Diacylglycerols from Butterfat: Production by Glycerolysis and Short-Path Distillation and Analysis of Physical Properties

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ABSTRACT: The aim of this paper was to develop a process for the production of DAG from butterfat through glycerolysis and short-path distillation and to evaluate the physical properties of the DAG in comparison with the original butterfat. Chemical glycerolysis produced a mixture of acylglycerols containing DAG together with MAG and TAG. From the mixture of glycerolysis products, MAG were removed through three consecutive distillations (vacuum < 0.001 mbar) at 150°C. TAG were separated from DAG by distillation at 210°C, which gave a product with more than 80% DAG in the distillates. Distillation temperatures had significant effects on acyl migration. The formation of desirable 1,3-DAG was favored at higher temperatures. Under 210°C distillation, the equilibrium ratio of 6:4 was obtained between 1,3-DAG and 1,2(2,3)-DAG. The FA profile of the DAG product was relatively similar to the original butterfat. The total DAG recovery was around 77% in the pilot-scale production. The different patterns of m.p. were observed between butterfat and the DAG fraction produced as well as the MAG fraction collected. Solid fat content profiles of the DAG fraction and its mixtures with rapeseed oil possessed trends similar to those of the corresponding butterfat and its mixtures with rapeseed oil. Compared with butterfat, the DAG fraction behaved differently in its thermal profiles, crystallization patterns, and rheological properties; for example, the dropping point was 13°C higher for the latter than for the former, and the crystal pattern was mostly β form for the latter, whereas the former was the β′ form.

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KEY WORDS: Acyl migration, butterfat, diacylglycerols, dropping point, DSC, glycerolysis, short-path distillation, solid fat content, thermal behavior, X-ray.

Fats and oils are essential ingredients of human food. They are important sources of energy, EFA, and fat-soluble vitamins, and they impart excellent flavor, texture, and palatability to the food. However, more and more studies have documented the health concerns associated with diets high in fats. A high intake of fats has been blamed for the high incidence of cardiovascular disease, hypertension, and obesity, especially in industrialized countries (1). Obesity is the result of an imbalance between energy intake and energy expenditure, by which surplus energy intake is stored as TAG in adipose tissues (2). Although many kinds of food with fat substitutes have been available on supermarket shelves, consumers do not seem to be willing to compromise taste for health (3). Furthermore, most of the fat substitutes focus on limiting fat digestion and/or absorption or enhancing fat catabolism, which result in fatty stools and low absorption of fat-soluble vitamins. DAG, new healthy oils, are digested and metabolized in a different way, which significantly affects the body weight increase (4).

DAG are esters of glycerol in which two of the hydroxyl groups are esterified with FA. DAG can be in different isomers, and commercial products often contain the positional isomers of *sn*-1,2(2,3)- and *sn*-1,3-DAG. Studies in animals and humans suggest that DAG, especially *sn*-1,3-DAG, have a number of beneficial effects on lipid metabolism. Compared with traditional cooking oils (rich in TAG), intake of DAG can lead to body weight reduction and reduce visceral fat accumulation in rats and humans (4). Consumption of DAG oil may also produce less postprandial elevation in plasma TAG levels in humans and lower fasting serum TAG concentrations in rats and humans (4). The beneficial functions of DAG are probably attributable to differences in DAG and TAG absorption and metabolism. Unlike TAG, *sn*-1,3 DAG are not hydrolyzed to *sn*-2 MAG but produce *sn*-1(3)-MAG in the intestine. It has been proposed that the 1(3)-MAG formed are a relatively poor substrate for TAG synthesis in the intestinal mucosal cells and also that a substantial oxidation of FA occurs in these cells following digestion of DAG (5). This may account for the reduction in postprandial lipemia after digestion and absorption. Consumption of DAG in place of TAG may slightly increase the resting metabolic rate and also increase fat oxidation, which may contribute to the reported beneficial health effects. Notably, DAG have the same bioavailability and physiological fuel value as TAG (4). Moreover, the taste and processing functions in foods made with DAG oil are comparable to those made with traditional edible plant fats and oils (2,6,7).

So far, more than 63,000 tons of DAG cooking oil have been sold in Japan since its introduction in February 1999, and a similar product is being test-marketed in the United States (4). The primary components of DAG oil are about 80% *sn*-1,3 and *sn*-1,2(2,3)-DAG (at a ratio of 7:3), 20% others including TAG, and less than 5% MAG. Commercial DAG oils are manufactured through the esterification of FA with either glycerol or MAG in the presence of lipase or through the chemical glycerolysis of oils and fats in the presence of a chemical catalyst (6). In the production of DAG oil,

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especially through chemical glycerolysis, purification of DAG is an essential process to improve the content of DAG in the products (>80%). Because the b.p. of DAG are high and they are heat-sensitive, leading to thermal decomposition at very high temperatures under atmosphere pressure, short-path distillation (SPD) with high vacuum is a feasible way to purify DAG from the acylglycerol mixture after glycerolysis.

Butterfat ranks third in the worldwide production of edible oils (8). Because of its higher content of saturated FA, butterfat has received special attention regarding the negative effect on human consumption. The potential effects of DAG on human consumption will improve the uses of butterfat in foods dramatically. So far, few reports have been found for the preparation of DAG from butterfat.

The physical characteristics substantially influence the uses of oils and fats, especially for plastic fats. For example, the formulation of margarines, shortenings, and other fat-based products must be primarily based on an understanding of the relationships between specific properties and the composition of the oil blends (9). It is reported that the DAG cooking oil from rapeseed and soybean oils has application properties similar to the original TAG oils (4). Again, no detailed reports have been found on the properties of the butterfat DAG product and its blends with other oils.

