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Effects of Polyunsaturated Phospholipids on the Thermal and Physical Properties of Starch in Dough and Bread

Tri Agus SISWOYO, Narumi MUKOYAMA and Naofumi MORITA*

Department of Applied Biological Chemistry, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1, Gakuen-cho, Sakai 599-8531, Japan

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The effects of polyunsaturated phospholipids on changes in the thermal and physical properties of starch systems during mixing, baking and storage were studied. In the presence of phospholipids (PLs) or polyunsaturated fatty acid-containing PLs (PUFA-PLs), the viscoelastic properties of starch dough, such as stress, modulus of elasticity and viscosity coefficient were decreased, and the gelatinization enthalpy (Δh_g) of dough also decreased slightly, but the starch-lipid complexes ($\Delta h_{s\text{-}l}$) of dough and bread increased distinctly. Twenty-one days after its storage, the retrogradation rate (k) that was estimated by the change of re-gelatinization enthalpy (Δh_t) of starch containing PLs or PUFA-PLs, was slowed by a factor of 1.98 or 3.28 for dough; and 1.60 or 2.07 for bread, respectively, when compared with that of the control. The changes in retrogradation value of starch in bread seemed to correlate with the retrogradation value of starch in dough in differential scanning calorimetric data (r^2=0.93). These changes in thermal and physical properties of dough and bread appeared to be caused by the formation of complexes between starch and the PLs.

Keywords: polyunsaturated fatty acid, phospholipids, starch gelatinization, retrogradation, differential scanning calorimetry, starch-lipid complexes

Clarification of physical properties of starch such as gelatinization, pasting, and retrogradation is important for effective use of starch in food systems and other industrial application. After gelatinization, starch normally retrogrades depending on the storage conditions (Lin & Czuchajowska, 1998). Retrogradation contains the process in two crystallization stages: in the first stage, the rigidity and crystallinity of starch gels develop quickly by amylose gelation. In the second stage, the crystallinity develops slowly by amylopectin (Biliaderis, 1991). Formation of the starch-lipids complex has been used to explain the positive effect of emulsifiers on the retrogradation of starch either directly by preventing the amylose molecules from crystallization, or indirectly, by changing the water distribution in starch. The physicochemical behavior of starch also depends on the type of starch and emulsifier as well as the time-share history during gelatinization and storage (Hua-liu *et al.*, 1997). The differential scanning calorimeter (DSC), farinograph and rheometer have been used to investigate the physicochemical properties of starch in dough and bread (Lin & Czuchajowska, 1998; Yoshimura *et al.*, 1999; Klucinec & Thompson, 1999).

Phospholipids (PLs) composed of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid have attracted attention as important minor nutrients. Though PUFAs can be emulsified with emulsifiers, their applications to processed food have been considerably restricted, because PUFAs are not soluble in water. Recently, PLs have been extensively used as an emulsifier, and PUFA-PLs can also improve the quality of bread. Supplementation of bread with the PUFA-PLs give people who dislike eating fish another way to obtain these nutrients.

PLs are used throughout the world to improve the functional properties in food processing: improvement in bread texture and volume, dough tolerance, baking properties, and to retard bread becoming state (Kweon *et al.*, 1994; Lin & Czuchajowska, 1998). For this reason, the present study focused on the usefulness of PUFA-PLs as a bread ingredient affecting the thermal and physical properties of starch in dough and bread during mixing, baking, and maintaining an excellent quality.

Material and Methods

Materials The wheat flour used was a hard type "Hermes", provided by Okumoto Flour Milling Co., Ltd. (Osaka). The protein and ash contents of the flour were 11.8 and 0.38%, respectively, both expressed as a 13.5% moisture basis. PLs of egg lecithin origin (Wako Pure Chem. Ind., Osaka) were purified according to the method described by Sridhar and Lakshminarayana (1994), and the concentrate of PUFAs was obtained from menhaden fish oil (Sigma Chemical Co., St. Louis, MS), using the urea-complex method described by Wanasundara and Shahidi (1999). Transesterification of PLs with immobilized lipase was carried out with shaking on a rotary shaker at 150 rpm and at 40°C in an optimized reaction mixture consisting of PLs (0.6 g), PUFA concentrates (2 g), hexane (18 ml) and immobilized lipase (21×10^3 units/g), according to the method described by Totani and Hara (1991); Siswoyo and Morita (2000). The fatty acid compositions of purified PLs *per se* and transesterified PUFA-PLs are summarized in Table 1.

