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Effect of Glycolipid Fraction on Fat Bloom in Dark and Milk Chocolates

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We earlier reported that glyceroglycolipid extracted from pumpkin significantly retarded the polymorphic transformation from Form V to VI of cocoa butter. We therefore investigated the effects of adding glyceroglycolipid on the fat bloom stability in dark and milk chocolates. Glycolipid fraction containing glyceroglycolipid was extracted with ethanol from pumpkin purée and partially purified on column chromatography. The apparent viscosity of dark and milk chocolates with the addition of the gained glycolipid fraction instead of lecithin significantly decreased up to 0.2% (w/w) according to a rotational viscometer at 45°C, and then decreased slightly at concentrations above that. The glycolipid fraction was then added to dark and milk chocolates up to 2% in place of lecithin. Each chocolate sample was evaluated for fat bloom resistance through two thermo-cycle tests given at an interval of 12 h between 30°C and 20°C, and between 32°C and 20°C. The accelerated tests indicated that the glycolipid fraction inhibited fat bloom compared to the control (0.4% lecithin). In dark and milk chocolates with the addition of 2.0% glycolipid fraction the polymorphic transformation from Form V to VI of cocoa butter was significantly retarded when measured by a differential scanning calorimeter. On the basis of these results, the quality of chocolate can be improved more effectively using the glycolipid fraction.

Keywords: chocolate, fat bloom, glycolipid, lecithin, viscosity

Chocolate consists of solid particles of cocoa, sugar, and milk solids that are dispersed in a continuous fat phase. In dark chocolate, the milk solids are absent. The properties of chocolate are influenced by the size and distribution of the above solid particles, and by the crystallographic properties of the fat phase. Fats influence such qualities of chocolate as heat resistance, snap, gloss, quick and sharp melting in the mouth and fat bloom resistance. In particular, the formation of fat bloom on the surface of chocolate is a well-known phenomenon and also the most troublesome problem. Accordingly, fat structures in chocolate have been investigated from various points of view (Lovegren *et al*., 1976; Timms, 1980; Chaiseri & Dimick, 1989).

The major fat in chocolate is cocoa butter. About eighty percent of cocoa butter is composed of specific triacylglycerols of a Sat-O-Sat (saturated-oleic-saturated) type: POP (1, 3-dipalmitoyl-2-oleoyl-glycerol), POS (2-oleoyl-palmitoyl-stearoyl-glycerol), and SOS (1, 3-distearoyl-2-oleoyl-glycerol). Cocoa butter, as a mixture of POP, POS and SOS, is polymorphic, revealing six forms (Form I, II, III, IV, V and VI), as identified by Wille and Lutton (1966). The polymorphic form of cocoa butter in chocolate is Form V, since this form has the most desirable melting and solidification behavior among other polymorphs. Form V is not the most stable polymorph, and Form VI is; therefore Form V can be transformed to Form VI either through a solid-state or a melt-mediated transformation. This transformation to Form VI gives rise to undesirable physical properties of the end products, and particularly causes fat bloom.

To prevent the polymorphic transformation from Form V to VI of cocoa butter, 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. Some emulsifiers are known to be useful agents to control the polymorphic behaviors of fats and oil (Kawamura, 1980; Aronhime *et al*., 1988).

In a previous report (Nakae *et al*., 2000), we described that monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and trigalactosyldiacylglycerol (TGDG) from pumpkin significantly retarded the polymorphic transformation from Form V to VI of cocoa butter. In particular, MGDG had the most retardation effect on this polymorphic transformation. MGDG is one of the glyceroglycolipids widely found in plants, in animal tissues and in bacteria. It consists of hydrophilic carbohydrate group and two hydrophobic fatty acid moieties that bind through a glycerol. We felt that MGDG could be a new fat substitute highly compatible with cocoa butter.

In this report, we sought a simple procedure to prepare the glycolipid fraction containing glyceroglycolipid from pumpkin. We also investigated the effects of adding this glycolipid fraction on the fat bloom stability of dark and milk chocolates.

Materials and Methods

Chemicals Pumpkin purée was purchased from KAGOME Co., Ltd. (Nagoya). Soya lecithin was purchased from AJINOMOTO Co., Ltd. (Tokyo). All other chemicals used were commercially available and of chemically pure grade.

