Distribution of Starch Lysophosphatidylcholine in Pasting and Gelation of Wheat Starch Suspensions

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The amount and fatty acid composition of lysophosphatidylcholine (LPC) in the gelatinization, pasting and gelation of wheat starch were measured under a specific temperature program of a Rapid Visco Analyzer. The amount of LPC extracted from native starch in boiling water (HOT-LPC) with water-saturated *n*-butanol (WSB) was higher than that extracted at room temperature (RT-LPC). However, in the starch suspension at the beginning of gelatinization and in starch paste with increasing viscosity, the amounts of RT-LPC were higher than HOT-LPC. However, in starch paste with decreasing viscosity and starch gel, the amount of HOT-LPC was again higher than RT-LPC and similar to that of the native starch. The proportion of palmitic acid (PA) in HOT-LPC of starch paste with increasing viscosity was over 50%. However, in HOT-LPC of native starch and starch gel, the proportion of PA was lower than that of linoleic acid. These results indicated that the distribution of starch LPC changed during gelatinization, pasting and gelation of wheat starch.

Keywords: wheat starch, amylose-lipid, lysophosphatidylcholine, fatty acid composition, pasting process

Introduction

The effects of amylose-lipid complexes on swelling, gelatinization, and retrogradation of starch have been studied using defatted starch (Eliasson and Krog, 1985; Eliasson and Wahgren, 2004; Gelders and Goesart *et al.*, 2006; Mira and Eliasson *et al.*, 2005; Siswoyo and Morita, 2003; Takahashi and Seib, 1998; Tufvesson and Wahlgren *et al.*, 2003). The effects of added emulsifiers are due to the formation of inclusion complexes between the amylose and emulsifiers. Although there have been many studies on the effects of amylose-lipid complexes on the rheological and thermal properties of starch, no study has examined starch phospholipids (PL) during gelatinization, pasting, and gelation of starch suspensions.

Over 90% of the internal starch lipids of wheat are lysophospholipid (LPL), of which lysophosphatidylcholine (LPC) accounts for 60 – 80% (Chung and Ohm, 2000; Morrison and Mann *et al.*, 1975). It is well known that LPL forms inclusion complexes with amylose (Eliasson and Wahgren, 2004;

Morrison, 1995; Tester and Morrison, 1990). The proportion of palmitic (PA) and linoleic (LA) acids in LPC is 35% and 54%, respectively (Chung and Ohm, 2000). However, studies on the addition of these lipids to defatted starch indicate that the stability of saturated fatty acid complexes is higher than that of unsaturated fatty acid complexes (Eliasson and Krog, 1985; Tufvesson and Wahlgren *et al.*, 2003). Therefore, lipids composed of saturated fatty acids are used to form such complexes (Eliasson and Krog, 1985; Gelders and Goesart *et al.*, 2006; Siswoyo and Morita, 2003; Tufvesson and Wahlgren *et al.*, 2003). These results do not reflect the fatty acid composition of the native complexes, and research into why there is such a discrepancy has not been undertaken.

On the other hand, lipids from amylose-lipid complexes of internal starch lipids can be extracted with hot aqueous alcohol (methanol, propanol, or *n*-butanol) in boiling water, but cannot be extracted at room temperature (Morrison and Mann *et al.*, 1975; Morrison and Coventry, 1985). In a previously reported study, we showed that this does not apply to wheat flour paste (Ishinaga and Tuohuti *et al.*, 2010). That is, in flour paste of increasing viscosity, the amount of LPC extracted at room temperature (RT-LPC) with water-saturated

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n-butanol (WSB) was greater than that of LPC thermally extracted in boiling water (HOT-LPC).

Furthermore, after the viscosity decreased, the amount of HOT-LPC was again greater than that of RT-LPC. The proportion of PA in HOT-LPC in the flour paste of increasing viscosity was 57%, whereas that of HOT-LPC in flour was 43%. Ours was the first demonstration of such a phenomenon.

The purpose of this study was to investigate whether the above-mentioned findings originate in the wheat protein or in the starch. That is, the distribution of LPL between RT- and HOT-lipid in gelatinization, pasting, and gelation of wheat starch was investigated.