Preparation of dough and bread For the physicochemical measurement of dough, wheat flour (280 g) and water (210 ml) containing PLs or PUFA-PLs were used after mixing for 30 min in a breadmaker to clearly determine the effect of PUFA-PLs (Sharp & Kitchens, 1990; Morita *et al.*, 1996; Morita *et al.*, 1997). The apparatus was an automatic bread maker (SD-BT-3

* To whom correspondence should be addressed.
E-mail: morita@biochem.osakafu-u.ac.jp

Table 1. Fatty acid compositions of purified PL *per se* and its further transesterified PLs with *Aspergillus niger* lipase.

Fatty acid	Fatty acid composition (%)	
	Purified PLs	Transesterified PLs[a)]
C20:4	6.6	13.3
C20:5	1.5	3.8
C22:6	4.1	11.8
Other	87.8	70.1
Transesterification efficiency (%)		20.2
Recovery of PLs (%)		59.0

[a)] Transesterification conditions: 0.6 g PLs, 2 g PUFA concentrates, and immobilized lipase (21×10^3 units/g) in18 ml hexane; transesterification time, 24 h at 50°C.

Home Bakery, Matsushita Electric Co., Osaka), which required a total processing time of 2 h 45 min to make bread as follows: the first mixing for 25 min, the second mixing for 5 min after addition of yeast (for the preparation of dough sample, yeast was not used), fermenting for 90 min, and baking for 45 min. The ingredients for breadmaking were wheat flour (280 g), sodium chloride (5 g), sugar (17 g), dry yeast (3 g) (from Asahi Kasei, Tokyo) and water (210 ml) in which various amounts of PLs or PUFA-PLs were added per kg of flour.

Rheological test The amounts of PLs or PUFA-PLs used for determination of physical properties of dough were 2% (w/w) of flour, unless otherwise stated. Data were obtained in a Brabender farinograph equipped with a 50 g stainless steel mixer; the standard speed of mixing was 63 rpm at 30°C. The farinograph data including arrival time, development time, stability time, the percentage water absorption and valorimeter value were obtained on maximum resistance, centered on 500 B.U.

Viscoelastic properties of dough and bread crumbs were measured using a Fudoh rheometer (Rheotech Co., Ltd., Tokyo). For the dough sample obtained by mixing for 30 min in the bread baker, a 2-cm diameter plunger for viscoelastic measurement and a 5-cm i.d.×5 cm sample were used, and the penetration depth was controlled at 2 cm. A 3-cm diameter plunger for compression stress and a 4×4×3 cm^3 sample were used for the bread crumbs, and the compression depth was controlled at 7 mm. For both samples, the speed of the plunger was 6 cm/min, and the data were processed using the computer program Rheosoft TR-06 (Rheotech Co., Ltd.).

Loaf volume was measured by the rapeseed displacement method. The computerized image analysis of gas cells of bread crumbs was carried out by the methods of Gohtani *et al.* (1992) and Morita *et al.* (1996), using a PIAS LA555 Pias computer image analyzer equipped with a PX-380 CCD camera an AV-M160S Victor color monitor and photocopies (Canon NP5020, Tokyo) of bread crumbs.

DSC The DSC measurements were done with a Shimadzu DSC apparatus (Model DSC-60, Kyoto), as reported by Morita *et al.* (1996) and Siswoyo *et al.* (1999) using liquid paraffin as a reference. Gelatinization temperatures of initial (T_i), peak (T_p) and recovery (T_r) of starch and starch-lipid complex, and the gelatinization enthalpies for starch (Δh_g) and starch-lipid complex ($\Delta h_{s\text{-}l}$) were measured to characterize the thermal properties of starch in dough and bread. The starch in dough and bread samples was analyzed for starch retrogradation after storage for 0–21 days in aluminum capsules at 22°C under a hermetically sealed condition. The temperature was raised from room temperature to 120°C at the rate of 5°C per min.