Our aim was to develop a process for the production of DAG from butterfat on a large scale and to investigate the effects of the process steps on DAG formation and purification. The physical properties of the products were compared with butterfat to provide information for the future exploitation of butterfat DAG products in food applications.

TABLE 1
Proportion of Acylglycerols and FA Composition of Butterfat and the Product after Glycerolysis

Items	Glycerolysis mixture	Butterfat	
Profile		wt%	
MAG	50.70		0.04
DAG	40.30		3.37
TAG	8.40		95.88
FFA	0.20		0.71
Glycerol	0.30		0.00
		mol%	
FA composition	Total	Total	*sn*-2
C4:0	4.19	3.32	0.04
C6:0	4.07	3.02	0.02
C8:0	2.97	1.82	0.02
C10:0	3.53	3.23	2.33
C12:0	3.78	3.78	4.82
C12:1	0.36	0.37	0.01
C14:0	10.74	11.00	23.26
C14:1	0.94	0.87	0.02
C15:0	1.68	1.84	0.25
C16:0	27.40	29.02	34.26
C16:1	1.70	1.75	2.61
C17:0	1.60	1.47	0.04
C18:0	10.00	10.43	6.37
C18:1n-9	22.27	23.74	16.76
C18:1n-7	0.00	0.00	3.26
C18:2n-6	2.58	2.75	2.27
C18:3n-3	0.95	0.90	2.05
C20:0	0.15	0.16	0.00

MATERIALS AND METHODS

Materials. Butterfat was donated by Arla Food (Götene, Sweden). The FA compositions of butterfat and the *sn*-2 position are given in Table 1. The rapeseed oil was obtained from a local supermarket and had an FFA content of 0.05%, PV of 0.3 meq/kg, and water content of 0.05%. Its FA composition is (mol%) C16:0, 4.7; C16:1, 0.3; C18:0, 1.7; C18:1n-9, 58.4; C18:1n-7, 3.0; C18:2n-6, 22.7; C18:3n-3, 6.2; and others, 3.0. FAME and TAG standards were purchased from Sigma (St. Louis, MO). All other reagents and solvents were of analytical grade.

Glycerolysis of butterfat. Dried butterfat was blended with dry glycerol (16%, w/w) and NaOH (0.1%, w/w) in the vessel (10). The reaction was conducted at 200°C with constant stirring and a N_2 flow of 100 mL/min for 2 h. After cooling to 80°C, the mixture was washed twice with water and dried under vacuum.

SPD. A KD6 SPD system (UIC GmbH, Alzenau-Hörstein, Germany) was used. The process is described elsewhere (11). The major part of the equipment was constructed from stainless steel. The vacuum system contained a diffusion pump and two vamp pumps. The heating of the evaporator was provided by the jacket circulated with the heated oil from an oil bath.

Isomerization of DAG. The purified DAG product (20 g) was heated to 80°C and stirred at 300 rpm for 29 h with and without the addition of 2 g of silica gel as catalyst. Samples were withdrawn at 1, 3, 5, 7, 22, and 29 h for the analysis of 1,2(2,3)- and 1,3-DAG contents. Triplicate determinations for each sample were made and the average was used (SD < ±3%).

Analysis of FFA, MAG, DAG, and TAG contents by TLC. Samples were developed on thin-layer Silica gel G plates (Merck Co., Darmstadt, Germany) impregnated with 3% boric acid with chloroform/acetone (90:10, vol/vol) as the developing solvent. The bands containing acylglycerols or FFA were scraped off separately. After adding and mixing with weighed standards (15:0 TAG, DAG, MAG, and FFA), the bands were extracted with hexane. The solvent was removed under a stream of N_2 after extraction. The residuals were used for methylation and analysis of FA compositions. Weights of each lipid species were calculated according to the FA compositions of TAG, DAG, MAG, and FFA with the internal standard method by the standards added. The content of each species was expressed as the weight percentage after normalized calculation including the four species. Triplicate determinations were made and the average was used (SD < ±5%).

Methylation and GC analysis. FAME were prepared through saponification of TAG and esterification with methanol in the presence of boron trifluoride (12). The FAME were analyzed by GC with an HP 6890 series chromatograph and a fused-silica capillary column (SUPELCOWAX-10, 60 m × 0.25 mm i.d., 0.25 mm film thickness; Supelco Inc., Bellefonte, PA). The carrier gas was helium with a flow rate of 40 mL/min. The injector was used in split mode with a ratio of 1:20. The oven temperature was programmed from

70 to 160°C at a rate of 15°C/min, increased to 180°C at a rate of 1°C/min, further to 185°C at a rate of 0.5°C/min, and finally to 200°C at a rate of 20°C/min and held for 10 min. The injector and detector temperatures were 250 and 260°C, respectively. The FAME were identified by comparing their retention times with authentic standards from Sigma Chemical.

Dropping point. Dropping point was determined according to AOCS Method Cc 18-80 (13). Triplicate determinations for each sample were made and the average was used (SD < ±3%).

Thermal analysis by DSC. A differential scanning calorimeter (model SSC/5200; Seiko SII, Chiba, Japan) was used to determine the thermal profiles. Nitrogen was the carrier gas, and calibration was conducted with cyclohexane. A 10-mg sample was sealed in an aluminum pan, with an empty pan serving as a reference, and was stored at 5°C overnight. The sample was heated to 90°C at a rate of 5°C/min and held for 10 min (to ensure that no residual nuclei remained), then cooled to −10°C (5°C/min) while the endothermal and exothermal profiles were recorded.

