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Preparation of Glyceroglycolipids from Pumpkin and Their Effects on Polymorphic Transformation of Cocoa Butter

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Monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and trigalactosyldiacylglycerol (TGDG) were extracted from pumpkin, then hydrolyzed to the corresponding galactosylmonoacylglycerols by 1, 3-specific lipase from *Rhizopus arrhizus*; MGDG was then hydrolyzed to diacylglycerol (DG) by β-galactosidase from *Aspergillus oryzae*. The kinetics of polymorphic transformation on cocoa butter and the effect caused by the addition of these seven glyceroglycolipids were investigated. Each glyceroglycolipid could be incorporated at the level of 5% into the cocoa butter without changing the crystal lattice. The polymorphic transformation on cocoa butter was induced by continuous temperature cycling between 32 and 20°C at 12 h intervals and monitored with a differential scanning calorimeter. The result indicated that MGDG, DGDG, TGDG and DG significantly retarded the polymorphic transformation from Form V to VI of cocoa butter compared to the control, while the other glyceroglycolipids had no effect. The addition of MGDG was the most effective. These results indicate that the number of the acyl chain and galactose of glyceroglycolipid strongly influence the polymorphic transformation of cocoa butter.

Keywords: cocoa butter, glyceroglycolipid, polymorphic transformation, DSC

The polymorphic behavior of cocoa butter is an important factor for chocolate manufacturers, because cocoa butter is a major solid fat in chocolate (Minifie, 1989). The qualities of chocolate like gross, snap, texture, heat resistance and fat bloom resistance are decided by the polymorphic structure of cocoa butter. In particular, fat bloom, which manifests itself as a white film and generally dulls the characteristic surface gloss, is the most troublesome problem. Therefore, the polymorphism of cocoa butter in relation to the crystallization behavior has been studied extensively. Wille and Lutton (1966) using X-ray diffraction spectroscopy described the six polymorphic forms of cocoa butter. They suspected that the polymorphic transformation from Form V to VI of cocoa butter caused fat bloom because they found Form VI whenever they found fat bloom. The polymorphic transformation from Form V to VI is generally found in well-tempered chocolate during storage. Storage temperature above the melting point of cocoa butter or its fluctuation below the melting point will increase the rate of transformation because of melting and recrystallization. Abusive storage temperatures can cause the solid state transformation from Form V to VI on cocoa butter. To prevent this transformation, there have been attempts to develop substitute fat which is highly compatible with cocoa butter and to develop a fat bloom inhibitor as an additive. It is well known that some emulsifiers are useful agents for controlling the polymorphic behaviors of fats and oil, for example, sorbitantristearate (STS) and sucrose fatty acid polyester (SPE) (Kawamura, 1980; Aronhime *et al*., 1988; Suwa & Matsuda, 1993).

Glyceroglycolipids are widely found in plants, in animal tissues and in bacteria; their structures are shown in Fig. 1. Glyceroglycolipids consist of hydrophilic carbohydrate groups and hydrophobic fatty acid moieties which bind to a glycerol moiety, and are known to be part of the major components of biomembranes (Kates, 1990). Glyceroglycolipids are also believed to play important roles such as enhancing membrane stability (Chapman *et al*., 1983); some glyceroglycolipids have also been reported to exhibit anti-tumor-promoting activity (Murakami *et al*., 1995) or biological activities (Katsuoka *et al*., 1990; Sakata & Ina, 1983). In a previous report (Nakae *et al*., 1998), we described the extraction of digalactosyldiacylglycerol (DGDG) and trigalactosyldiacylglycerol (TGDG) from pumpkin, and their hydrolyzation to digalactosylmonoacylglycerol (DGMG) and trigalactosylmonoacylglycerol (TGMG) using 1,3-specific lipase for the purpose of obtaining hydrophilic emulsifiers.