Materials and Methods

Materials Wheat starch (moisture, 14%) was kindly donated by the Association for Separation and Processing of Wheat Flour of Japan. The sample was divided (20 g each) into reagent bottles, and preserved at 5°C. Authentic phosphatidylcholine (PC), phosphatidylserine, phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and their lysoforms were purchased from Sigma-Aldrich (St. Louis, MO, USA), Avanti Polar Lipid Inc. (Alabaster, AL, USA), Serdary Res. Lab. (Toronto, ON, Canada). TLC plates (Silica gel 60;1.05721.0009) were obtained from Merck (Darmstadt, Germany). Standard fatty acid methyl esters (grain fatty acid methyl ester mix) and the methylation reagent ($\text{BF}_3/\text{CH}_3\text{OH}$, 14% solution) were obtained from Supelco Inc. (Bellefonte, PA, USA).

RVA Sampling for PL analysis at the beginning of gelatinization, pasting and gelation of the starch suspensions were carried out using a Rapid Visco Analyzer RVA 3D (RVA; Newport Scientific Pty. Ltd., NSW, Australia). The starch-water suspension contained 4 g of wheat starch in 26 mL of water. The starch suspension was stirred at 960 rpm for 10 s and then maintained at 160 rpm during the subsequent heating and cooling cycle. The starch suspension was stirred at 40°C for 5 min, and subsequently heated to 95°C at a heating rate of 4°C/min, held at 95°C for 5 min, cooled at a constant rate (4°C/min) to 50°C, and held at 50°C for 5 min. A starch pasting viscosity curve, corresponding to the extended temperature program, is shown in Figure 1. This profile was similar to the usual RVA profiles of wheat starch, though the program was different (Zeng and Morrison *et al.*, 1997).

The starch suspension was freshly prepared prior to each use, and the RVA program was restarted. Sampling was performed at 40°C for 5 min (suspension), at 60°C (approximate beginning temperature of gelatinization), at 80°C and 90°C (early and late stages of increasing viscosity), while holding at 95°C for 5 min (decreasing viscosity), and at 50°C for 5 min (maximum viscosity during cooling). When the RVA

completed at the programmed sampling times, 7.5 g (corresponding to 1 g native starch) of the suspension, paste, or gel was removed from the canister and placed in a round-bottomed tube.

Lipid analysis

(1) Lipid extraction Lipid extraction was basically carried out according to the method of Morrison and Mann *et al.* (1975). An 8-mL aliquot of WSB, containing 0.05 mL of 1% butylated hydroxytoluene, was added to a 50 mL round-bottom tube containing 7.5 g starch suspension, paste or gel, vigorously mixed for 30 min at RT (approx. 23°C), and centrifuged for 20 min at $2500 \times g$. After removal of the supernatant, the same volume of extraction solvent was again added to the residue, and the lipid (RT-lipid) was re-extracted in the same manner. The RT-lipid extraction was performed three times in total. After RT extraction three times, the same volume of extraction solvent was added to the residue, the lipids were extracted for 30 min in boiling water with occasional shaking (HOT-lipid), and following centrifugation the supernatant was withdrawn. An 8-mL aliquot of extraction solvent was again added to the residue, and the lipid re-extracted in boiling water. The extraction of the HOT-lipid was performed three times in total. The combined WSB fractions were dried by rotary vacuum evaporation and the extracted lipids were washed with 15 mL chloroform, 15 mL of methanol and 12 mL of 2% NaCl. The crude lipids were dissolved in 7 mL of chloroform, and stored at -80°C . In native starch samples, 1 g of starch was used for lipid extraction. The preparation of samples was performed in triplicate or quadruplicate.

(2) Determination of phospholipid phosphorus The content of phospholipid phosphorus (PL-Pi) in the crude lipid was determined using the method of Bartlett (1959).

Fig. 1. Pasting profile of wheat starch water suspension (0.154 starch/water ratio) from a Rapid Visco Analyzer.

Phospholipids were prepared from crude lipids separated by TLC with chloroform/methanol/acetic acid/water (180:150:30:10, v/v) as the developing solvent (Ishinaga, and Sato *et al.*, 1982; Ishinaga and Tuohuti *et al.*, 2010). After developing with iodine vapor to visualize the spots, the spots were then carefully scraped from the plate with a razor blade. The PL-Pi content was determined using the method of Keenan and Schmidt *et al.* (1968).