Solid fat content (SFC). SFC was determined by low-resolution NMR according to AOCS Method Cd 16b-93 (13). The direct method was used. A tube with 2.5 mL of oil sample was first heated for 5 min at 60°C, then quickly transferred to 0°C and maintained there for 60 min. The tube was then transferred to the measuring temperature for SFC determination and maintained there for 30 min before NMR determination. Triplicate determinations were made for each sample and the average was used (SD < ±5%).

Consistency by penetrometry. A TA.XT2 texture analyzer with a 45° conical probe (P/45C; Stable Micro Systems, Surrey, United Kingdom) was used to measure the firmness of fats or their derivatives by measuring the distance the 45° conical probe penetrated the fat according to AOCS Method Cc 16-60 (13). The samples (liquid) were placed in the refrigerator at 5°C overnight and kept at that temperature prior to testing. The distance of the penetration used was 5 mm (into the sample) at a speed of 2 mm/s. The hardness value was represented by the peak force of the first compression of the product. Cohesiveness was represented by how well the product withstood a second deformation relative to how it behaved under the first deformation. It was measured as the area of work during the second compression divided by the area of work during the first compression (Area 2/Area 1). Triplicate determinations for each sample were made and the average was used.

X-ray analysis. A Siemens D5000 diffraction meter with rotating X-ray tube and detector was used (Bruker AXS, Congleton, United Kingdom). The apparatus comprised a standard generator producing Cu Ka radiation with a wavelength of 0.154 nm. Approximately 60 mg of fat was melted at 80°C and stored at 5°C overnight. X-ray measurements were carried out at room temperature. Polymorphic forms were determined by short spacing, and chain packing was measured by long-spacing determinations. The α form is a 2θ of about 21°, which is equivalent to a short spacing of 4.15Å. The β′ form is a 2θ at 20.8° and 23.0°, which are equivalent to short spacings of 4.2 and 3.8 Å. The β crystal had a very strong peak at 4.6 Å, which is a 2θ of approximately 19.1° (14).

RESULTS AND DISCUSSION

Glycerolysis. The acylglycerol composition and FA composition (FAC) of the glycerolysis product of butterfat are indicated in Table 1. Chemical glycerolysis did not result in a significant change in the FAC of butterfat. Chemical glycerolysis has been widely used in industry for the production of MAG, and the reaction is basically conducted in a random manner, in which the content of each species in the reaction mixture is randomly distributed. Therefore, the content of each species from the reaction between fats and glycerol can be theoretically calculated (15). The maximum content of DAG from the reaction is around 40–45% from the substrate ratio used. This agrees well with the result in Table 1. MAG, TAG, and others can be removed to improve the content of DAG by SPD.

Separation of MAG from the reaction mixture at different temperatures. Very little distillate was obtained below 100°C (Fig. 1), and most of the distillate was FFA and glycerol. The distillate yield was increased with the increase in temperature, and most of the distillate was MAG. At distillation temperatures over 130°C, FFA and glycerol were not present in the distillates and were fully removed under the distillation conditions. This agrees with previously published results (16). With the increase in temperature, the FAC of the residuals were slightly affected. In general, the amounts of short- and medium-chain FA (C_4–C_{10}) were not significantly

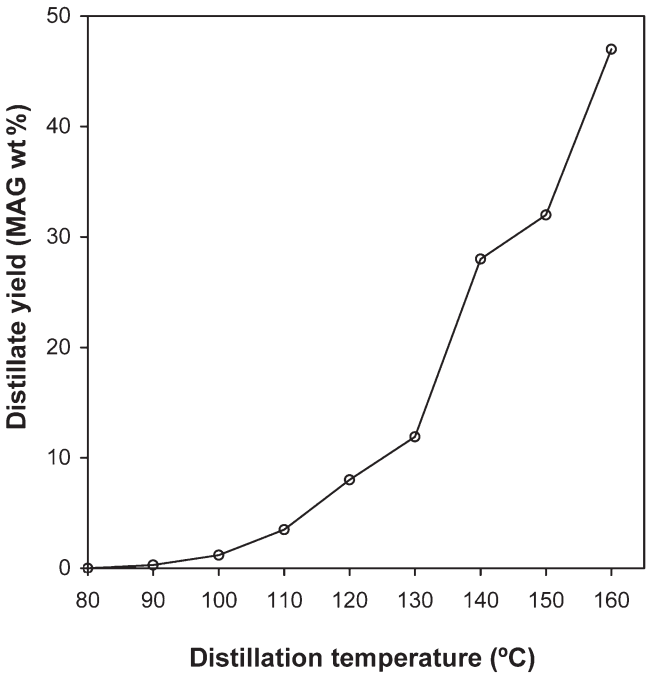

FIG. 1. Relationship between yield of distillates (MAG, wt% of starting materials) and distillation temperatures. Other conditions: heating exchange temperature 80ºC, condenser temperature 80ºC, feeding flow 200–250 mL/h, and vacuum <0.001 mbar.