About eighty percent of cocoa butter is composed of specific triacylglycerols of a Sat-O-Sat (saturated-oleic-saturated) type, such as POP (1, 3-dipalmitoyl-2-oleoyl-glycerol), POS (2-oleoyl-palmitoyl-stearoyl-glycerol), and SOS (1, 3-distearoyl-2-oleoyl-glycerol). In pumpkin, linoleic acid, linolenic acid, palmitic acid, oleic acid and stearic acid were the major glyceroglycolipid components. Accordingly, the fatty acid chain length of glyceroglycolipid is the same as that of cocoa butter, and both glyceroglycolipid and cocoa butter are a kind of glyceride. Therefore, we suspected that glyceroglycolipid would be structurally better compatible with cocoa butter than STS and SPE described above.

In this report, we isolated MGDG, DGDG and TGDG from pumpkin, and then hydrolyzed them to monogalactosylmonoacylglycerol (MGMG), DGMG and TGMG by lipase; we then hydrolyzed MGDG to diacylglycerol by β-galactosidase for the purpose of obtaining various types of glyceroglycolipids. We investigated the performance of their seven glyceroglycolipids as a dynamic control of the polymorphic transformation of cocoa but-

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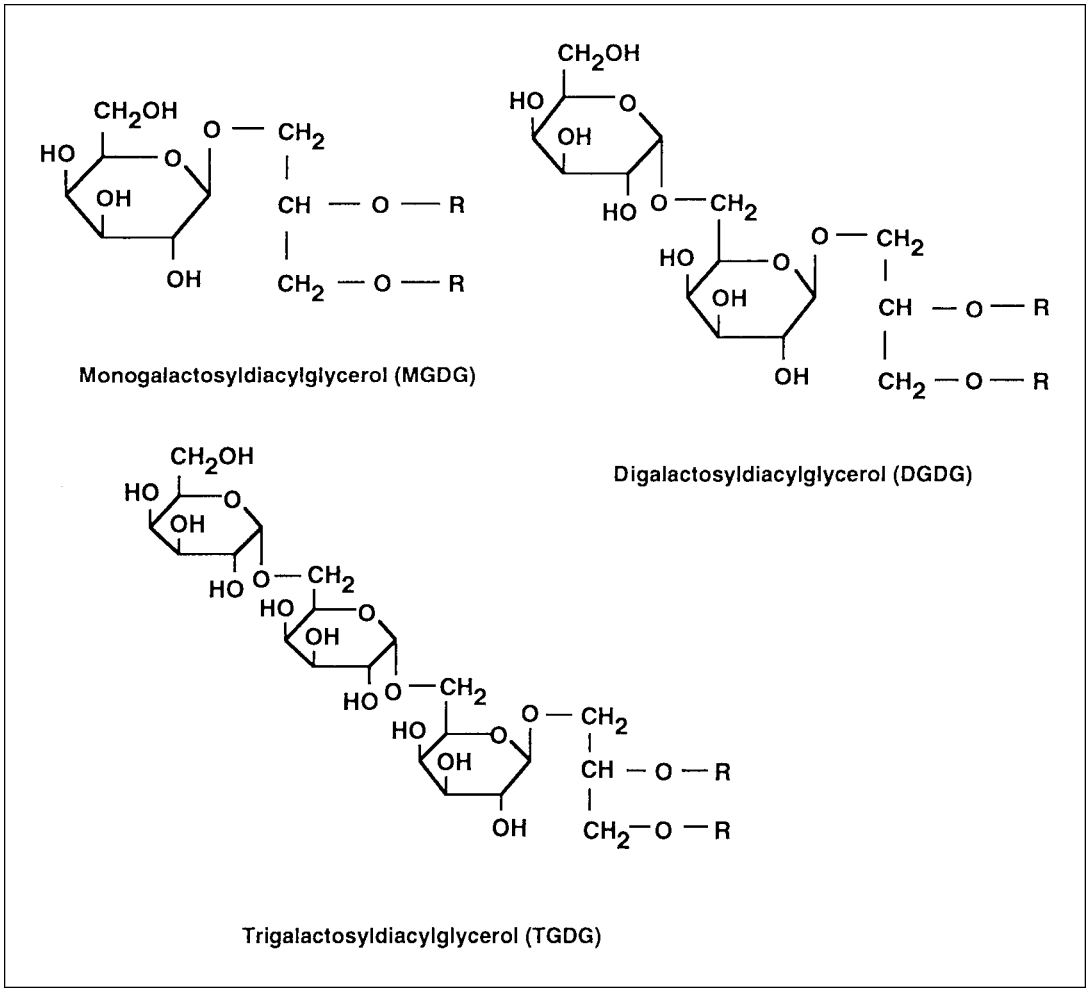

Fig. 1. Structures of MGDG, DGDG and TGDG. R represents acyl residue.

ter hoping to understand this phenomenon which could serve as basic knowledge for more complex systems of cocoa butter like chocolate.