Glycolipid fraction Twenty kilograms of pumpkin purée was weighed into a stainless tray and then was freeze-dried. Twenty five liters of ethanol was added to the resulting powder and the mixture was extracted by stirring for 16 h at 20°C. After filtration and diluting with the same amount of water, the eluate was run through Amberlite XAD-7 column (Organo Co. Ltd., Tokyo) to obtain a glycolipid fraction. After washing with 50% ethanol, the fraction containing glyceroglycolipid was eluted

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with 75% ethanol. The eluate was concentrated and the glycolipid fraction was obtained as a yellowish paste by lyophilization.

Purity of the glycolipid fraction was 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 glyceroglycolipid in the gained glycolipid fraction 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, with 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.

Chocolate manufacturing Types of chocolate studied in this report are shown in Table 1. All of the materials in each chocolate were refined and conched before the solidification process. Cacao mass was produced from cocoa beans roasted in our laboratory. The particle sizes of ingredients were reduced with a refining roll to about 25 μm.

Viscosity measurement As the chocolate showed non-Newtonian behavior, the viscosity measurement referred to in this study is apparent viscosity. The apparent viscosity was measured using a rotational viscometer (DVH-B-4II; TOKIMEC INC., Tokyo) fitted with a rotor No.6 at 4 rpm. All viscosity measurements were made at 45˚C.

Evaluations of fat bloom stability The fat bloom stability of solidified dark and milk chocolates were examined through a thermo-cycle test by the following methods. For dark chocolate, each sample at 60˚C was cooled to 27˚C by stirring, and was held, then reheated to 31˚C by stirring (hand tempering). For milk chocolate, each sample at 60˚C was cooled to 26˚C by stirring, and was held, then reheated to 30˚C by stirring (hand tempering). After the hand tempering, 20 g of dark and milk chocolates were put in a mold made of ethylene chloride resin; they were immediately cooled and solidified at 15˚C for 15 min in a cooling box. Thereafter, the demolded samples were stored at 20˚C for five days (aging), after which, the thermo-cycle test was administered using a thermostat chamber (SANYO Co., Ltd., Osaka): one cycle involves heating at 30 or 32˚C for 12 h and cooling at 20˚C for 12 h. The fat bloom was observed visually and measured by changes in whiteness of the sample surface using an image analyzer (SUPER ASPECT; Mitani Corporation, Fukui). To observe the increase of bloom whiteness over time, whiteness values at zero time were subtracted from whiteness values at increasing cycling times for each chocolate (expressed as ΔWhiteness).

Determination of polymorphism of dark and milk chocolates The polymorphic modification of dark and milk chocolates was determined with DSC (Seiko Co., Ltd.: SSC220C type) at a scanning rate of 5˚C/min after the two solidification processes: (a) after the aging of solidified samples at 20˚C for five days and (b) after the thermo-cycle test. The melting point was defined as peak temperature of the DSC melting peak.

Table 1. Type of chocolate studied.

	Dark chocolate	Milk chocolate
Cacao mass	35	20
Cocoa butter	10	10
Vegetable fat	10	15
Sugar	45	42
Whole milk powder	—	13

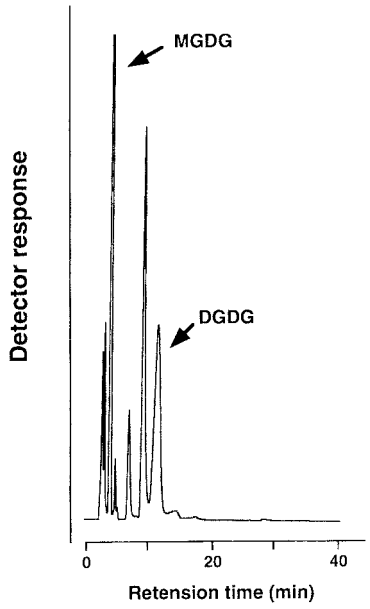

Fig. 1. Chromatograms of glycolipid fraction of pumpkin purée. The glycolipid fraction was analyzed on Lichrosorb DIOL (5 μm, 250 mm×4 mm) column.