Individual phospholipids were identified using Dittmer reagent and Ninhydrin spray, and by comparison with phospholipid standards as previously described (Ishinaga and Tuohuti *et al.*, 2010). n-Acyl phosphatidylethanolamine (Acyl PE) and n-acylsphosphatidylethanolamine (AcylLPE) were also identified as previously described (Ishinaga and Tuohuti *et al.*, 2010).

(3) Analysis of fatty acid composition of LPC The crude lipid containing 0.2 – 0.3 mg of PL-Pi was developed on TLC in parallel with phospholipid standards. When the total PL-Pi was 0.1 mg or less, the crude lipid, which derived from 2 – 3 g of starch, was used. After evaporating the solvent from the plate *in vacuo* for 6 h, the LPC standards were identified by exposing the plate to iodine vapor, and the sample spot corresponding to LPC was carefully scraped from the plate, placed in a screw-capped glass tube fitted with a Teflon cap, and 14% BF₃/methanol was added for trans-methylation (Dougherty and Galli *et al.*, 1987; Ishinaga and Tuohuti *et al.*, 2010).

The fatty acid composition was analyzed by gas chromatography (GC14A; Shimadzu Corporation, Kyoto, Japan) with a DB-225 capillary column (length, 30 m; i.d., 0.25 mm; film, 0.25 µm; J&M Scientific, Folsom, CA, USA). The column temperature was maintained at 180°C for the first min, then raised to 220°C at a rate of 3°C/min, and kept at this temperature for 25 min. Identification of each fatty acid was done by comparison with standards (Ishinaga and Tuohuti *et al.*, 2010). The system was controlled with the gas chromatography software CLASS-GC10 ver. 1.3 (Shimadzu Corporation, Kyoto, Japan).

Statistical analysis Measurements were performed in triplicate or quadruplicate and results expressed as mean ± SD. Differences among sampling points were determined by one-way ANOVA ($P < 0.05$). Individual differences between sampling points were determined using the Tukey-Kramer test. Differences of fatty acid composition in the sampling points between RT- and HOT-LPC were determined by Student's *t* test ($P < 0.05$). Statistical analyses were performed using Stat View 5.0 for Windows (SAS Institute).

Results

Pasting profile Figure 1 shows the pasting profile of

the wheat starch-water suspension measured by a Rapid Visco Analyzer. Since the swelling of wheat starch granules begins at 45 – 50°C (Tester and Morrison, 1990) and at the same time amylose leaches from the granules (Eliasson and Wahgren, 2004; Morrison, 1995; Tester and Morrison, 1990), the starch suspension was held at 40°C for 5 min. Though the temperature range at which the gelatinization occurs is generally 53 – 64°C (Cornell, 2004; Soulaka and Morrison, 1985) and pasting is defined as the phenomenon following gelatinization (Batey, 2007), it was estimated from the profile that the beginning temperature for gelatinization was approx. 60°C (Fig. 1). The starch paste showed peak viscosity when held at 95°C for 1 min and minimum viscosity at 25 min running time. Retrogradation began at approx. 4 min (27 min running time) after the starch paste began cooling from 95°C to 50°C. The maximum viscosity was obtained at 40 min (holding for 5 min at 50°C).

Phospholipid distribution in gelatinization, pasting and gelation of wheat starch-water suspension The amount of phospholipid extracted at RT (RT-PL) and the amount thermally extracted (HOT-PL) with WSB were measured during the beginning of gelatinization, pasting and gelation of wheat starch suspensions (Fig. 2).

The amounts of HOT-PL-Pi from native and starch sus-

Fig. 2. The distribution of PL extracted from wheat starch pastes during gelatinization, pasting, and gelation of wheat starch suspension (mg/g wheat starch).