Statistical analysis Values were obtained as the means± SD of 3 determinations, following ANOVA and were analyzed by Duncan's multiple range test. Differences among samples were considered significant at $p<0.05$. Retrogradation of starch during storage was estimated from the DSC curve. The degree of retrogradation was expressed as the ratio of the re-gelatinization enthalpy (Δh_t) in the second run DSC heating to that of gelatinization enthalpy (Δh_g).

The data of retrogradation fitted to an exponential first-order equation (Baker & Rayas-Duarte 1998; Russell & Oliver 1989) by regression:

$$\frac{\Delta h_\infty - \Delta h_t}{\Delta h_\infty - \Delta h_0} = \exp(-kt), \qquad (1)$$

where Δh_0 and Δh_t are the enthalpies at time 0 and time t, respectively, Δh_∞ is the limiting enthalpy, and k is the rate constant. Δh_∞ was taken to be the limiting enthalpy change at infinite time ($t \to \infty$) obtained from the plot of $1/\Delta h_t$ against $1/t$ (Mita, 1992). The coefficient of correlation (r^2) of relationship between starch in dough and bread during storage was calculated by linear regression analysis.

Results and Discussion

Rheological properties of dough Additions of PLs and PUFA-PLs reduced the viscoelastic properties of dough such as stress, modulus of elasticity, and viscosity coefficient compared with those of the control, whereas the relaxation time was longer than that of the control, as shown in Table 2. The farinograph data are shown in Table 3: development time of the doughs con-

Table 2. Viscoelastic properties of dough.

	Stress (10^3 Nm^{-2})	Modulus of elasticity (10^4 Nm^{-2})	Relaxation time (s)	Viscosity coefficient (10^4 Nsm^{-2})
Control	3.4±0.20[a+]	1.3±0.14[a]	0.8±0.001[c]	1.3±0.13[a]
PLs (%)				
0.5	3.1±0.40[ab]	1.2±0.17[ab]	1.0±0.06[b]	1.1±0.19[ab]
1.0	2.9±0.10[ab]	1.2±0.09[ab]	1.0±0.06[b]	1.1±0.11[ab]
2.0	2.3±0.10[cd]	1.0±0.30[bc]	1.0±0.12[b]	1.0±0.45[ab]
PUFA-PLs(%)				
0.5	2.9±0.10[ab]	1.1±0.19[abc]	1.1±0.00[a]	1.1±0.24[ab]
1.0	2.7±0.20[bc]	1.1±0.19[abc]	1.0±0.12[b]	1.0±0.32[ab]
2.0	2.1±0.20[d]	0.8±0.08[c]	0.9±0.06[b]	0.8±0.12[b]

Data are average of 3 replications. [+]Values followed by the same letter in the same column are not significantly different ($p<0.05$). Abbreviations: PLs, phospholipids; PUFA-PLs, polyunsaturated fatty acid-phospholipids.

Table 3. Farinograph data

	Arrival time (min)	Development time (min)	Stability time (min)	Water absorption (%)	Valorimeter value (unit)	Weakness (B.U)
Control	3.5	29.0	54.0	67.0	81	45
PLs	2.5	22.0	38.3	67.6	83	30
PUFA-PLs	3.4	23.5	49.0	67.2	82	40

Amount of PLs and PUFA-PLs added: 2% (w/w) of flour. Abbreviations: PLs, phospholipids; PUFA-PLs, polyunsaturated fatty acid-phospholipids.

Table 4. Effect of PLs and PUFA-PLs on bread properties.

	Firmness (g/cm^2)	Specific volume (cm^3/g)	Gas cells (mm)
Control	$15.5\pm0.7^{a+}$	4.35 ± 0.03^{a}	1.08 ± 0.01^{a}
PLs	10.7 ± 0.6^{b}	5.02 ± 0.21^{b}	0.87 ± 0.004^{a}
PUFA-PLs	13.5 ± 0.5^{c}	4.65 ± 0.31^{b}	1.02 ± 0.01^{a}

Data are average of 3 replications. $^{+}$Values followed by the same letter in the same column are not significantly different ($p<0.05$). Amount of PLs and PUFA-PLs added: 2% (w/w) of flour. Abbreviations: PLs, phospholipids; PUFA-PLs, polyunsaturated fatty acid-phospholipids.