Results

Preparation of glycolipid fraction The fraction containing glyceroglycolipids was prepared by the method described Materials and Methods. The yield was 37.3 g for the gained fraction. In TLC analysis, a water-soluble fraction and most pigments were detected in 50% ethanol eluate on a XAD-7 column, glyceroglycolipids were detected in 75% ethanol eluate and part of the neutral lipids and phospholipids were detected in 100% ethanol eluate. Figure 1 shows the HPLC chromatogram of the fraction eluted with 75% ethanol; it still contained some neutral lipids, pigments and phospholipids but the main part was glycolipids containing glyceroglycolipids. There were MGDG and digalactosyldiacylglycerol (DGDG) in this fraction but no TGDG. Purity of glyceroglycolipids in the gained fraction was about 40% (MGDG 24.2%, DGDG 15.4%) by measuring for HPLC. We therefore called the gained fraction the glycolipid fraction.

In a previous report (Nakae *et al*., 2000), we showed that MGDG, DGDG and TGDG significantly retarded the polymorphic transformation from Form V to VI of cocoa butter. Accordingly, we thought that the glycolipid fraction would be sufficient to retard this transformation, and for this reason we used the glycolipid fraction in this study.

Change in apparent viscosity Figure 2 shows the reduction in apparent viscosity on dark and milk chocolates to the concentration of the glycolipid fraction and lecithin as a reference. Without an emulsifier the apparent viscosity was 45,500 mPa·s and 44,200 mPa·s, respectively. All the apparent viscosity significantly decreased up to 0.2% concentration, and then decreased

slightly at concentrations above that. The apparent viscosity on dark chocolate in 0.4% glycolipid fraction and lecithin was 23,800 mPa·s and 19,200 mPa·s, respectively, while that on milk chocolate was 20,900 mPa·s and 17,400 mPa·s, respectively.

From these results, reduction of the apparent viscosity of the glycolipid fraction was at least equivalent to that of lecithin.

Fat bloom stability after thermo-cycle test We examined the degree of demolding and the occurrence of the fat bloom of the tempered dark and milk chocolates just after solidification at 15°C. These chocolates to which the glycolipid fraction had been added instead of lecithin showed easy demolding and did not induce fat bloom formation by changing the crystal lattice.

Figure 3 shows the surface appearance of the tempered dark chocolate after 18 cycle tests of 32/20°C, in which the fat bloom is revealed in white-color areas. Table 2 shows fat bloom stability of the tempered dark chocolate with the addition of lecithin or glycolipid fraction through the thermo-cycle tests. The addition of lecithin caused the fat bloom at all concentrations through the 30/20°C and 32/20°C cycle tests. In contrast, addition of the glycolipid fraction gave rise to significant fat bloom stability through the 30/20°C and 32/20°C cycle tests, as the concentration was increased. In particular, the samples with the addition of 2.0% concentration did not produce the fat bloom even through the 20 cycle tests of 32/20°C.

Figure 4 shows the surface appearance of the tempered milk chocolate after 25 cycle tests of 32/20°C, with the fat bloom shown as the white-color areas. Table 3 shows fat bloom stability of milk chocolate with the addition of lecithin or glycolipid fraction through the thermo-cycle tests. The addition of lecithin caused the fat bloom at all concentrations through the 32/20°C cycle tests. Control (0.4% lecithin) also caused the fat bloom through the 30/20°C cycle tests. By contrast, the addition of glycolipid fraction gave rise to significant fat bloom stability

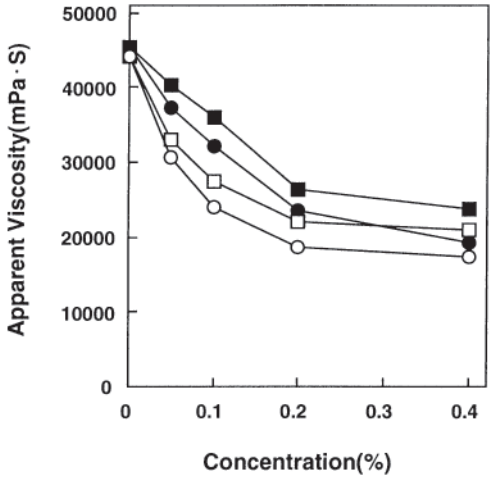

Fig. 2. Apparent viscosity of dark and milk chocolates with the addition of glycolipid fraction. The apparent viscosity was measured using a rotational viscometer fitted with a rotor No.6 at 4 rpm. All viscosity measurements were made at 45°C. Lecithin (milk chocolate), (○); Lecithin (dark chocolate), (●); Glycolipid fraction (milk chocolate), (□); Glycolipid fraction (dark chocolate), (■).