Values are expressed as mean ± SD ($n = 3 - 4$) of mg/g wheat starch. Samples were extracted with WSB at room temperature (▲; RT-PL), and in boiling water (○; HOT-PL) from the residue. Letters indicate significant differences ($p < 0.05$). Sampling was carried out at 5, 10, 15, 17.5, 23.8, and 40 min running time, and the states of the starch suspensions correspond to: suspension (40°C for 5 min), beginning of gelatinization (60°C), early and late stages of increasing viscosity (80°C and 90°C), decreasing viscosity (state holding at 95°C for 5 min), and maximum viscosity during cooling (holding at 50°C for 5 min), as described in the text. *NS: native starch.

pension (0.34 mg/g starch) were over four times that of RT-PL-Pi (0.06-0.08 mg/g starch). However, the amounts of RT-PL-Pi from the starch suspension at the beginning of gelatinization and during the starch paste with increasing viscosity were higher than those of HOT-PL-Pi. In the increased viscosity starch paste, RT-PL-Pi (0.28-0.31 mg/g starch) increased to about double that of HOT-PL-Pi (0.13-0.16 mg/g starch). When the viscosity of starch paste decreased (holding for 5 min at 95°C), HOT-PL-Pi was again higher than RT-PL-Pi. In the gel, RT- and HOT-PL-Pi were similar to that of native starch.

These results suggest that there were two forms of internal LPL in wheat starch granules.

Distribution of the phospholipid classes in gelatinization, pasting and gelation of wheat starch-water suspension Table 1 shows the phospholipid classes and amounts in RT- and HOT-PL. The major phospholipids were LPC and LPE, with LPC accounting for greater than 85% of the total phospholipid. LPC distribution is shown in Figure 3.

Fig. 3. The distribution of LPC extracted from wheat starch pastes during gelatinization, pasting, and gelation of wheat starch suspension (mg/g wheat starch).

Values are expressed as mean $\pm$ SD ($n = 3 - 4$) of mg/g wheat starch from the data in Table 1. See legend of Figure 2.

Table 1. Composition of phospholipid extracted from the starch paste during gelatinization, pasting, and gelation (PL-Pi μ g/g starch).

Native Starch		Running time of RVA program						
		5 min (40 °C)		10 min (60 °C)		15 min (80 °C)		
Phospholipid	RT*	HOT	RT	HOT	RT	HOT	RT	HOT
LPC***	54.8 $\pm$ 0.4	281.3 $\pm$ 5.1	71.4 $\pm$ 4.7	282.1 $\pm$ 5.3	215.0 $\pm$ 4.3	164.8 $\pm$ 4.4	264.6 $\pm$ 7.1	109.1 $\pm$ 4.3
PC + LPS	T**	T	T	T	T	T	T	T
LPE	4.4 $\pm$ 0.7	40.8 $\pm$ 3.5	6.2 $\pm$ 0.9	45.7 $\pm$ 2.0	20.9 $\pm$ 1.9	22.6 $\pm$ 1.0	34.8 $\pm$ 3.7	11.4 $\pm$ 1.4
LPG + PI	T	5.0 $\pm$ 0.2	T	6.4 $\pm$ 0.2	4.7 $\pm$ 0.2	5.2 $\pm$ 0.4	4.3 $\pm$ 0.2	4.4 $\pm$ 0.2
PE + PG	T	T	T	T	T	T	T	T
Acyltype PE	T	T	T	T	T	T	T	T

Running time of RVA program						
17.5 min (90 °C)		23.8 min (holding at 95°C for 5min)		40 min (holding at 50 °C for 5 min)		
Phospholipid	RT	HOT	RT	HOT	RT	HOT
LPC***	243.1 $\pm$ 5.0	130.0 $\pm$ 3.8	84.6 $\pm$ 5.3	275.3 $\pm$ 8.2	61.3 $\pm$ 16.4	297.8 $\pm$ 19.3
PC + LPS	T	T	T	T	T	T
LPE	31.5 $\pm$ 2.0	18.1 $\pm$ 1.3	7.5 $\pm$ 1.0	37.5 $\pm$ 4.4	4.4 $\pm$ 2.5	43.5 $\pm$ 4.7
LPG + PI	4.9 $\pm$ 0.0	6.3 $\pm$ 0.6	T	9.2 $\pm$ 1.7	T	9.7 $\pm$ 0.9
PE + PG	T	T	T	T	T	T
Acyltype PE	T	T	T	T	T	T

Values are expressed as means $\pm$ SD ($n=3 - 4$).

*: See legend of Figure 2.

**: T, less than 4 μ g (PL-Pi)/g starch. This value corresponds to approx. 1% of the total amount of PL-Pi (about 400 μ g/g starch).

***: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; Acyltype PE, n-acylphosphatidylethanolamine and n-acyllysophosphatidylethanolamine.