Materials and Methods

Chemicals Pumpkin purée was purchased from KAGOME Co., Ltd. (Nagoya). Lipase (from *Rhizopus arrhizus*), β-galactosidase (from *Aspergillus oryzae*) and STS were purchased from Sigma Co., Ltd. (St Louis, MO). Refined cocoa butter was provided by Daito Cacao Co., Ltd. (Tokyo). All other chemicals used were commercially available and of chemically pure grade.

Preparation of MGDG, DGDG and TGDG The extraction of MGDG, DGDG and TGDG from pumpkin was done by the previously reported method of Nakae *et al.* (1998). Pumpkin purée was suspended in chloroform-methanol (2 : 1, v/v) solution and the mixture homogenized using a Physcotron mixer (MICROTECH NITION Co. Ltd., Chiba), then extracted by stirring at room temperature. The lipid fraction was subjected to silica gel (Wakogel C-200; 1×15 cm) column chromatography by sequential elution with chloroform, acetone, and methanol. The glycolipid fraction was then subjected to silica gel column chromatography by stepwise elution with chloroform-acetone. MGDG was eluted with chloroform-acetone (40 : 60, v/v); DGDG was also eluted with chloroform-acetone (20 : 80, v/v); while TGDG was eluted with acetone only. Purities of the fractions containing MGDG, DGDG and TGDG were confirmed by thin-layer chromatography (TLC) (Merck, art. 5626, Darmstadt, Germany) using chloroform-methanol-H_2O (65 : 25 : 4, v/v/v), and were detected by spraying 50% (v/v) H_2SO_4 followed by heating at 130°C for 5 min. The purity of each extracted glycero-glycolipid was confirmed by high performance liquid chromatography (HPLC) using DIOL column (4.6×250 mm, Merck) eluted with *n*-hexane/2-propanol/1-butanol/water (60/30/7/3, v/v/v/v) at a flow rate of 1.0 ml/min at 25°C and detection by an evaporative light scattering detector (ELSD; DDL-31, EUROSEP Instruments, Cergy, France). Detector temperature was 60°C and the inlet nebulizer pressure was set to 1.5 bar.

Preparation of MGMG, DGMG and TGMG by lipase Preparation of MGMG, DGMG and TGMG from pumpkin was done by the previously reported method of Nakae *et al.* (1998). The reaction mixture containing 1.0% MGDG (or DGDG, or TGDG), 0.4% Triton X-100 and 4000 units/ml of lipase (*Rhizopus arrhizus*) in 25 mM Tris-HCl buffer (pH 7.5) was stirred at 37°C for 16 h. The reaction mixture of MGDG was subjected to silica gel column chromatography by elution with chloroform-methanol (7 : 1, v/v); and the reaction mixture of DGDG was subjected to silica gel column chromatography by elution with chloroform-methanol-H_2O (70 : 30 : 10, v/v/v, lower

layer); while the reaction mixture of TGDG was subjected to silica gel column chromatography by elution with chloroform-methanol-H_2O (65 : 35 : 10, v/v/v, lower layer). Each eluate was analyzed by TLC (Merck, art. 5626) using chloroform-methanol-H_2O (65 : 35 : 10, v/v/v, lower layer), and was detected by spraying 50% (v/v) H_2SO_4 followed by heating at 130°C for 5 min. Each eluate was also detected by HPLC as described above.

Preparation of diacylglycerol by β-galactosidase The reaction mixture containing 1.0% MGDG, 50% acetone and 50 units/ml of β-galactosidase (*A. oryzae*) in 25 mM Tris-HCl buffer (pH 7.5) was stirred at 37°C for 48 h. The mixture was then subjected to silica gel (Wakogel C-200; 1×15 cm) column chromatography by elution with chloroform. Eluate was analyzed by TLC (Merck, art. 5626) using heptane-isopropyl ether-acetic acid (60 : 40 : 4, v/v/v), and was detected by spraying 50% (v/v) H_2SO_4 followed by heating at 130°C for 5 min. Eluate was also detected by HPLC as described above.

