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Cooperation of phosphatidylcholine with endogenous lipids of wheat flour for an increase in dough volume

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Abstract

Phosphatidylcholine (PC) increases the gas-retaining ability of dough, the dough volume on fermentation and the loaf volume of bread. The cooperation of wheat flour endogenous lipids with PC was examined. More than 90% of the total wheat flour lipids were extracted with chloroform, the extracted lipids comprising glycolipids (33 wt%), non-polar lipids (56 wt%), and phospholipids (11 wt%). The increase in the specific volume of dough with delipidated wheat flour by the addition of PC was smaller than the increase in the specific volume of dough with native wheat flour. The addition of the extracted lipids to delipidated wheat flour restored the increase in dough volume by the addition of PC. The glycolipid fraction of the extracted lipids was most effective for enhancing the action of PC. The results suggest that interaction of PC with wheat flour glycolipids may synergistically increase foam stability to enhance the gas-retaining stability of dough.

Keywords: Bread; Dough; Phosphatidylcholine; Glycolipid; Wheat flour

1. Introduction

The addition of exogenous food proteins such as soy proteins to dough reduces the loaf volume of bread (Finney, Bode, Yamazaki, Swickard, & Anderson, 1950; Serna-Saldivar, Lopez-Ahumada, Ortega-Ramirez, & Dominguez, 1988). However, the presence of lecithin (Adler & Pomeranz, 1959; Mizrahi, Zimmermann, Berk, & Cogan, 1967; van Nieuwenhuyzen & Szuhaj, 1998), glycolipids (Pomeranz, Shogren, & Finney, 1969a; Pomeranz, Shogren, & Finney, 1969b) or some detergents (Chung, Tuen, & Robinson, 1981; Serna-Saldivar et al., 1988; Tsen & Tang, 1971) prevents the reduction. Among soy phospholipids, so-called soy lecithin, phosphatidylcholine (PC) was determined to have the most effective action (Urade et al., 2003). The function of PC could not be replaced by other phospholipids such as phosphatidylethanolamine, phospatidic acid, or lysoPC. The addition of PC was shown to have little effect on the rheological properties of dough. PC did not bind to starch granules but to gluten fibers, and increased the gas-retaining ability of dough. However, it remains unknown how PC increases the gas-retaining ability of dough to restore the reduced loaf volume. The gas-retaining ability of dough is thought to depend on the foaming properties and stability of the aqueous phase in dough (Wilde, 2003). Wheat proteins and lipids may be responsible for the foam stability of the aqueous phase. Hence, it is necessary to examine the function of PC with those components.

In this paper, we describe the relationship between wheat lipids and the enhancement of the gas-retaining ability of dough by PC, and the cooperation of wheat lipids with PC.

2. Materials and methods

2.1. Materials

Spring wheat flour (Super King™) (13.8% protein, 0.42% ash, 14% water) was obtained from Nisshin Flour

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Milling Inc. (Tokyo, Japan). Dried active yeast (*Saccharomyces cerevisiae*) was purchased from S.I. Lesaffre (Marcq-en-Baroeul, France). Purified β-conglycinin was a gift from Fuji Oil Co. (Osaka, Japan). Soy PC and di-palmitoyl PC (DPPC) were obtained from NOF Co. (Tokyo, Japan). Silica gel 60-precoated thin layer chromatography (TLC) plates and silica gel 60 (0.063–0.2 mm) were purchased from Merck & Co., Inc. (NJ, USA). Other chemicals were of reagent grade.

2.2. Delipidation of wheat flour

One hundred grams of wheat flour was suspended in 120 ml of chloroform, followed by standing at room temperature overnight and then filtration. The filtered wheat flour was resuspended in 120 ml chloroform and then filtered again. These procedures were repeated five times. The delipidated wheat flour was dried in air. The chloroform filtrates containing lipids were combined, and then equal volumes of methanol and distilled water were added. The solution was shaken vigorously, and then centrifuged at 3000 rpm and room temperature for 10 min. The chloroform layer was taken and evaporated in an evaporator. The dried lipids were weighed and analyzed. In large-scale experiments, 1 kg of wheat flour was delipidated by the same procedures. The lipids extracted with chloroform were fractionated by silicic acid gel column chromatography. A slurry of 80 g silica gel 60 was poured into a 2.5×50 cm chromatography column and then equilibrated with chloroform. The lipid extract (2.5 g) was loaded, and eluted sequentially with chloroform (700 ml), acetone (2800 ml), and then methanol (700 ml). Each fraction was evaporated, and the remaining lipids were dissolved in chloroform and stored at −20 °C.