TABLE 2
FA Compositions (mol%) of the Distillation Residuals at Different Distillation Temperatures

FA	80°C	90°C	100°C	110°C	120°C	130°C	140°C	150°C	160°C
C4:0	2.95	2.95	2.81	3.04	3.75	4.49	3.52	3.12	2.96
C6:0	2.38	1.52	2.25	1.78	1.61	1.96	1.98	2.42	3.93
C8:0	1.69	1.21	1.66	1.40	1.18	1.14	1.18	1.76	2.94
C10:0	3.71	3.36	3.48	3.26	2.56	2.01	2.16	2.84	4.05
C12:0	4.58	4.54	4.43	4.32	3.40	2.64	2.39	2.77	3.49
C14:0	13.18	13.45	13.20	13.25	12.10	10.97	8.53	8.43	9.25
C14:1	1.09	1.10	1.09	1.09	0.98	0.89	0.71	0.54	0.79
C15:0	1.00	1.04	1.03	1.04	1.00	0.97	0.84	0.78	0.77
C16:0	32.28	33.23	32.77	33.31	33.69	33.77	32.05	29.68	26.58
C16:1	1.53	1.48	1.47	1.48	1.51	1.48	1.36	1.25	1.17
C17:0	0.47	0.48	0.46	0.47	0.48	0.49	0.55	0.54	0.46
C18:0	10.77	11.09	10.99	11.21	11.87	12.36	14.46	15.19	14.52
C18:1n-9	21.10	21.47	21.46	21.65	22.92	23.64	27.07	27.40	25.94
C18:2n-6	1.51	1.52	1.52	1.53	1.61	1.64	1.93	1.92	1.78
C18:3n-3	0.48	0.48	0.49	0.48	0.52	0.53	0.62	0.63	0.59
C20:0	0.09	0.00	0.09	0.10	0.12	0.13	0.17	0.22	0.28

reduced (Table 2). The content in the residues was first slightly reduced and then slightly increased again after reaching a temperature of 150°C. The reason for this was that a small amount of DAG with short- and medium-chain FA was removed to the distillates when a temperature lower than 150°C was used (results not shown), but when a temperature higher than 150°C was used, long-chain FA DAG started to be distilled off also, leading to the increase in short- and medium-chain FA in the residues.

Changes in the acylglycerol profiles with an increase in distillation temperature are shown in Figure 2. The content of DAG increased over the temperature range studied, whereas the MAG content was reduced. The content of TAG remained stable until 150°C, at which point it began to decrease.

Changes in the acylglycerol profiles of the residues also can be calculated by a simple mass balance, if we assume that the distillation was conducted as we expected (i.e., that no DAG were distilled off and that no FFA and glycerol were left in the residues). The discrepancies between the calculated results and those measured in Figure 2 showed us how well justified our assumptions were so as to provide information for the process evaluation. The calculation equations are given below for the contents of MAG, DAG, and TAG, respectively, in the distillation residuals according to the theoretical mass balance:

$$\text{cal} - \text{MAG} = \frac{51.2 - \text{MAG}_{\text{Fig. 1}}}{100 - \text{MAG}_{\text{Fig. 1}}} \quad [1]$$

$$\text{cal} - \text{DAG} = \frac{40.30}{100 - \text{MAG}_{\text{Fig. 1}}} \quad [2]$$

$$\text{cal} - \text{TAG} = \frac{8.40^{14}}{100 - \text{MAG}_{\text{Fig. 1}}} \quad [3]$$

Here, $\text{MAG}_{\text{Fig. 1}}$ is the MAG yield shown in Figure 1. The numbers 40.3 and 8.4 are DAG and TAG contents in the starting materials of distillation, respectively, and 51.2 is the sum of MAG, FFA, and glycerol contents in the starting materials (see Table 1).

The dotted lines in Figure 2 show the calculated MAG, DAG, and TAG contents in the residuals over the distillation temperature range. At low temperatures, the difference was minimal and could be neglected. However, there was a pronounced difference between the observed content of DAG and that from the calculation at higher temperatures, in particular 160°C, even though the MAG content was significantly reduced. This means the assumptions were not fully correct under higher temperatures. We measured the FFA content and the glycerol content in the residuals (less than 0.03% FFA in the residuals and no glycerol detected), which indicated that the assumption for this aspect was correct. The other assumption was therefore the major reason for the discrepancies under higher temperatures. This is because some smaller DAG molecules were also distilled off with the MAG at higher temperatures. For the sake of MAG removal and the

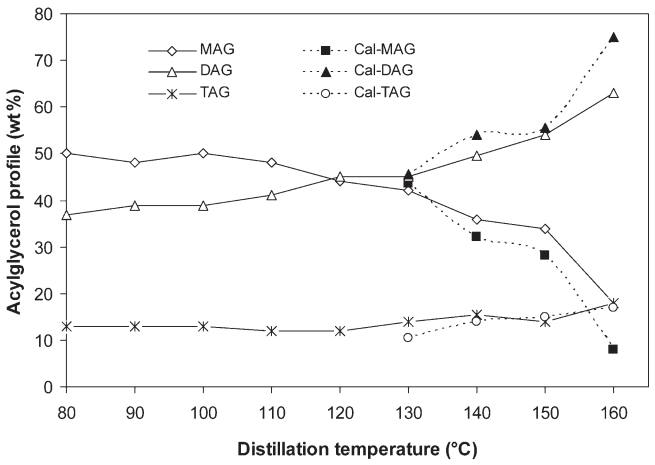

FIG. 2. Relationship between the acylglycerol profiles of residuals and distillation temperatures. Cal-, calculated (for method see text). Conditions the same as in Figure 1.

low loss of smaller DAG molecules, 150°C was taken as a suitable temperature for the separation of MAG from the mixture.