taining 2% PLs and PUFA-PLs was strongly delayed compared with that of the control. Also, the stability and arrival times of the dough prepared with PLs and PUFA-PLs were shorter than those of the control. Additions of PLs and PUFA-PLs caused the flour to absorb a little more water than the control. This phenomenon might be due to an increase in gluten solubility (Tanaka & Bushusk, 1973; Pomeranz *et al.*, 1968). The valorimeter value did not change among all samples tested, but those of the PLs and PUFA-PLs doughs were slightly weaker than the control.

Effects of PLs and PUFA-PLs on bread properties To determine the optimal amount for baking, concentrations of PLs up to 4% of the amount of flour were used in the baking tests. These experiments showed that concentrations exceeding 2% did lead to a further increase in the loaf volumes (data not shown). Therefore, the following baking tests were performed with the PL concentration of 2%. The effects of PLs and PUFA-PLs on the firmness of bread crumbs, specific volume and size of gas cells are presented in Table 4. Additions of PLs and PUFA-PLs significantly decreased the bread crumbs firmness but did not change the size of gas cells, whereas both treatments increased the specific volumes of the bread (by 1.15 fold and 1.07 fold, respectively, as compared with the control). PL and PUFA-PL doughs did not significantly differ in specific volume however. It is likely that the PL and PUFA-PL doughs react to form starch-lipid complexes (as described later in the DSC section), before they are introduced into the gluten matrix, and this could improve the bread properties (Eliasson & Ljunger, 1988).

Thermal properties of starch in dough and bread As shown in Table 5, increasing the amounts of PLs and PUFA-PLs added to the dough formulation tended to decrease the area of the first endothermic peak in the DSC curves due to the enthalpy of gelatinization (Δh_g) of starch granules, and tended to increase the second peak due to the enthalpy of starch-lipid complexes ($\Delta h_{s\text{-}l}$) (Eliasson & Ljunger, 1988). The increase in the amount of lipids is believed to accelerate the formation of starch-lipid complexes, and thereby to inhibit the starch gelatinization (Larsson, 1980). Additions of PLs increased the $\Delta h_{s\text{-}l}$ as compared with the control. Furthermore, additions of PUFA-PLs increased the $\Delta h_{s\text{-}l}$ by 16% over that obtained with the PL treatment, suggesting that PUFA-PLs with long fatty acid had more affinity to starch than PLs with the short chains. It is likely that PUFA-PLs have more favorable configuration for forming starch-lipid complexes than PLs with short fatty acid chains.

The $\Delta h_{s\text{-}l}$ in dough and bread were compared (Table 6) and were 1.22 and 1.18 J/g, respectively in the control. Addition of PLs increased the $\Delta h_{s\text{-}l}$ for dough and bread by 1.05 and 1.15 fold, respectively, as compared with the control. The $\Delta h_{s\text{-}l}$ for bread containing PUFA-PLs was significantly greater than the control. Thus, PUFA-PLs had a distinctly positive effect in form-

Table 6. The enthalphy of starch-lipid complexes ($\Delta h_{s\text{-}l}$) of dough and bread.

	Dough (J/g)	Bread (J/g)
Control	$1.22\pm0.10^{ab+}$	1.18 ± 0.16^{a}
PLs	1.28 ± 0.02^{bc}	1.36 ± 0.12^{c}
PUFA-PLs	1.48 ± 0.13^{d}	1.66 ± 0.09^{e}

Amounts of PLs and PUFA-PLs added: 2% (w/w) of flour. Data are average of 3 replications. $^{+}$Values followed by the same letter are not significantly different ($p<0.05$). Abbreviations: PLs, phospholipids; PUFA-PLs, polyunsaturated fatty acid-phospholipids.

Table 5. Effects of PLs and PUFA-PLs concentrations on thermal properties of dough.