Fatty acid analysis Preparation of the methyl esters from each glyceroglycolipid was followed by a slight modification of the method previously reported by Metcalfe and Schmitz (1961). The glyceroglycolipids were deacylated with 0.5 N sodium methylate in methanol at 80°C for 15 min. Fatty acids were methylated with 14% boron trifluoride in methanol at the same temperature for 10 min. Fatty acid methyl esters were extracted into hexane and analyzed by gas chromatography (GC) (GC-14A; Shimadzu Co., Ltd.) using a DEGS (25% chromsorb WAW-DMCS 80/100 mesh) column with nitrogen gas as a carrier at the flow rate of 40 ml/min at 170°C with detection by a flame ionization detector (FID). Methylpentadecanoate was used as an internal standard.

Determination of crystallization rate of cocoa butter We examined the crystallization time of Form V of cocoa butter. The procedures of DSC isothermal analysis were followed by a slight modification of the method reported by Kawamura (1979). Each sample was tempered with 1.0% seed crystal (β_1 stable crystal of SOS; Fuji Oil Co., Ltd., Osaka) at 30°C, rapidly cooled at a constant rate of −20°C/min and held at the programmed temperatures of 17, 20 and 22°C for the isothermal crystallization.

Determination of polymorphic transformation of cocoa butter We examined the polymorphic transformation from Form V to Form VI of cocoa butter through a thermo-cycle test by the following methods. Ten grams of cocoa butter at 60°C was cooled to 30°C within 10 min. The seven glyceroglycolipid samples were added at different concentrations when the temperature of the butter had reached 30°C. The glyceroglycolipid sample concentration ranged from 0.5 to 5%. Then, 0.25% seed crystal (as above) was added to make a uniform crystal type to Form V. After the seeding, the cocoa butter was agitated for 5 min at 30°C in order to homogeneously disperse the glyceroglycolipid and seed crystal, then cocoa butter in a conical tube (28×100 mm) was immediately cooled and solidified at 15°C for 15 min in a cooling box. Thereafter, the glyceroglycolipid samples were stored at 20°C for one day (aging). A thermo-cycle test was then conducted using a thermostatic chamber (SANYO INCUBATOR): one cycle involved heating at 32°C for 12 h and cooling at 20°C for 12 h. The polymorphic transformation of cocoa butter was determined with a differential scanning calorimeter (SSC-220C type: Seiko Instrument Co., Ltd.) at a scanning rate of 5°C/min after this test. The melting point was defined as the peak temperature of the DSC melting peak.

Results

Preparation of glyceroglycolipids Each glyceroglycolipid was prepared as described in Materials and Methods, and the purity of each was confirmed by TLC and HPLC. The R_f values of MGDG, DGDG and TGDG coincided with the results previously reported (Nakae *et al*., 1998). We examined the hydrolyzate of MGDG by β-galactosidase. After β-galactosidase treat-ment, the spot of MGDG disappeared and a new spot appeared. The new spot at the R_f=0.28 position was determined to be 1,2-diacylglycerol by comparing it with the standard sample.

The fatty acid compositions of glyceroglycolipids The fatty acid compositions of MGDG, DGDG, TGDG, MGMG, DGMG, TGMG and DG from pumpkin are shown in Table 1. In all glyceroglycolipids, linoleic acid, linolenic acid, palmitic acid, oleic acid and stearic acid were the major components, with linoleic acid and linolenic acid accounting for more than 60% of the fatty acid composition of MGDG, DGDG and TGDG. In