For extraction of all lipids from wheat flour, procedures were carried out by Bligh and Dyer's method (Bligh & Dyer, 1959). Briefly, 100 g of wheat flour was suspended in 120 ml chloroform–methanol mixture (1:2, in volume) and then stood overnight at room temperature. The delipidated flour was resuspended in 120 ml chloroform–methanol mixture (1:2, in volume) and then filtered. These procedures were repeated five times. The filtrates were combined. To separate the chloroform layer, distilled water was added to the filtrate, followed by mixing and centrifugation at 3000 rpm and room temperature for 10 min. The chloroform layer was taken and washed once with 2 M KCl. Chloroform was evaporated off in the evaporator, and the dried lipids were weighed and analyzed.

2.3. Lipid analysis

For quantification, the extracted wheat flour lipids were separated by TLC with a solvent system of chloroform/methanol/acetone/acetic acid/distilled water (200:150:100:15:10, by volume). The TLC plate was dried and sprayed with 0.0002% 2′,7-dichlorofluorescein in 95% ethanol, and then scanned with a fluorescence detection apparatus, Typhoon 8600 imaging system from Amersham Biosciences (NJ, USA), with excitation at 532 nm and emission at 560 nm. The relative intensity of each spot was quantified with ImageQuant analysis software (Kishimoto, Urade, Ogawa, & Moriyama, 2001).

For identification of wheat flour lipids, the lipids eluted with chloroform, acetone and methanol from the silicic acid gel column were analyzed by TLC with solvent system I (diethyl ether/benzene/ethanol/acetic acid (40:50:2:0.2, by volume)), air-dried, and then redeveloped in the same direction with solvent system II (diethyl ether/hexane (6:94, by volume)). In other analysis, the lipids were analyzed by TLC with solvent system III (chloroform/acetone/distilled water (30:60:20, by volume)) or solvent system IV (chloroform/methanol/30% ammonium water/distilled water (60:30:5:2.5, by volume)) (Clayton, MacMurray, & Morrison, 1970). Spots on the plate were visualized by charring with 50% sulphuric acid. To detect amino-lipids, the TLC plate was sprayed with 0.25% ninhydrin in acetone-lutidine (9:1) and then heated at 11 °C (Marinetti, 1964). Glycolipids were detected by spraying 0.2% 5-methylresorcinol in 11.4% H_2SO_4 and then heating at 110 °C (Bevenue & Williams, 1951). Sterols and sterol esters were detected by spraying a 0.05% $FeCl_3 \cdot 6H_2O$ solution in 5% acetic acid/5% H_2SO_4 and then heating at 100 °C for 3 min (Lowry, 1968).

2.4. Scanning electron microscopy (SEM)

Control and delipidated wheat flours were observed by SEM. The flour was mounted on specimen holders. The mounted specimens were coated with a thin layer of gold/palladium. The samples were viewed by scanning the entire surface under a Hitachi S4100 scanning electron microscope from Hitachi Science Systems (Ibaragi, Japan). A representative area was photographed on Fuji FP-500B_{45} film.

2.5. SDS-polyacrylamide gel electrophoresis (PAGE)

SDS–PAGE was carried out by Laemmli's method (Laemmli, 1970). Twenty milligrams of control or delipidated wheat flour was suspended in 0.5 ml of sample buffer and then boiled for 2.5 min. The sample was centrifuged at 14,000 rpm for 10 min at room temperature. The supernatant (2.5 μl) was loaded onto a 12.5% polyacrylamide gel. The proteins on the gel were stained with Coomassie Brilliant Blue R-250.