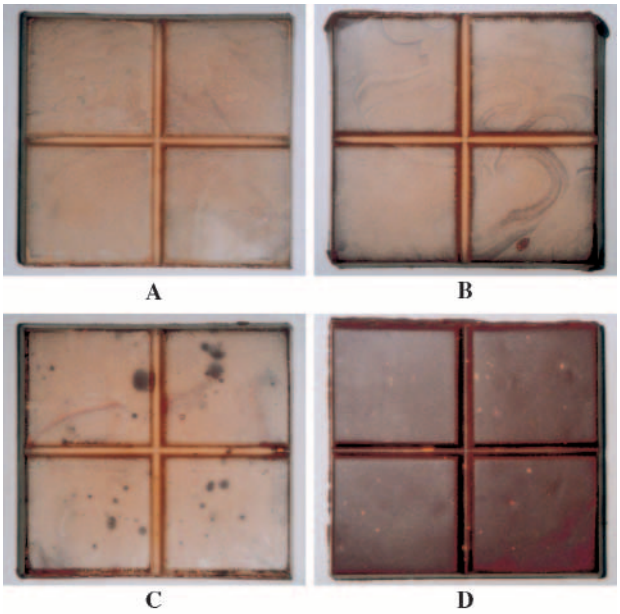

Fig. 3. Photographs showing dark chocolate, with the addition of glycolipid fraction at three concentrations, during the 18 repetitions of the 32/20°C thermo-cycle tests. A, Control (0.4% lecithin); B, 2.0% lecithin; C, 0.4% glycolipid fraction; D, 2.0% glycolipid fraction.

Table 2. Stability of fat bloom of dark chocolate with the addition of lecithin or glycolipid fractions.

	Control (no addition)		Lecithin concentration (wt %)						Glycolipid concentration (wt %)					
			0.4		1.0		2.0		0.4		1.0		2.0	
	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness
Start	−	93	−	98	−	99	−	99	−	99	−	99	−	98
30°C/20°C														
10	−	92	−	97	−	98	−	101	−	98	−	101	−	100
20	−	92	−	96	−	96	−	100	−	96	−	99	−	96
30	±	97	+	106	±	96	±	101	±	97	±	99	−	98
40	+	106	++	140	+	112	+	113	+	107	±	104	−	95
50	+	110	++	139	+	111	+	116	+	106	+	109	−	100
32°C/20°C														
5	−	96	−	97	−	95	−	97	−	96	−	100	−	99
10	+	104	++	139	+	112	+	115	++	119	++	135	−	99
15	+++	167	+++	171	+++	186	++	142	+++	171	+++	166	±	104
20	+++	186	+++	192	+++	204	+++	168	+++	191	+++	180	±	110
25	+++	186	+++	190	+++	220	+++	190	+++	204	+++	190	+	127

Fat bloom occurrence evaluated by visual observation: −, no fat bloom; ±, weak bloom; +, bloom; ++, strong bloom. +++, intensive bloom. Whiteness value measured with an image analyzer.