	Gelatinization				Starch-lipid complexes			
	T_i (°C)	T_p (°C)	T_r (°C)	Δh_g (J/g)	T_i (°C)	T_p (°C)	T_r (°C)	$\Delta h_{s\text{-}l}$ (J/g)
Control	54.4±0.4	64.1±0.2	71.7±0.9	9.8±1.4	102.4±0.9	111.3±0.8	118.9±0.8	1.22±0.1
PLs (%)								
0.5	56.4±0.8	65.7±0.6	69.1±0.8	9.8±1.4	98.7±0.6	108.1±0.8	117.4±0.1	1.22±0.1
1.0	57.0±0.7	65.3±0.4	68.6±0.8	8.4±0.8	99.6±0.3	108.4±0.3	118.5±1.3	1.27±0.1
2.0	57.0±0.1	64.7±0.3	69.4±0.6	8.3±0.6	99.3±0.6	108.8±0.7	118.2±0.2	1.28±0.0
PUFA-PLs (%)								
0.5	55.1±0.2	63.8±0.3	70.9±0.1	8.2±1.4	101.6±0.9	111.3±0.5	118.2±0.9	1.29±0.1
1.0	59.2±0.4	63.9±0.8	70.4±1.4	6.9±0.9	101.8±2.3	108.3±0.7	117.2±0.6	1.35±0.1
2.0	57.9±0.1	65.5±0.5	70.7±1.0	6.3±1.4	99.4±0.7	108.2±0.4	117.6±1.2	1.48±0.1

Data are average of 3 replications. Abbreviations: PLs, phospholipids; PUFA-PLs, polyunsaturated fatty acid-phospholipids; T_i, initial temperature; T_p, peak temperature; T_r, recovery temperature; Δh_g, enthalpy of starch gelatinization; $\Delta h_{s\text{-}l}$, enthalpy of starch-lipid complexes.

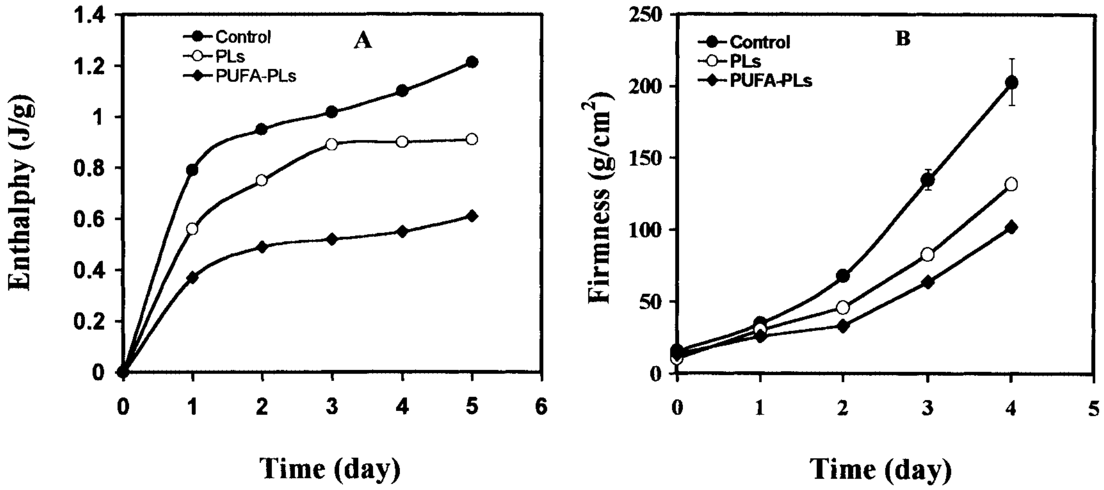

Fig. 1. Effects of PLs and PUFA-PLs on retrogradation of bread crumbs, as measured by DSC (A) and rheometer (B). Vertical bars indicate standard deviation.

Table 7. Thermal properties and retrogradation degree of starch in dough and bread during storage for 21 days.