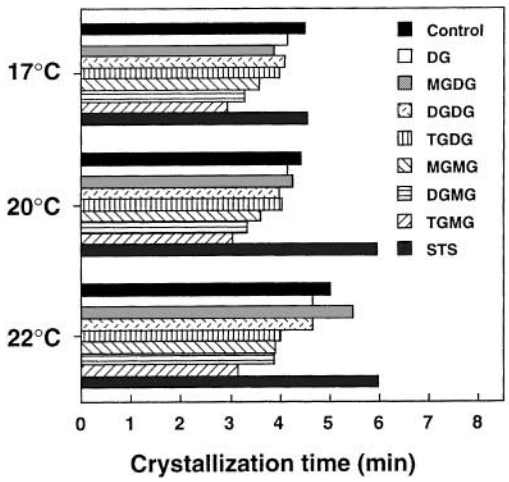

Fig. 2. The effect of glyceroglycolipids on *tc* of isothermal DSC. The values of *tc*, the period of appearance of the second DSC exothermic peak, in isothermal crystallization at 17, 20 and 22°C were measured under the conditions described in Materials and Methods.

Table 1. Fatty acid compositions of MGDG, DGDG, TGDG, MGMG, DGMG and TGMG from pumpkin.

Fatty acid	MGDG	DGDG	TGDG	MGMG	DGMG	TGMG	DG
Palmitic acid (C16:0)	15.3	12.2	11.8	5.3	1.9	1.5	15.3
Stearic acid (C18:0)	8.2	1.4	0.9	2.7	0.3	–	8.2
Oleic acid (C18:1)	15.0	5.9	6.0	4.7	4.5	0.7	15.0
Linoleic acid (C18:2)	30.1	29.0	30.9	34.6	28.9	28.1	30.1
Linolenic acid (C18:3)	31.4	51.5	50.4	52.7	64.4	69.7	31.4

MGMG, DGMG and TGMG, the ratio of palmitic acid, stearic acid and oleic acid decreased and that of linolenic acid increased compared with MGDG, DGDG and TGDG, respectively, and together linoleic acid and linolenic acid accounted for more than 90% of the fatty acid composition of MGMG, DGMG and TGMG. The ratio of palmitic acid, stearic acid and oleic acid in MGDG was the highest among the glyceroglycolipids

Effects of glyceroglycolipids on crystallization rate in cocoa butter The effects of glyceroglycolipids on the crystallization kinetics in cocoa butter were examined by DSC iso-thermogram. The cooling of the seed tempered cocoa butter and subsequent incubation at the programmed crystallization temperatures (17, 20 and 22°C) gave two exothermic peaks due to the heat of crystallization. We defined the period of appearance of the second exothermic peak, crystallization time (*tc*), measured after the sample reached the programmed crystallization temperature. The *tc* values during the incubation at 17, 20 and 22°C are shown in Fig. 2. The addition of 5% MGMG, DGMG and TGMG shortened *tc* in comparison to the original cocoa butter without glyceroglycolipids. The addition of 5% MGDG did not cause any difference at lower temperatures, but prolonged *tc* at a higher temperature. The addition of 5% DG, DGDG and TGDG did not result in any difference, but the addition of 5% STS prolonged *tc*.

Effects of glyceroglycolipids on polymorphic transformation in cocoa butter Figure 3 shows DSC melting peaks of samples with the addition of 5% DG, MGDG, DGDG, TGDG, MGMG, DGMG and TGMG through the 32/20°C thermo-cycle tests. In the case of control (no addition), the solidified samples showed a large endothermic peak at 35.7°C which corresponds to the melting of Form V. In the case of 5% DG, MGDG, DGDG, TGDG, MGMG, DGMG and TGMG, their solidified samples showed a large endothermic peak at 34.9 to 35.6°C which corresponds to the melting of Form V, respectively. From these results, the crystal lattices of cocoa butter were not affected by the addition of 5% glyceroglycolipids (Fig. 3-A). After the 10 cycle tests of 32/20°C (Fig. 3-B), the control (no addition) sample showed a small melting peak of Form V at 33.8°C and a large melting peak of Form VI at 38.4°C. The samples with the addition of 5% MGMG, DGMG and TGMG also showed the same results. Hence, the samples with the addition of 5% MGDG, DGDG, TGDG and DG showed two almost equal melting peaks of Form V and Form VI. Table 2 shows the ratio of vertical division of DSC melting peak area between Form V and Form VI of samples with the addition of 5% DG, MGDG, DGDG, TGDG, MGMG, DGMG and TGMG after 1 to 10 cycles of the 32/20°C thermo-cycle tests. After the 10 cycle tests of 32/20°C, the control (no addition) sample showed 54.0% of Form V and 46.0% of Form VI. The sample with the addition of 5% MGDG showed 67.2% of Form V and 32.8% of Form VI, and that of DGDG showed 62.9% and 37.1%, and that of TGDG showed 61.2% and 38.8%, and that of DG showed 62.1% and 37.9%. From these results, MGDG, DGDG, TGDG and DG retarded the polymorphic transformation from Form V to Form VI of cocoa butter in comparison with control (no addition), while the other glyceroglycolipids did not change it.