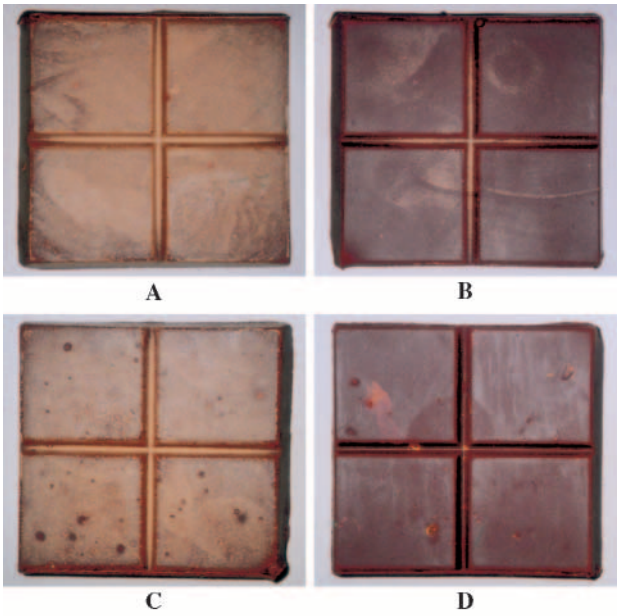

Fig. 4. Photographs showing milk chocolate, with the addition of glycolipid fraction at three concentrations, during the 25 repetitions of the 32/20°C thermo-cycle tests. A, Control (0.4% lecithin); B, 2.0% lecithin; C, 0.4% glycolipid fraction; D, 2.0% glycolipid fraction.

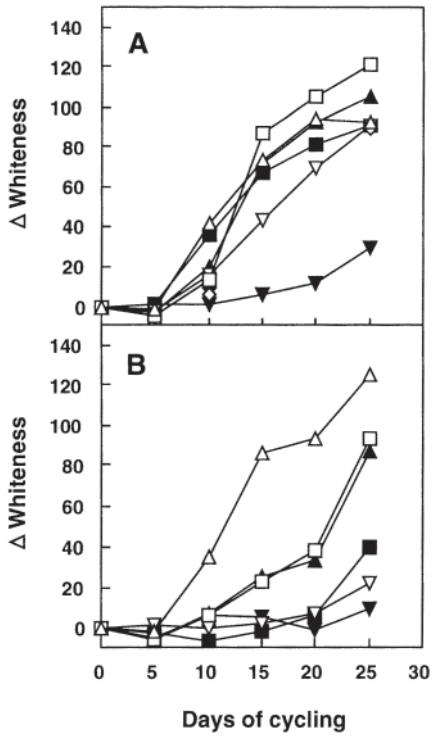

Fig. 5. Bloom development of dark and milk chocolates containing glycolipid fraction or lecithin at various concentrations during the 32/20°C thermo-cycle tests. ΔWhiteness represented in terms of the value subtracted whiteness at zero time from whiteness with increasing cycling times for each chocolate. A, dark chocolate; B, milk chocolate. Control (0.4% lecithin), (△); 1.0% lecithin, (□); 2.0% lecithin, (▽); 0.4% glycolipid fraction, (▲); 1.0% glycolipid fraction, (■); 2.0% glycolipid fraction, (▼).

through the 30/20°C and 32/20°C cycle tests, as the concentration was increased. In particular, the samples with the addition of 2.0% concentration did not produce the fat bloom even through the 25 cycle tests of 32/20°C.

Figure 5 shows whiteness change on the surface of dark and milk chocolates with the addition of lecithin or glycolipid fraction during the 32/20°C thermo-cycle tests. In both chocolates, addition of the glycolipid fraction caused less development of fat bloom than that of lecithin.

Polymorphism of bloomed chocolates Figure 6 shows DSC melting peaks of tempered dark chocolates with the addition of various concentrations of lecithin and glycolipid fraction through the thermo-cycle tests. All solidified samples showed a large endothermic peak at 33.4 to 34.4°C which corresponds to the melting of Form V, respectively. After the 25 cycle tests of 32/20°C, the control (0.4% lecithin) sample showed a small melting peak of Form V at 33.3°C and a large melting peak of Form VI at 37.6°C. Hence, the sample with the addition of 2% glycolipid fraction had two melting peaks of Form V and Form VI, but its polymorphic transformation from Form V to VI was lower than control. DSC melting peaks of tempered milk chocolates with the addition of various concentrations of lecithin and glycolipid fraction showed the same results. We examined the ratio of DSC melting peak area between Form V and Form VI of dark

Table 3. Stability of fat bloom of milk chocolate with the addition of lecithin or glycolipid fractions.