2.6. Dough

For measurement of the specific volume of dough, dough was prepared from 10 g wheat flour, 0.2 g yeast, 0.5 g sucrose, 0.2 g sodium chloride, and 6.5 g distilled water. For the wheat flour-β-conglycinin combination, 5% of the wheat flour was replaced with β-conglycinin. For supplementation of PC or wheat flour lipids, lipids

were dispersed in 6.5 g distilled water by sonication and then mixed with the other ingredients at 25 °C for 20 min with a Microfarinograph RSM 65 NG (Duisburg, Germany) (Urade et al., 2003). The dough was fermented at 28 °C for 40 min under 100% humidity and then rested at 23 °C for 20 min. Fifteen grams of dough after resting was placed in a graduated cylinder (100 ml). The second fermentation was carried out under 100% humidity for 40 min at 36 °C. In the case of DPPC-supplemented dough, the second fermentation was carried out at 36 °C or 46 °C. After the second fermentation, the volume of the dough in the graduated cylinder was measured.

3. Results and discussion

3.1. Wheat flour lipids

In order to determine whether or not wheat lipids affect the function of PC, delipidated wheat flour was prepared using chloroform to prevent deterioration of the wheat flour (MacRitchie & Gras, 1973). The extraction with a mixture of chloroform and methanol (1:2) was also performed to determine the efficiency of the extraction with chloroform alone. From 100 g wheat flour, 1 and 1.1 g lipids were extracted with chloroform, and the mixture of chloroform and methanol, respectively. The extraction efficiency was determined by comparing seven spots on the plate by TLC (Fig. 1A). More than 85–100% of each lipid extracted with the mixture of chloroform and methanol was found to be extracted with chloroform (Fig. 1B). The lipids were separated by silicic acid gel chromatography. Non-polar lipids, glycolipids and phospholipids were eluted with chloroform, acetone and methanol, respectively (Fig. 1C). The separated lipids were analyzed by TLC with three solvent systems according to Clayton et al. (1970) (Fig. 2). Sterol esters, triacylglycerols, diacylglycerols, and fatty acids were identified in the chloroform effluent. 6-O-Acyl monogalactosyldiglyceride, 6-O-acyl sterylglucoside, monogalactosyldiglyceride, monogalactosylmonoglyceride, digalactosyldiglyceride, and sterylglycoside were found in the acetone effluent. The methanol effluent contained lysophosphatidylethanolamine, N-acyl phosphatidylethanolamine, phosphatidylethanolamine, and phosphatidylcholine.

Whether or not delipidation treatment affected the microstructure of wheat flour was examined, SEM analysis being carried out. Starch granules were embedded in a continuous matrix, that is, coalesced protein bodies in mature endosperm cells (Amend, 1995) (Fig. 3A). The chloroform treatment appeared not to damage the structure in comparison with the native structure (Fig. 3B). On the other hand, smooth surfaces of starch granules and nicks between coalesced protein bodies were observed after the treatment with the mixture of chloroform and methanol (Fig. 3C).

When wheat proteins were analyzed by SDS–PAGE, proteins presumed to be α/β and γ-gliadins decreased on treatment with the mixture of chloroform and methanol. The treatment with chloroform had apparently no effect on the protein composition (Fig. 3D).

3.2. Effect of soy PC on the specific volume of dough prepared with delipidated wheat flour

The delipidation of wheat flour did not affect the dough volume after the second fermentation compared with that of the untreated wheat flour (Fig. 4A). However, the increase caused by PC of the dough volume of native wheat flour was reduced in the case of delipidated wheat flour. The addition of 2% PC to the native wheat flour increased the specific dough volume 1.4-fold compared with that without PC. On the other hand, the addition of 2% PC to the delipidated flour increased the specific dough volume 1.25-fold compared with that without PC. More than 2% PC did not increase the dough volume any more.

Previously, we showed that the addition of soy proteins decreased the loaf volume of bread and that this adverse effect of soy proteins could be counteracted by the addition of PC (Urade et al., 2003). Thus, the effects of the addition of PC and β-conglycinin, which is a major component of soy proteins and is known to decrease the plasma triacylglycerol level in humans (Aoyama et al., 2001), on the specific dough volume was determined. When 5% of the native wheat flour was substituted with β-conglycinin, the dough volume decreased to 69% of that of the dough without β-conglycinin (Fig. 4B). To this dough, the addition of 2% PC increased the specific dough volume 1.88-fold. When β-conglycinin was added to delipidated wheat flour, the specific dough volume decreased to 87% of that without β-conglycinin. The effect of PC on this delipidated wheat flour containing β-conglycinin was lower than that on the native wheat flour containing β-conglycinin. From these results, it was suggested that PC alone has the ability to increase the specific dough volume, and that wheat flour lipids may enhance its effect.