The amounts of RT- and HOT-LPC-Pi from native starch and starch suspension at 40°C were 55 – 71 and 281 – 282 µg/g starch, respectively. When gelatinization of the starch suspension occurred at approx. 60°C, the relative amount of RT- and HOT-LPC-Pi from the starch paste was reversed. RT-LPC-Pi increased to 215 µg/g starch, and HOT-LPC-Pi decreased to 165 µg/g starch. In the early and late stages of increasing viscosity, the RT-LPC-Pi of the paste increased to 265 and 243 µg/g starch, respectively. These values were twice those of HOT-LPC. Furthermore, when the viscosity of the starch paste decreased (holding for 5 min at 95°C), the amount of HOT-LPC-Pi (275 µg/g starch) again increased to more than that of RT-LPC-Pi (85 µg/g starch). In the maximum viscosity starch gel, the amounts of RT- and HOT-LPC-Pi were 61 and 298 µg/g starch, respectively, and these values were similar to those of the native starch. As shown in Figure 3, the changes in the amounts of RT-LPC and HOT-LPC in paste were inversely proportional during the starch

suspension pasting process.

The fluctuations in the amounts of LPE and LPG (starch LPL contains LysoPG rather than PI) were also almost the same as the LPC fluctuations (Table 1).

Fatty acid composition of LPC in gelatinization, pasting and gelation of wheat starch-water suspensions The fatty acid composition of RT-LPC and HOT-LPC during the pasting process of the starch suspension is shown in Table 2.

The LPC fatty acids consisted of PA, stearic, oleic, LA, and linolenic acids. The proportions of PA and LA accounted for more than 80% of total fatty acid composition. In the gelatinization, pasting, and gelation stages, the proportions of PA and LA in RT-LPC from starch paste were 32 – 35% and 52 – 57%, respectively. These values were almost the same as those in native starch. However, the proportions of PA in HOT-LPC in the starch suspension at the beginning of the gelatinization, and in the starch paste of increasing viscosity, increased to 47 – 53%, whereas the proportions of LA

Table 2. Composition of phospholipid extracted from the starch paste during gelatinization, pasting, and gelation (PL-Pi µg/g starch).

Running time of RVA programm								
Native Starch			5 min (40 °C)		10 min (60 °C)		15 min (80 °C)	
Fatty acid	RT	HOT	RT	HOT	RT	HOT	RT	HOT
16:0	37.0 ± 1.0	37.7 ± 1.0	36.9 ± 0.6	38.7 ± 0.3	32.1 ± 0.6	47.2 ± 0.4*	32.9 ± 0.6	52.7 ± 1.2*
18:0	0.8 ± 0.2	1.0 ± 0.1	0.8 ± 0.0	1.4 ± 0.4*	0.6 ± 0.0	1.9 ± 0.0*	0.5 ± 0.0	2.5 ± 0.1*
18:1	7.0 ± 2.6	8.2 ± 1.3	5.8 ± 0.0	9.0 ± 0.1*	5.8 ± 0.3	12.3 ± 0.1*	7.2 ± 0.1	11.5 ± 0.4*
18:2	51.4 ± 3.0	49.1 ± 1.9	52.4 ± 0.3	46.9 ± 0.3*	57.4 ± 0.4	35.3 ± 0.3*	55.3 ± 0.6	30.0 ± 0.8*
18:3 (n3)	2.5 ± 0.3	2.3 ± 0.2	2.6 ± 0.1	2.1 ± 0.0	2.9 ± 0.1	1.1 ± 0.0*	2.7 ± 0.0	1.0 ± 0.0*
Unkown	0.4 ± 0.0	0.9 ± 0.3	0.7 ± 0.0	0.9 ± 0.0	0.7 ± 0.1	1.2 ± 0.0	0.7 ± 0.1	1.2 ± 0.0
Other**	0.9	0.9	1	0.8	0.5	1	0.7	1.2