Repeated distillations at 150°C. A better separation efficiency can be obtained by repeated distillations at a constant temperature, especially when the distillates are present in large amounts (16). The residue from one distillation was used as the starting material for the next distillation at the same conditions. In this case, the residue from the first distillation (at 150°C) was distilled a second time under the same conditions and an additional 15% distillate was produced; a third distillation of the residue from the second yielded another 10%. Therefore, the two additional distillations increased the MAG yield from 30 to 55%. Considering 51.2% MAG, FFA, and glycerol in the starting material (Table 1), we calculated that about 4% DAG containing short-chain FA was removed into the distillates after three sequential distillations. This figure was roughly in agreement with the results observed for the DAG content in the distillates. A further slight reduction of short- and medium-chain FA was also observed with an increase in distillation times (data not shown). This suggests that the fourth distillation at 150°C was not necessary to remove more MAG from the residual since the loss of DAG containing shorter-chain FA would be increased.

Separation of DAG from TAG. After three sequential distillations at 150°C, the residual consisted mainly of TAG and DAG. Another distillation stage at higher temperatures (180–210°C) was conducted to separate DAG from TAG. The effect of temperature on the separation of DAG from TAG is shown in Figure 3. With the increase in temperature, the MAG and TAG contents in the distillates decreased and the DAG content increased. At 210°C, there was more than 80% DAG in the distillate, and the purity was higher than the current commercial DAG cooking oil made from soybean and rapeseed oils.

Effect of temperature on acyl migration. Intramolecular acyl migration is a well-known reaction in acylglycerols. Acyl migration is affected by temperature, acyl chain length, catalysts, and other factors (17). Acyl migration is initiated by the nucleophilic attack of a lone pair of electrons of the free hydroxyl oxygen on the ester carbonyl carbon to form an unstable five-member ring intermediate orthoester. Ring opening by the cleavage of the original ester carbon–oxygen single bond results in acyl migration (18). The primary hydroxyl oxygen is a better nucleophile than the secondary hydroxyl; therefore, the acyl shift from a secondary hydroxyl to a primary hydroxyl is favored. The shorter the acyl chains in DAG, the more readily the reaction reaches equilibrium (17).

The ratio between contents of 1,3- and 1,2(2,3)-DAG was also increased with the increase in distillation temperature (Fig. 4). Chemical glycerolysis is a random reaction, and isomers will be in equal proportions after the reaction. However 1,3-DAG are much more stable than 1,2(2,3)-DAG, as already discussed; therefore, the latter will migrate to the former ones. The result confirms that higher temperature increased the migration rate. For the ratio of 1,3- to 1,2(2,3)-DAG, the increase with temperature can be treated linearly in the relationship as $y = 0.0028x + 0.79$ with a coefficient of determination of $R^2 = 0.91$ (generated from Fig. 4 by regression), where x and y stand for the distillation temperature and the ratio between 1,3- and 1,2(2,3)-DAG, respectively. Under a temperature of 210°C, the ratio between the 1,3- and 1,2(2,3)-DAG was approximately 6:4 (Figs. 3, 4).

The equilibrium ratio between 1,3- and 1,2(2,3)-DAG has been reported to be 6:4 or 7:3 (17,19). Commercial DAG oil is reported to have a 7:3 ratio between the two major isomer groups. For nutritional considerations, 1,3-DAG are the target product. Therefore, the product formed under distillation at 210°C should be checked to confirm whether it has reached equilibrium. Silica gel could dramatically accelerate acyl migration because it lowers the activation energy barrier through

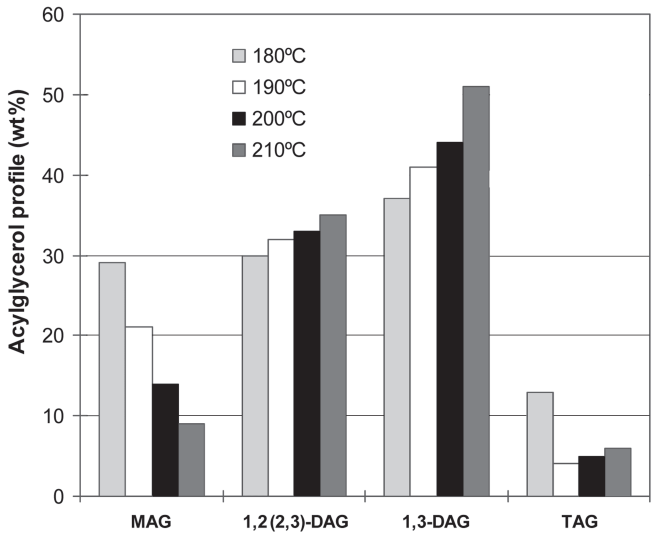

FIG. 3. Effect of temperature on the acylglycerol profiles of the distillates from short-path distillation (SPD). Other conditions the same as in Figure 1.

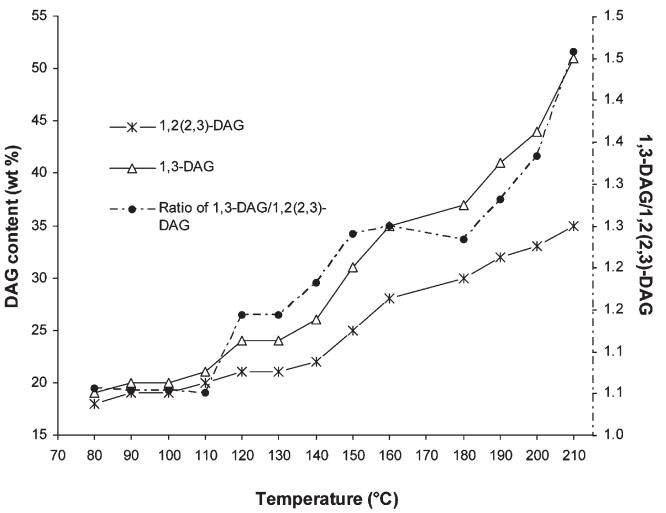

FIG. 4. Effect of temperature on the ratio between 1,3- and 1,2(2,3)-DAG during SPD. For abbreviation see Figure 3. Other conditions the same as in Figure 1.