3.3. Function of PC dependent on the physical state

PC is composed of a phosphocholine polar head and two non-polar fatty acyl chains. PCs composed of different combinations of fatty acyl moieties are referred to as molecular species. The gel (L_β) to liquid-crystalline (L_α) phase transition temperature (T_c) of each PC molecular species changes the nature of the two fatty acyl chains of PC (Cullis, Fenske, & Hope, 1996; Koynava & Caffrey, 1998). Previously, it was shown that the addition of PC molecular species to increase the dough volume differed in degree (Urade et al., 2003). PC molecular species with a lower T_c than the fermentation temperature (36 °C), such as di-oleoyl PC, di-linoleoyl PC and di-myristoyl PC, increased the dough volume. The T_c of soy PC (−38.2 °C) is also lower than the fermentation temperature (36 °C), since most fatty acyl residues of soy PC comprise unsaturated fatty acids such as oleic acid and linoleic acid (Urade et al., 2003). On the other hand, molecular species

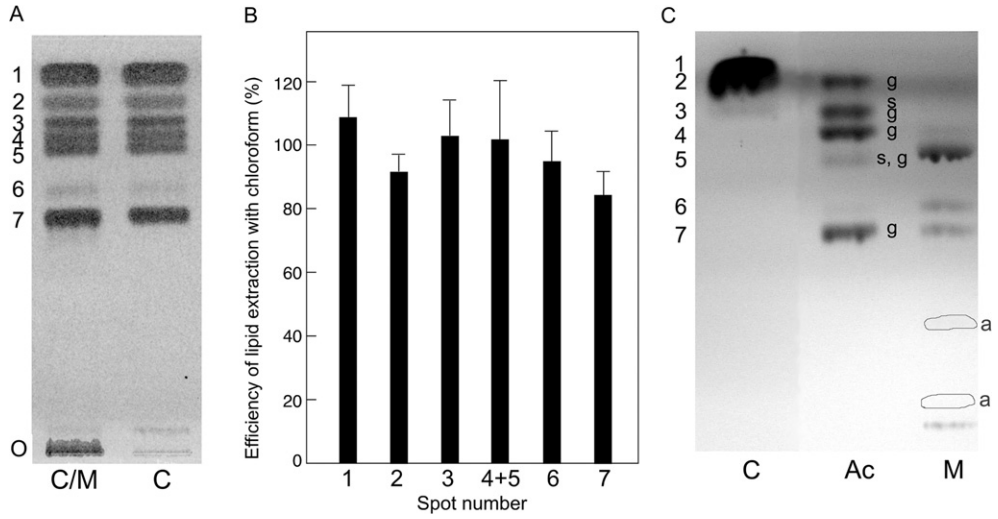

Fig. 1. Extraction and fractionation of wheat flour lipids. (A) Wheat flour lipids were extracted with a chloroform–methanol mixture (C/M) or chloroform (C). Lipids corresponding to the amount extracted from 45.5 mg of flour were separated by TLC with a solvent system of chloroform/methanol/acetone/acetic acid/distilled water. Spots are numbered from the front of development. (B) The efficiency of the extraction of lipids from the flour with chloroform. The lipids separated by TLC in A were quantified as described under Section 2. The numbers on the abscissa indicate the spot numbers in A. One hundred percent corresponds to the amount of total lipids extracted with the chloroform–methanol mixture. Error bars indicate standard deviation for five experiments. (C) wheat flour lipids extracted with chloroform were fractionated by a silicic acid column chromatography. The lipid fractions eluted with chloroform (C), acetone (Ac), and methanol (M) were separated by TLC and then stained with iodide vapour. The numbers indicate the spot numbers in A. g, s, and a indicate spots, positively stained for glycolipids, sterol or sterol esters, and amino-lipids, respectively. O, origin.