Running time of RVA programm						
17.5 min (90 °C)		23.8 min (holding at 95°C for 5min)		40 min (holding at 50 °C for 5 min)		
Fatty acid	RT	HOT	RT	HOT	RT	HOT
16:0	31.8 ± 0.6	52.1 ± 0.5*	34.6 ± 3.1	41.5 ± 1.5*	32.7 ± 0.3	42.9 ± 1.2*
18:0	0.5 ± 0.0	2.4 ± 0.0*	0.5 ± 0.0	1.3 ± 0.1*	0.5 ± 0.0	1.5 ± 0.1*
18:1	7.3 ± 0.5	11.7 ± 0.1*	6.7 ± 0.2	9.3 ± 0.7*	6.9 ± 0.1	10.3 ± 0.5*
18:2	56.4 ± 0.6	30.5 ± 0.5*	53.6 ± 2.4	44.6 ± 1.2*	55.5 ± 0.3	42.1 ± 1.3*
18:3 (n3)	2.8 ± 0.0	1.0 ± 0.0*	3.1 ± 0.6	1.9 ± 0.1*	2.9 ± 0.0	1.7 ± 0.1*
Unkown	0.7 ± 0.0	1.2 ± 0.0	0.7 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	1.0 ± 0.0
Other**	0.7	1.2	0.8	0.8	0.7	0.9

Values are expressed as means ± SD (n=3 – 4).

*: Significant different between RT and HOT (p < 0.05).

**: Fatty acids present at levels of <1% in both RT and HOT were 14:0, 15:0, 17:0 and 20:1.

See legends of Figure 2 and Table 1.

in HOT-LPC decreased to 30 – 35%. In the starch paste of decreasing viscosity through to the peak viscosity and in the starch gel, the proportions of PA and LA in HOT-LPC were 42 – 43% and 42 – 45%, respectively, and these values were similar to those in native starch.

Discussion

In a previous study, we were the first to report that during the pasting process of a wheat flour-water suspension, changes in the amounts of RT- and HOT-LPC were inversely proportional and their fatty acid composition, especially the proportions of PA and LA, also changed (Ishinaga and Tuohuti *et al.*, 2010). Furthermore, we demonstrate in this paper that this phenomenon originates not in the wheat protein but in the starch.

The distribution between RT- and HOT-lipid, of PL and LPL in starch paste during the pasting process, is shown in Figure 2 and Table 1. More than 95% of the amount of PL in starch was comprised of LPL. More than 85% of LPL was comprised of LPC, with approx. 10% being LPE. These results are in accordance with other reports (Chung and Ohm, 2000).

It is well known that the amount of HOT-PL extracted with hot WSB from native starch is markedly higher than that of RT-PL extracted with WSB at RT (Fig. 2 and Table 1) (Chung and Ohm, 2000; Morrison and Mann *et al.*, 1975). The HOT-PL is an internal starch lipid and is generally thought to exist as an amylose-LPL complex (Morrison, 1995). However, as shown in Figures 2 and 3, in the starch suspension at the beginning of gelatinization and in the starch paste with increasing viscosity, the amounts of RT-PL and -LPC significantly exceeded those of HOT-PL and -LPC. This phenomenon is contrary to current thinking, since the internal starch lipid amylose-LPL complexes are thought to be extractable only with hot alcohol (Morrison and Coventry, 1985). On the other hand, when the starch-water suspension was held at 40°C for 5 min, the amounts of RT- and HOT-PL in the suspension were similar to those in native starch. Therefore, it is necessary for the starch to be gelatinized for the LPC of the HOT-PL to be recovered as RT-PL. We draw the following inferences from this phenomenon.

Continuous heating of native starch granules above a certain temperature in excessive water results in its gradual swelling and the leaching of amylose (Morrison, 1995; Morrison and Tester *et al.*, 1993). At that time, the rigid and organized structure of the native starch granules (Gallant and Bouchet *et al.*, 1997; Pérez and Bertoft, 2010) will change into a loose structure, and the LPC that is enclosed in the rigid structure may become easily accessible and thus can easily be extracted at RT. LPC enclosed in the rigid structure,

not the inclusion complexes with LPC and amylose, may not be detectable in crystallographic patterns with X-ray diffraction. Although Morrison and Tester *et al.* (1993) showed that the internal starch lipids of barley are fully formed complexes with amylose, this has not been demonstrated in wheat starch.