silica gel coordination with the transition state to form the intermediate orthoester (17). Therefore, the DAG product was exposed to silica gel under 80°C for 29 h and compared with the control experiment. The ratio between 1,3- and 1,2(2,3)-DAG in the product could not be improved by the treatment (results not shown). This was most probably because the equilibrium had been reached during distillation at 210°C. This result implies that the equilibrium ratio in this case for the two groups of isomers was 6:4, which is similar to the result obtained by Kodali *et al.* (17). It also implies that the SPD procedure is sufficient to cause the migration into equilibrium.

Large-scale purification of DAG from the glycerolysis mixture. A DAG production process was based on the above studies. After chemical glycerolysis, the mixture was subjected to three sequential SPD at 150°C to remove MAG. The residue from this operation was subject to distillation at 210°C to recover the DAG. With this process, around 2 kg of DAG oil was produced from butterfat for application studies. The yield of the DAG product (DAG oil/starting butterfat, w/w) was 36%. The MAG product was a co-product of the process, with a yield of around 52%. The rest (12%) was mostly TAG with some remaining DAG, which mainly contained long-chain FA. The total DAG recovery was around 77%, suggesting that part of the DAG was lost to either the MAG fraction or the TAG fraction (the last residual). Since butterfat is a very complicated fat with high amounts of short- and medium-chain FA, this can contribute to complexities in the distillation process. The acylglycerol profile and FA composition of the DAG product are indicated in Table 3. 1,3-DAG was the main component of the final DAG product. The FA profile of the DAG product was relatively similar to the original butterfat. In the following description, the DAG and MAG fractions produced are referred to as Butterfat DAG and Butterfat MAG, respectively.

TABLE 3
Proportion of Acylglycerols and the FA Composition of the DAG Product from Butterfat

Acylglycerols	wt%
MAG	7.0
1,2(2,3)-DAG	35.0
1,3-DAG	51.0
TAG	7.0
FA	**mol%**
C4:0	6.78
C6:0	4.65
C8:0	2.54
C10:0	5.10
C12:0	5.00
C12:1	0.21
C14:0	13.22
C14:1	1.06
C15:0	1.31
C16:0	28.7
C16:1	1.20
C17:0	1.27
C18:0	9.00
C18:1n-9	17.11
C18:2n-6	1.19
C18:3n-3	0.39
C20:0	0.12

Melting and crystallization profiles. The melting behavior of acylglycerols depends on their FA compositions, acylglycerol compositions, crystals, and tempering history (20). The m.p. (dropping points) of butterfat, butter DAG, butter MAG and their mixtures with rapeseed oil are indicated in Figure 5. The change patterns of m.p. of butterfat, Butterfat DAG, and Butterfat MAG with the increase of their contents in the mixture were similar to those of other TAG, DAG, and MAG (21). Obviously, butterfat MAG had a higher m.p. (10°C) than Butterfat DAG, and Butterfat DAG had a higher m.p. (13°C) than butterfat. For butterfat, Butterfat DAG, and Butterfat MAG, there were good linear relationships between dropping points and their percentages in mixtures with rapeseed oil (butterfat: $y = 0.13x + 21.07$, $R^2 = 0.92$; DAG: $y = 0.13x + 35.32$, $R^2 = 0.86$; and MAG: $y = 0.051x + 51.07$, $R^2 = 0.94$). Butterfat DAG shared the same trend as butterfat when blended with rapeseed oil.

Melting and crystallization profiles of butterfat, Butterfat DAG, and Butterfat MAG and their mixtures with rapeseed oil from DSC are presented in Figure 6. Melting profiles were consistent with the dropping points in Figure 5. The melting range of MAG was wider than those of butterfat and Butterfat DAG (Fig. 6A). This is presumably because there are C_6–C_{18} MAG in Butterfat MAG and there is a large difference in the m.p. between $C_{6:0}$-MAG (19.4°C) and $C_{18:0}$-MAG (81.5°C) (21). The blending of 30% rapeseed oil obviously reduced the crystallization temperature (onset temperature) by 2–3°C (Fig. 6B), probably due to the reduction of SFC, whereas the difference varied for the melting temperature (offset temperature) when rapeseed oil was blended (Fig. 6A). The two smaller peaks in Figure 6B, (2) and (5), were mostly due to crystal formation for some of the species in the DAG product with, for example, higher unsaturation and shorter chain length. For a detailed explanation, further study is needed.

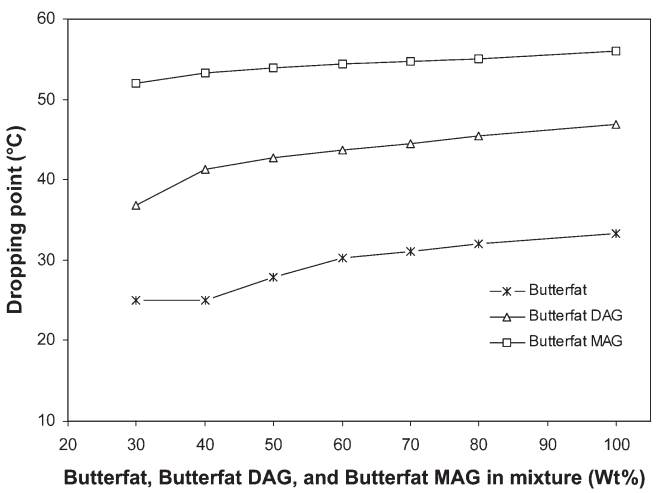

FIG. 5. Dropping points of mixtures of butterfat, Butterfat DAG, and Butterfat MAG with rapeseed oil under different proportions.