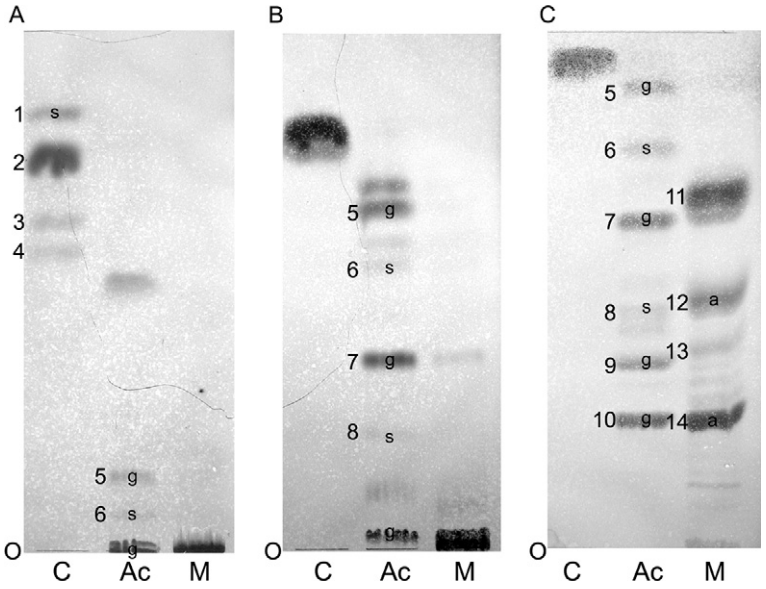

Fig. 2. TLC of the fractionated wheat flour lipids. (A) The lipid fractions eluted with chloroform (C), acetone (Ac), and methanol (M) were developed first with solvent system I, air-dried, and then redeveloped in the same direction with solvent system II. (B) The lipid fractions were developed with solvent system III. (C) The lipid fractions were developed with solvent system IV. g, s, and a indicate spots positively stained for glycolipids, sterol or sterol esters, and amino-lipids, respectively. 1, sterol ester; 2, triglyceride; 3, diglyceride; 4, free fatty acid; 5, 6-O-acyl monogalactosyldiglyceride; 6, 6-O-acyl sterylglucoside; 7, monogalactosyldiglyceride; 8, sterylglucoside; 9, monogalactosylmonoglyceride; 10, digalactosyldiglyceride; 11, N-acyl phosphatidylethanolamine; 12, phosphatidylethanolamine; 13, phosphatidylcholine; 14, lysophosphatidylethanolamine. O, origin.

with higher T_c values than 36 °C, such as DPPC ($T_c = 44.3$ °C), could not increase the dough volume. Hence, in order to examine the relationship between the effect on the dough volume and the physical state of PC, the function of DPPC at 36 or 46 °C was examined (Fig. 5). At 36 °C, DPPC hardly increased the specific dough volume with either native or delipidated wheat flour. However, at 46 °C, DPPC increased the specific dough volume up to 118% with native wheat flour, but had little effect on the specific volume with delipidated wheat flour.

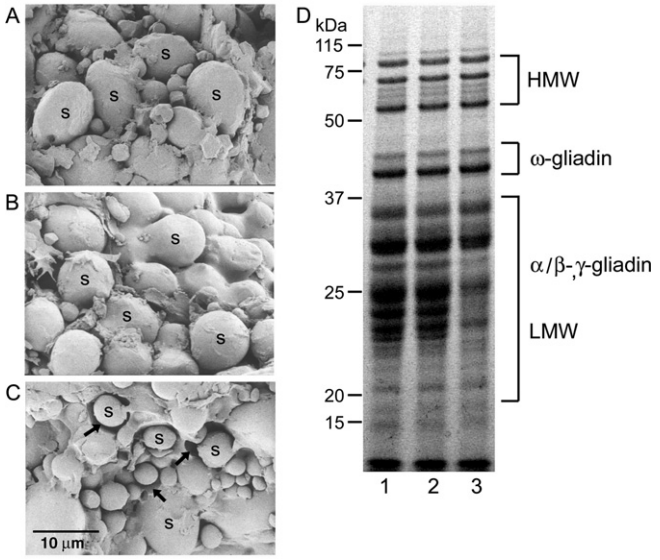

Fig. 3. Effects of delipidation-treatment on the morphology and protein composition of wheat flour. SEM of native wheat flour (A), and delipidated wheat flour with chloroform (B) and the chloroform–methanol mixture (C). s, starch granule. Arrows indicate nicks between starch granules and matrix. (D) proteins of untreated wheat flour (lane 1) or delipidated wheat flour obtained with chloroform (lane 2) or the chloroform–methanol mixture (lane 3) were separated by SDS–PAGE and then stained with Coomassie Brilliant Blue R-250. HMW, high molecular weight subunits of glutenin; LMW, low molecular weight subunits of glutenin.