Figure 4 shows DSC melting peaks of samples with the addition of MGDG between 0.5% to 5.0% through the 32/20°C thermo-cycle tests. In the case of control (no addition), the solidified samples showed a large endothermic peak at 34.6°C which corresponds to the melting of Form V. In MGDG between 0.5% to 5.0%, the solidified samples showed a large endothermic peak at 34.9 to 35.6°C which corresponds to the melting of Form V, respectively (Fig. 4-A). After the 10 cycle tests of 32/20°C (Fig. 4-B), the control (no addition) sample showed a small melting peak of Form V at 34.2°C and a large melting peak of Form VI at 38.4°C. Hence, the sample with the addition of 5% MGDG has two melting peaks of Form V and Form VI, which were almost

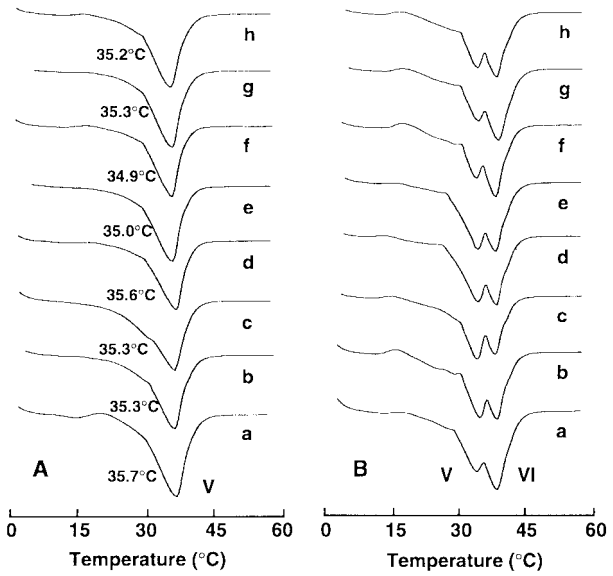

Fig. 3. DSC peaks of cocoa butter with the addition of 5% of various glyceroglycolipids through the 32/20°C thermo-cycle tests. A, after solidification at 15°C for 15 min; B, after 10 cycles of the 32/20°C thermo-cycle tests. a, control (no addition); b, DG; c, MGDG; d, DGDG; e, TGDG; f, MGMG; g, DGMG; h, TGMG.

Table 2. Polymorphism of cocoa butter after thermo-cycle tests between 32 and 20°C adding MGDG, DGDG, TGDG, MGMG, DGMG, TGMG and DG.

	Thermo-cycle condition (32°C/20°C)					
	Start		4 cycles		10 cycles	
	Form V	Form Vl	Form V	Form Vl	Form V	Form VI
Control (no addition)	100	0	63.7	36.3	54.0	46.0
5% DG	100	0	69.4	30.6	62.1	37.9
5% MGDG	100	0	75.1	24.9	67.2	32.8
5% DGDG	100	0	67.0	33.0	62.9	37.1
5% TGDG	100	0	66.6	33.4	61.2	38.8
5% MGMG	100	0	63.0	37.0	54.4	45.6
5% DGMG	100	0	63.7	36.3	53.6	46.4
5% TGMG	100	0	65.8	34.2	56.8	43.2

