the DMG. This supports the opinion that reduction of the crystallization temperature is dependent on the influence of water on the high energy hydrogen bonds in the crystallized monoglyceride.

Emulsion Properties

The results are highly relevant to the emulsifying process and to the properties of emulsions. The presence of liquid crystalline phases is of importance for the stability of emulsions and for the crystallization and orientation phenomena in the vicinity of emulsion drop surfaces. This fits well with the picture described earlier.

When preparing an emulsion with DMG of this type, the monoglyceride must be dissolved in a hot oil phase. When cold water is added, crystallization of the α-form takes place at the surfaces of the water droplets. The system then appears as a "mash" consisting of water and crystallized monoglyceride in oil. Gentle heating to a suitable temperature transforms the crystalline monoglyceride layers at the droplet surfaces to a liquid crystalline phase which will have a neat phase structure at DMG/water ratios of approximately 1:1. This treatment results in a stable emulsion with the water droplets protected by layers of liquid crystals.

It is essential that the temperature be kept in the region where the neat phase can exist. If the temperature is lowered the emulsion remains stable only as long as the solidified monoglyceride remains in the α-form. But as the transformation to β-form proceeds, the emulsion breaks down.

At high temperatures or with increased amounts of water a viscous isotropic phase is formed. This phase has a viscosity of a different magnitude from that of the neat phase, and this can give rise to handling problems when operating on a factory scale.

The neat phase region for this specific preparation was narrow but can be extended by various additives.

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