	Control (no addition)		Lecithin concentration (wt %)						Glycolipid concentration (wt %)					
			0.4		1.0		2.0		0.4		1.0		2.0	
	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness	Fat bloom	Whiteness
Start	−	105	−	108	−	109	−	103	−	110	−	105	−	105
30°C/20°C														
10	−	104	−	107	−	112	−	109	−	112	−	114	−	114
20	−	104	−	114	−	116	−	114	−	117	−	114	−	115
30	−	101	−	109	−	110	−	108	−	107	−	106	−	107
40	±	107	+	122	−	115	−	115	±	116	−	113	−	115
50	±	107	+	123	−	115	−	113	±	114	−	114	−	117
32°C/20°C														
5	−	102	−	106	−	103	−	104	−	104	−	102	−	100
10	±	108	++	143	±	115	−	113	±	117	−	112	−	111
15	++	165	+++	194	+	132	±	115	+	135	±	1 13	±	110
20	+++	190	+++	201	+	147	+	110	++	143	+	111	±	104
25	+++	236	+++	233	+++	202	+	125	+++	197	++	145	±	114

Fat bloom occurrence evaluated by visual observation: −, no fat bloom; ±, weak bloom; +, bloom; ++, strong bloom. +++, intensive bloom. Whiteness value measured with an image analyzer.

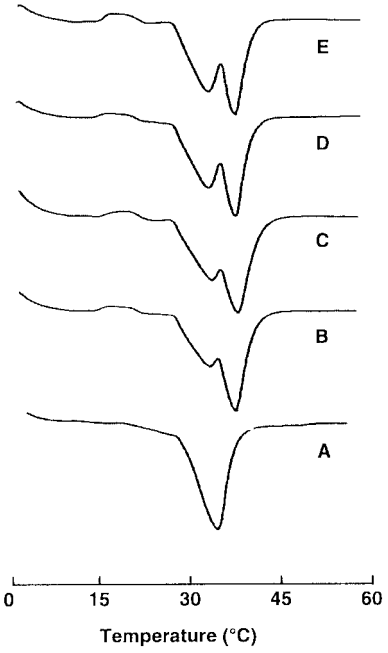

Fig. 6. DSC peaks of tempered dark chocolates with the addition of various concentrations of lecithin and glycolipid fraction through the 32/20°C thermo-cycle tests. A, Control (0.4% lecithin) after solidification at 15°C for 15 min; B, Control (0.4% lecithin) after 25 cycles of the 32/20°C thermo-cycle tests; C, 2.0% lecithin after 25 cycles of the 32/20°C thermo-cycle tests; D, 0.4% glycolipid fraction after 25 cycles of the 32/20°C thermo-cycle tests; E, 2.0% glycolipid fraction after 25 cycles of the 32/20°C thermo-cycle tests.

and milk chocolates with the addition of lecithin and glycolipid fraction after 25 cycles of the 32/20°C thermo-cycle tests. In dark chocolate, the control (0.4% lecithin) sample showed 44.0% of Form V and 56.0% of Form VI. Hence, the sample with the addition of 2.0% lecithin showed 47.9% and 52.1%, and that of 2.0% glycolipid fraction showed 55.9% and 44.1%. In milk chocolate, the control (0.4% lecithin) sample showed 58.3% of Form V and 41.7% of Form VI. Hence, the sample with the addition of 2.0% lecithin showed 61.9% and 38.1%, and that of 2.0% glycolipid fraction showed 63.4% and 36.6%. From these results, the glycolipid fraction retarded the polymorphic transformation from Form V to VI of cocoa butter in comparison to control (0.4% lecithin). It was obvious that milk chocolate had less polymorphic transformation from Form V to VI of cocoa butter than dark chocolate.

Discussion

Fat bloom has been a problem in chocolate manufacturing for many years because it significantly damages the commercial value of the products. There have been attempts to prevent fat bloom by, for example, improving the process for producing chocolate (Hachiya *et al*., 1989a, b), adding a substitute fat which is highly compatible with cocoa butter (Lohman & Hartel, 1994) and adding an emulsifier as a fat bloom inhibitor (Aronhime *et al*., 1988, Suwa & Matsuda, 1993).