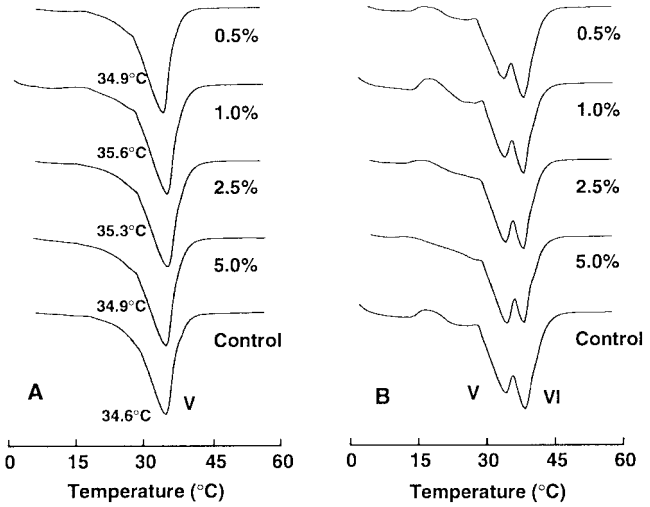

Fig. 4. DSC peaks of cocoa butter with the addition of various concentrations of MGDG through the 32/20°C thermo-cycle tests. A, after solidification at 15°C for 15 min; B, after 10 cycles of the 32/20°C thermo-cycle tests.

equal. Table 3 shows the ratio of DSC melting peak area between Form V and Form VI of samples with the addition of between 0.5% to 5.0% after 1 to 10 cycle tests of 32/20°C. During the 32/20°C thermo-cycle tests, the ratio of Form V of sample increased with MGDG content. After the 10 cycle tests of 32/20°C, the control (no addition) sample showed 55.6% of Form V and 44.4% of Form VI. The sample with the addition of 2.5% MGDG showed 61.3% of Form V and 38.7% of Form VI, and that of 5.0% MGDG showed 67.0% and 33.0%.

Discussion

Fat bloom has been a problem in chocolate manufacturing for many years because it significantly damages the commercial value of the chocolate products. It is believed that fat bloom is formed due to the polymorphic transformation of cocoa butter, making it necessary to control this transformation from Form V to VI to inhibit the fat bloom (Jana & Thakar, 1993; Hartel, 1999). Control has been proposed by, for example, adding a substitute fat highly compatible with cocoa butter (Lohman & Hartel, 1994) or adding an emulsifier as a fat bloom inhibitor (Aronhime *et al*., 1988; Suwa & Matsuda, 1993).

We investigated the performance of glyceroglycolipid as a dynamic controller of the polymorphic transformation of cocoa butter. It was obvious that one certain glyceroglycolipid retarded the polymorphic transformation from Form V to VI, and we therefore examined the structures of glyceroglycolipids to learn which one had the greatest retardation effect on this transformation. We extracted MGDG, DGDG and TGDG from pumpkin, hydrolyzed them to MGMG, DGMG and TGMG by lipase, and then hydrolyzed MGDG to DG by β-galactosidase. The seven glyceroglycolipids differ from the number of the acyl chain and galactose combined with glycerol. However, having the same fatty acid chain length as the major triacylglycerol components (POP, POS and SOS) in cocoa butter, they are expected to effectively form mixed crystals. The result indicated that MGDG, DGDG, TGDG and DG significantly retarded the polymorphic transformation from Form V to VI on cocoa butter compared to the control, while the other glyceroglycolipids had no effect (Fig. 3, Table 2). Furthermore, MGDG showed a tendency to have the greatest retardation effect among the seven glyceroglycolipids tested. These results indicated that the number of the acyl chain and galactose of glyceroglycolipid strongly influenced the polymorphic transformation of cocoa butter. In short, it was assumed that diglyceride was structurally better compatible with cocoa butter than monoglyceride, and that DGDG and TGDG which were more hydrophilic than MGDG were not present in as much quantity in the crystal lattice of cocoa butter. Like our results, Hernqvist and Anjou (1983) reported the retardation effect of DG on the polymorphic transformation of margarine, and Uragami *et al*. (1986) reported that DG inhibited the polymorphic transformation of fat. They proposed the following mechanism of the effect of DG : DG was incorporated into triacylglycerol of fat, and inhibited the polymorphic transformation. In addition, Aronhime *et al*. (1988) reported that the retardation of the polymorphic transformation on fat by the addition of STS is caused by the penetration of its molecules among monoacid saturated tria-

Table 3. Polymorphism of cocoa butter after thermo-cycle tests between 32 and 20°C adding various concentrations of MGDG.