Another possibility was discussed as follows. Eliasson (1980) demonstrated that when the water content of the wheat starch increases from 35% to 80%, endothermic transition temperatures of amylose-lipid complexes decreased from 106°C to 70°C and from 140°C to 100°C, respectively. These temperatures probably originate in the structure of type 1 and type 2 of the complexes, respectively (Eliasson and Krog, 1985; Eliasson, 1994; Biliaderis, 1992). Since the type 1 structure is an amorphous state, when wheat starch was gelatinized in excessive water (over 85% in our case), interactions between amylose and LPL in the complex will be weakened. As the result, LPL of amylose-LPL complexes of the type 1 form could be extracted with WSB at RT.

On the other hand, several studies have demonstrated the existence of complexes of the outer branches of amylopectin and polar lipids (Eliasson, 1994; Eliasson and Wahgren, 2004; Villwock and Eliasson *et al.*, 1999). These amylopectin-lipid complexes will not behave in a manner similar to that of the amylose-lipid complexes (Eliasson and Wahgren, 2004). Although a relationship seems to exist between amylopectin and increasing LPC in the RT-PL, further data is required to clarify this matter.

The proportions of PA (38%) and LA (49%) in native starch (Table 2) were in accordance with previously reported values (Chung and Ohm, 2000). However, as shown in Table 2, the fatty acid proportions of HOT-LPC from the starch suspension at the beginning of gelatinization and from starch paste of increasing viscosity were significantly different from the values of native starch and starch suspensions (40°C for 5 min). The proportion of PA increased from 38% to 47 – 53%, and, conversely, LA decreased from 49% to 30 – 35%. In these cases, the HOT-LPC (derived from the HOT-PL fraction) was recovered in the RT-PL fraction and was approx. three times that of RT-LPC derived from native starch. However, the fatty acid composition of RT-LPC from these starch pastes was similar to that of native starch. These results indicated that LPC with the same fatty acid composition as that from native starch transitioned from HOT-LPC to RT-LPC. On the other hand, the proportion of PA in HOT-LPC from starch paste with increasing viscosity was greater than that of RT-LPC, and the proportion of LA was lower. Therefore, HOT-LPC from starch paste during increasing viscosity forms stable amylose-lipid complexes because it mainly consists of saturated fatty acids (Eliasson and Krog,

1985; Tufvesson and Wahlgren *et al.*, 2003).

These results suggest that there are two forms of LPL in the internal lipid of starch granules. One is the stable LPL formed in polysaccharide-lipid complexes with the LPC containing saturated fatty acid. The other is the free or unstably bound LPL form that changes into a state that is easily extracted at RT from the starch suspension at the beginning of gelatinization and from the starch paste of increasing viscosity, in which LPC contains unsaturated fatty acid.

When the starch paste decreased in viscosity (holding at 95°C for 5 min) and gelled (holding at 50°C for 5 min), the amounts of PL and LPC from the starch paste and gel, and the fatty acid composition of the LPC, were similar to those of native starch (Figs. 2 and 3). This result suggested that the starch paste with decreasing viscosity might have gelled during the experimental procedure. The temperature of the sample decreases rapidly from 95°C to near RT, because a few minutes are required from the stop of the RVA program to the WSB addition. As a result, it is highly possible that the starch paste with decreasing viscosity, where high levels of amylose exist, has resulted in retrogradation. During holding at 95°C for 5 min, amylose-LPL complexes might again be reassociated between dispersed LPL and amylose (Singh and Singh *et al.*, 2008), or free LPL in the starch paste might be incorporated in polysaccharide aggregates or/and ghost granules during retrogradation. In the case of wheat flour, lipids other than LPL, such as AcylPE and AcylLPE, should also be incorporated in the aggregates (Ishinaga and Tuohuti *et al.*, 2010).

On the other hand, the amounts of RT-PL and -LPC from starch paste with decreasing viscosity and gel (maximum viscosity) did not decrease more than those of native starch (Figs. 2 and 3). This suggests that the RT-LPC from native starch, which is a starch surface lipid, binds to components, likely to be proteins, on the starch surface. This is supported by Wilde and Clark *et al.* (1993), who reported that puroindoline is associated with LPC molecules *in vitro*.

The observed re-distribution of LPC content between RT- and HOT-PL and the changes in fatty acid composition of LPC during gelatinization, pasting, and gelation of starch suspensions will help clarify the relationship between polysaccharide and LPL in the pasting process of wheat starch.

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