	Thermal properties			Retrogradation		
	T_i (°C)	T_p (°C)	Δh_t (J/g)	R^+	k^{-day} ++	r^{2+++}
Starch in dough						
Control	47.3	56.3	2.31	24	0.210	0.87
PLs	47.2	55.2	2.03	21	0.106	0.75
PUFA-PLs	49.7	57.2	1.75	18	0.064	0.91
Starch in bread						
Control	52.8	60.5	1.60	16	0.199	0.88
PLs	52.7	59.8	1.45	15	0.124	0.83
PUFA-PLs	52.5	60.5	1.35	14	0.096	0.96

Amount of PLs and PUFA-PLs added: 2% (w/w) of flour. Abbreviations: T_i, initial temperature; T_p, peak temperature; Δh_t, enthalpy; R, maximum retrogradation. $^+$ Calculated as: $(\Delta h_t/\Delta h_g)\times 100\%$, where t = 21 days, which were obtained from the data as described in Materials and Methods. $^{++}k^{-day}$: rate constant per day, obtained from equation (1) in Materials and Methods. $^{+++}r^2$, correlation coefficient value of regression from equation (1).

ing the starch-lipid complexes for both dough and bread. In the present data, the $\Delta h_{s\text{-}l}$ of bread containing PLs was about 6% higher than that of the dough, suggesting that the degradation or dissociation of starch-lipid complexes, or changes in the physical structure of protein, starch or other components occur during baking. On the contrary, in the case of PUFA-PLs, the $\Delta h_{s\text{-}l}$ of the bread was about 12% higher than that of the dough, suggesting that PUFA-PLs were better at promoting the formation of starch-lipid complexes than dissociating them during the early stage of baking.

Retrogradation of starch in dough and bread The firmness and enthalpy (Δh_t) of bread stored for 4 days are shown in Fig. 1. The additions of PLs and PUFA-PLs reduced the firmness and the enthalpy (Δh_t) of bread crumbs. The values of firmness of all samples tested were very small after baking, since the starch was completely gelatinized during heating. However, PLs tended to increase the $\Delta h_{s\text{-}l}$ of starch in bread as compared with the control, whereas PUFA-PLs increased these values more distinctly.

After twenty-one days of storage, the addition of 2% PLs showed a reduction in the Δh of the bread compared with the control; during the period of storage, the Δh_t was also decreased gradually. PUFA-PLs brought about a greater decrease in the Δh_t (Table 7), which suggests that the retrogradation of starch was retarded by the coexistence of PUFA-PLs. Table 7 shows the fitting of retrogradation data to an exponential first-order equation. The addition of 2% PLs slowed the retrogradation rate of starch in dough and bread by factors of 1.98 and 1.60 as compared with those of the control. The complex with PUFA-PLs was inferior to that with PLs or the control in retrogradation; the retrograda-

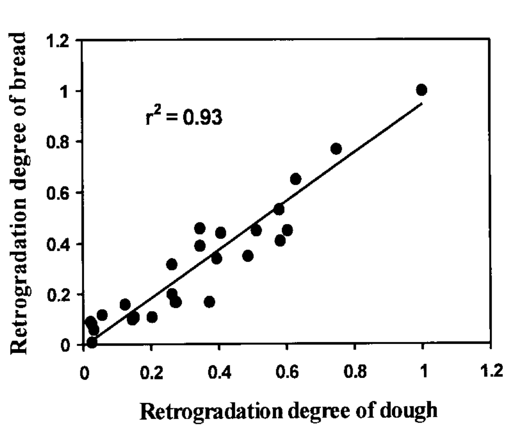

Fig. 2. Relationship of retrogradation value between starch in dough and bread during storage. Retrogradation value was calculated using the formula described in Material and Methods.

tion rates were slowed by factors of 1.65 or 3.28 for dough and 1.60 or 2.07 for bread, respectively. This result indicates that both PLs and PUFA-PLs play an important role in starch retrogradation in dough and bread. The retrogradation rates of starch in dough and bread seemed to be correlated as shown in Fig. 2. The value of r^2 was 0.93. These results suggest that the retrogradation rate of starch in bread during storage could be determined by the retrogradation rate of starch in dough.

In conclusion, this research has focused on the effects of PLs or PUFA-PLs on thermal and physical properties of starch in dough and bread. The results indicate that the changes caused by the PLs or PUFA-PLs were due to the formation of complexes between starch and lipids. PLs retarded the retrogradation of starch, and PUFA-PLs retarded the retrogradation by an even greater amount. However, further study is necessary to clarify the structural characteristics of amylose or amylopectin-lipid complexes, and especially the interaction between starch and PL molecular species having different in chain lengths.

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