	Thermo-cycle condition (32°C/20°C)					
	Start		4 cycles		10 cycles	
	Form V	Form VI	Form V	Form Vl	Form V	Form VI
Control (no addition)	100	0	63.5	36.5	55.6	44.4
0.5% MGDG	100	0	63.6	36.4	54.4	45.6
1.0% MGDG	100	0	64.5	35.5	58.7	41.3
2.5% MGDG	100	0	66.0	34.0	61.3	38.7
5.0% MGDG	100	0	71.0	29.0	67.0	33.0

Table 4. Effect of 5% glyceroglycolipids on rate of melting energy of cocoa butter. (%)

Temperature (°C)	Control	DG	MGDG	DGDG	TGDG	MGMG	DGMG	TGMG
20	0.1	2.3	2.1	0.5	1.3	0.9	0.8	0.9
25	5.9	9.2	9.0	6.4	7.0	6.4	5.3	6.1
30	25.0	27.9	28.3	26.0	23.6	23.4	20.5	21.6
35	80.9	81.1	75.8	83.7	79.8	83.7	75.5	74.7
40	97.6	98.2	97.8	98.9	98.7	99.0	97.8	97.3
45	99.8	100	99.9	100	100	100	100	99.8

cylglycerol, and the incorporation of the molecules into created vacancies. Our results showed that MGDG, DGDG and TGDG significantly retarded the polymorphic transformation of cocoa butter, while the other glyceroglycolipids did not. From these results it was suggested that the action of glyceroglycolipids in retarding the polymorphic transformation of cocoa butter were closer to that of STS. There are few reports on this retardation effect by glyceroglycolipids. Accordingly, this is the first report of MGDG, DGDG and TGDG as a controller of this action.

The major fatty acid compositions of glyceroglycolipids tested in this study were linoleic acid and linolenic acid which are unsaturated fatty acids (Table 1). Nevertheless, MGDG, DGDG, TGDG and DG retarded the polymorphic transformation from Form V to VI of cocoa butter. The compatibility of fat with cocoa butter is reportedly important for the inhibition of fat bloom (Suwa & Matsuda, 1993). From these results, it is assumed that MGDG which consists of palmitic acid, stearic acid and oleic acid would have a greater retardation effect on the polymorphic transformation of cocoa butter than MGDG tested in this study. We will investigate this effect by adding MGDG with palmitic acid, stearic acid and oleic acid; the results will be published in another paper.

It is also known that a certain emulsifier exerts some effect on the crystallization rate of fat. It was reported that STS prolonged the rate of crystallization on fat (Kawamura, 1979); in our results, the addition of 5% STS prolonged the rate of crystallization of cocoa butter (Fig. 2). Accordingly, the chocolate to which STS is added must have a longer cooling process in its production. The addition of 5% MGDG, however, had no remarkable effect on the rate of crystallization of cocoa butter because MGDG is structurally more compatible with cocoa butter than STS. We then assume that the cooling process chocolate to which MGDG has been added does not have to be changed.

Siew and Ng (1999) have reported that the addition of DG suppressed the solid fat content (SFC) of fat. SFC is an important factor in estimating the physical property of fat; we therefore calculated the rate of melting energy from DSC melting curves of cocoa butter with the addition of 5% glyceroglycolipids, as shown in Table 4. The addition of 5% DG and MGDG increased the rate of melting energy slightly in comparison with the original cocoa butter at lower temperatures, while the addition of the other glyceroglycolipids did not change the rate. These variations in rate with the addition of MGDG were not believed to greatly affect the snap of products made from cocoa butter, particularly chocolate.

This study, suggested that MGDG was a good inhibitor of polymorphic transformation on cocoa butter, and it is expected that in chocolate with MGDG added fat bloom can be inhibited. The addition of MGDG would not greatly affect the physical properties or taste of the chocolate. Furthermore, it was reported that the average daily intake of glyceroglycolipids was 90 mg MGDG and 220 mg DGDG in a human (Sugawara & Miyazawa, 1999). In short, it is suggested that the source of glycerolipids MGDG, DGDG and TGDG are a very useful and safe nutrition.

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