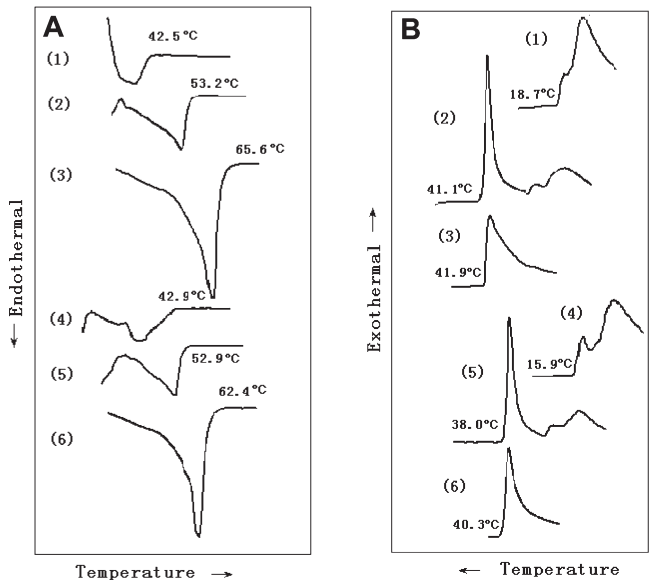

FIG. 6. DSC profiles of butterfat (1), Butterfat DAG (2), Butterfat MAG (3), butterfat/rapeseed oil (70:30, w/w) (4), Butterfat DAG/rapeseed oil (70:30) (5), and Butterfat MAG/rapeseed oil (70:30). (A) Melting profiles. The temperature above the melting curve is the fusion temperature for the corresponding sample, at which the whole sample was melted. (B) Crystallization profiles. The temperature above the crystallization curve is the crystallization temperature for the corresponding sample, at which the sample began to crystallize.

The butterfat, Butterfat DAG, and Butterfat MAG had different crystal behaviors. Figure 7 shows that the butterfat contained pure β′ crystals, which had two strong peaks at the 2θ value of 20.8° and 23.0°. However, Butterfat DAG and Butterfat MAG were β-form crystals, which had a very strong peak at the 2θ value of 19.1°. Double packing was observed for butterfat, Butterfat DAG, and Butterfat MAG by the long-spacings of X-ray analysis (40.8, 44.7, and 45.6Å, respectively). In the case of Butterfat MAG, some concurrent α crystals (at 21–22°) may also exist. Generally, the desirable polymorphic form of a plastic fat for margarine and butter products is the β′ form, which results in a smooth product texture. The β form (large crystal) gives the product a grainy structure and undesirable texture (22). This indicates that Butterfat DAG cannot be used in the same way as butterfat, such as in butter or margarine production, since the β′ form is required in these two applications. Therefore, application systems for Butterfat DAG should be studied further and developed to exploit their possible uses in the food industry. Advances in processing technology may also overcome inherent problems with regard to the unsuitability of the thermal properties of Butterfat DAG for normal plastic fat usage.

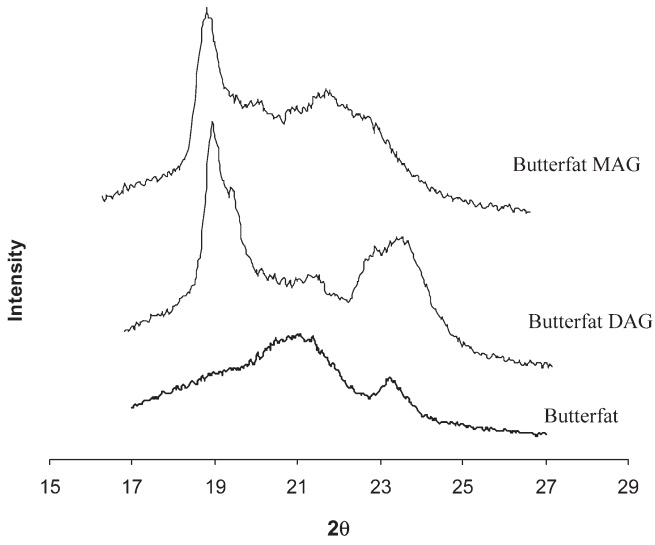

FIG. 7. X-ray diffraction patterns of butterfat, Butterfat DAG, and Butterfat MAG.

SFC and consistency. SFC is related to the spreadability at the refrigerator temperature, the resistance to oil-off at room temperature, and the mouthfeel of the fat product (20). SFC profiles of Butterfat DAG and its mixtures with rapeseed oil showed trends similar to those of the corresponding butterfat and its mixtures with rapeseed oil (Fig. 8). MAG and its mixtures with rapeseed oil had a series of solid, flat curves because of its high m.p. (>50°C). The profiles for butterfat and its 80:20 blend with rapeseed oil indicate little difference in SFC at 10°C (Fig. 8A), whereas for Butterfat DAG, the difference is much larger at the same temperature (Fig. 8B). This phenomenon could be due to the packing behavior of various acylglycerols. A eutectic phenomenon existed for the Butterfat MAG/rapeseed oil blend at 70:30 (Fig. 8C). This again could be due to the particular crystal structure. Clear understandings of all these phenomena need more detailed study.