There are two theories for fat bloom development on chocolate during storage (Hartel, 1999). The phase separation theory is based on separation of the high- and low-melting triacylglycerols in cocoa butter, in which the high-melting triacylglycerol group caused bloom. In short, it was thought that the low-melting, liquid-like triacylglycerols (lower melting point) in cocoa butter dissolved a portion of the high-melting triacylglycerols (higher melting point) and carried them to the chocolate surface, where they recrystallized in a purified form. The other polymorphic transformation theory is based on the formation of fat bloom in which cocoa butter crystals change from an unstable to a more stable polymorphic form. The mechanism of fat bloom development during storage of chocolate remains unclear, however, we believe it is important to control the polymorphic transformation of cocoa butter to inhibit this development. In our previous study, MGDG extracted from pumpkin had the greatest retardation effect on the polymorphic transformation of cocoa butter (Nakae *et al*., 2000). We speculated that the penetration of MGDG molecules among the triacylglycerol fatty acid chains and the incorporation of these molecules into the created vacancies caused this retardation. As MGDG extracted from pumpkin retarded the polymorphic transformation from Form V to VI on cocoa butter, it would be expected that MGDG prevented fat bloom development on chocolate during storage.

Table 4. Effects of lecithin and glycolipid on apparent SFC of dark chocolate.

Temperature (°C)	Control	Lecithin concentration (%)		Glycolipid concentration (%)	
		0.4	2.0	0.4	2.0
20	99.8	99.7	99.4	99.7	99.6
25	95.3	94.0	94.1	92.7	91.9
30	75.3	79.2	74.2	71.5	73.1
35	10.0	15.1	8.2	8.3	11.3
40	0.4	0.6	0.3	0.3	0.6
45	0.0	0.0	0.0	0.0	0.0

So, for the purpose of obtaining a new natural fat bloom inhibitor, we extracted the glycolipid fraction containing MGDG from pumpkin. The glycolipid fraction was gained by extracting with ethanol and partially purifying on column chromatography. The gained glycolipid fraction contained MGDG, DGDG and other glycolipids. Most of the water-soluble fraction, pigments, neutral lipids and phospholipids were not detected in the glycolipid fraction which eluted with 75% ethanol on Amberlite XAD-7 column. Accordingly, we believe that solid phase extraction and elution with 75% ethanol are a suitable procedure to selectively elute the glycolipids. We also believe that this glycolipid fraction will be sufficient to retard the polymorphic transformation of cocoa butter.

We prepared two types of chocolates; dark chocolate and milk chocolate. Firstly, we estimated to the ability of the gained glycolipid fraction as chocolate emulsifier instead of soya lecithin, which is an important factor in reducing the viscosity of chocolate. The apparent viscosity reduction of the gained glycolipid fraction was at least equivalent to that of soya lecithin. From these results it is possible to use the glycolipid fraction instead of soya lecithin as a viscosity reduction agent of chocolate.

Secondly, we calculated the apparent SFC from DSC melting curves of chocolates with the addition of 2% lecithin and glycolipid fraction. SFC is an important indicator of hardness. Though the addition of 2% glycolipid fraction suppressed apparent SFC slightly in comparison with the original cocoa butter at lower temperatures (Table 4), we did not believe these variations observed in the apparent SFC would greatly affect the properties of chocolate such as the snap.

Next, we examined fat bloom development on the surface of dark and milk chocolates with the addition of lecithin or glycolipid fraction through the 30/20˚C and 32/20˚C thermo-cycle tests. These tests showed that the development may be initiated by Form VI crystals of cocoa butter which are formed through solid-state transformation. The coarse crystals of Form VI were converted from Form V through thermal treatment. In dark and milk chocolates, it was obvious that addition of the glycolipid fraction showed less development of fat bloom than that of lecithin (Table 2, 3), and it was also obvious that the glycolipid fraction retarded the polymorphic transformation from Form V to Form VI of cocoa butter in comparison with control (0.4% lecithin) (Fig. 6). Accordingly, we concluded that this fraction could inhibit fat bloom development on chocolate from both visual observations and polymorphic transformation analysis.

We also examined fat bloom development on the surface of chocolate with the addition of sorbitantristearate (STS) or sucrose fatty acid polyester (SPE) through the 32/20˚C thermocycle tests. In these results, the ability of glycolipid fraction inhibiting the development was equal to that of STS or SPE (data not shown). These results will be published in another paper.

For the chocolate manufacturer, this may lead to new strategies to improve the quality of chocolate. This study suggested that the glycolipid fraction containing glyceroglycolipids was a good fat bloom inhibiting agent for chocolate. The quality of chocolate can be improved more effectively using the glycolipid fraction instead of lecithin which is now used industrially. Furthermore, as the glycolipid fraction is derived from a natural material such as pumpkin, chocolates to which the glycolipid fraction is added are safe.

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