Penetration hardness can provide relevant information for the textural properties of the spreads. Factors affecting the rheological properties of a fat-based spread are SFC, acylglycerol composition, and crystal modification (20). The data given in Table 4 are an indication of the hardness of the samples at 5°C. The smaller the value of the force, the softer the sample will be. Butterfat MAG had higher hardness and a more severe second deformation than Butter DAG and butterfat due to its high m.p. The hardness and cohesiveness of Butterfat DAG were similar to that of butterfat, although the SFC profile and dropping points were higher than those of butterfat. SFC is only partially related to penetration hardness (23). The rheological properties of plastic fats are consistent with a tightly packed structure of fat crystals. The smaller the crystal size, the firmer the fat will be (20). In small amounts,

TABLE 4
Penetration Hardness of Butterfat, Butterfat DAG, Butterfat MAG, and Their Mixtures with Rapeseed Oil Represented by Penetration Force and the Ratio Between Area 1 and Area 2

Samples	Force (N)	Area 1/Area 2
Butterfat	11.67 ± 0.38	11.54 ± 0.57
Butterfat/rapeseed oil (70:30, w/w)	6.17 ± 0.55	6.05 ± 0.39
Butterfat DAG	9.72 ± 0.73	9.28 ± 0.61
Butterfat DAG/rapeseed oil (70:30, w/w)	4.23 ± 0.21	4.02 ± 0.21
Butterfat MAG	30.32 ± 5.75	25.82 ± 1.74
Butterfat MAG/rapeseed oil (70:30, w/w)	14.53 ± 3.19	12.19 ± 1.76

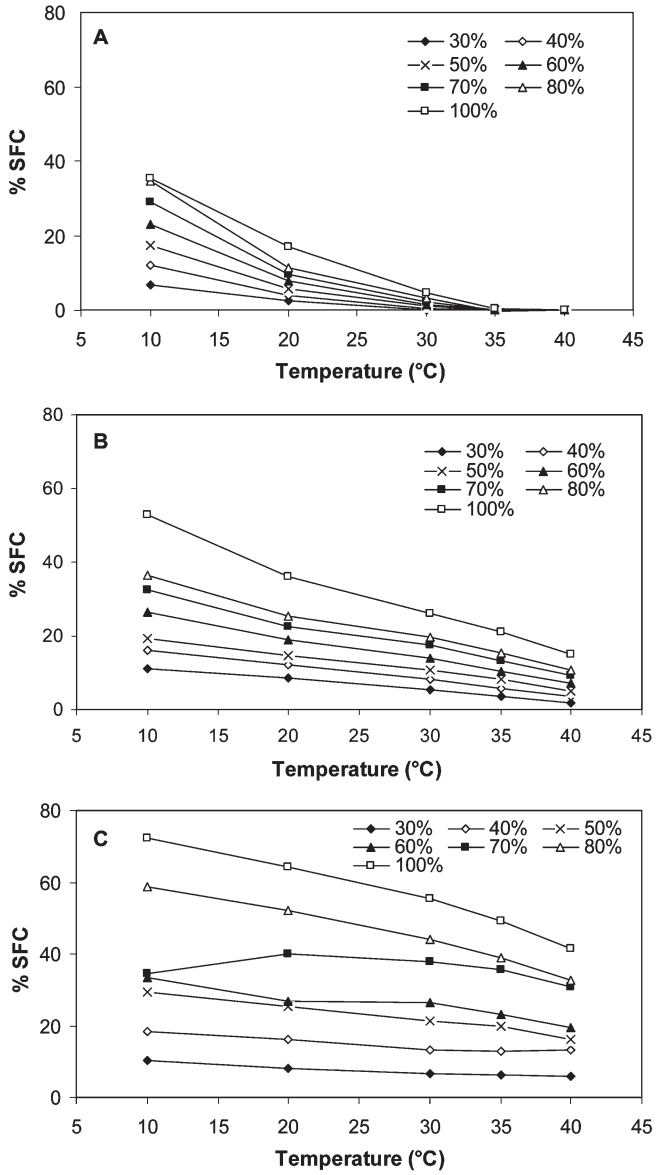

FIG. 8. Solid fat contents (SFC) of butterfat/rapeseed oil (A), Butterfat DAG/rapeseed oil (B), and Butterfat MAG/rapeseed oil (C).

DAG has been shown to inhibit the crystallization of fats. When DAG is the main product, the inhibition is not shown in the DSC profile (Fig. 6). Wright and Marangoni (24) also reported that DAG has a larger crystal size than TAG. This agrees in general with the results and phenomenon obtained in this study for Butterfat DAG, as shown in Figures 6 and 7 and in Table 4. The large, course DAG crystals that formed during crystallization will result in a loose structure of the Butterfat DAG product. For a potential application, this phenomenon has to be taken into consideration. Processing technology can be developed to optimize the effect of this phenomenon on application performance.

In conclusion, from butterfat with a very complicated FA composition, a potentially healthy DAG fat with high purity (86%) can be manufactured efficiently by chemical glycerolysis and the removal of MAG and TAG through SPD separation. Butterfat DAG behaved differently in its thermal profiles, crystallization patterns, and rheological properties compared with butterfat. Results from this study supply a clue to potential applications of DAG products from butterfat in the food industry.

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