These results may indicate that the physical state of DPPC changed at 46 °C, but not at 36 °C, consequently, the dough volume-increasing function of DPPC in the presence of wheat flour lipids appeared at 46 °C. It may be concluded that the physical state of PC is an important factor for the dough volume-increasing function in the presence of endogenous wheat flour lipids. However, the reason why DPPC did not function at 46 °C in the absence of endogenous lipids is unclear.

3.4. Cooperation of PC and wheat flour lipids to increase the specific dough volume

The ability of PC to increase the specific dough volume was shown to be more effective with the native wheat flour than with the delipidated wheat flour. Hence, it was expected that the addition of extracted wheat flour lipids to the delipidated wheat flour would enhance the effect of PC alone. This was confirmed by experiments based on the above idea (Fig. 6). The addition of wheat flour lipids enhanced the effect of PC on the specific volume of dough. However, wheat flour lipids alone decreased the specific dough volume. Thus, wheat flour lipids were assumed to increase the specific dough volume in cooperation with PC.

In order to identify the components of wheat flour lipids effective for the function of PC, lipids were separated by silicic acid gel chromatography (Fig. 1C), non-polar lipids, glycolipids and phospholipids being found to comprise 56 wt%, 33 wt% and 11 wt% of the total lipids obtained. The addition of glycolipids alone to the delipidated wheat flour decreased the specific dough volume to 75% of the specific volume of the control dough (Fig. 6).

However, the coexistence of PC and glycolipids increased the specific volume to higher than that of the dough with PC alone. Under these conditions, glycolipids and PC function synergistically. Non-polar lipids or phospholipids had lower effects on the specific volume of the dough than glycolipids. The gas-retaining ability of the

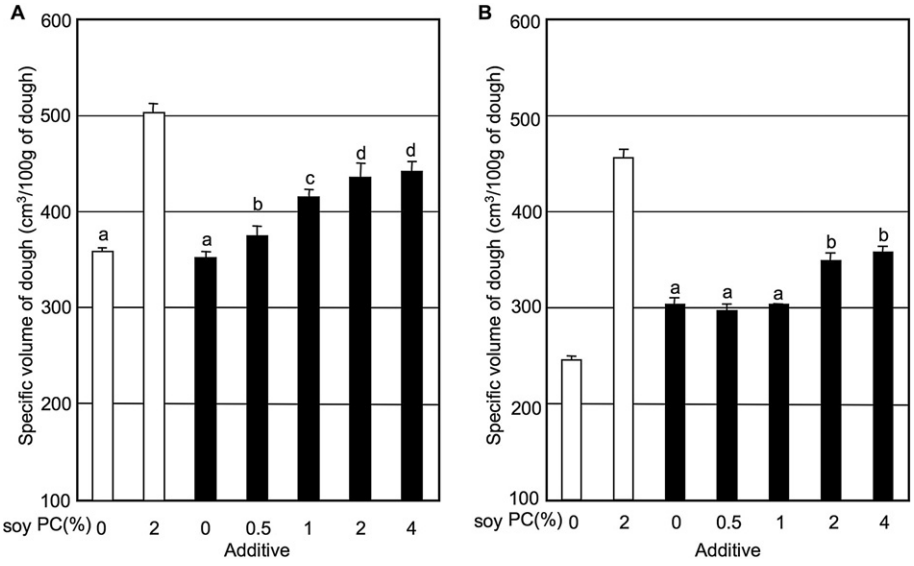

Fig. 4. Effect of soy PC on the specific volume of dough prepared from delipidated wheat flour. Dough was prepared from native (white bars) or delipidated flour (black bars) without (A) or with added β-conglycinin (B). Soy PC was added at the indicated wt% of flour. Error bars indicate standard deviation for the three doughs. Different letters (a, b, c...) indicate significant differences among the doughs ($p < 0.01$).

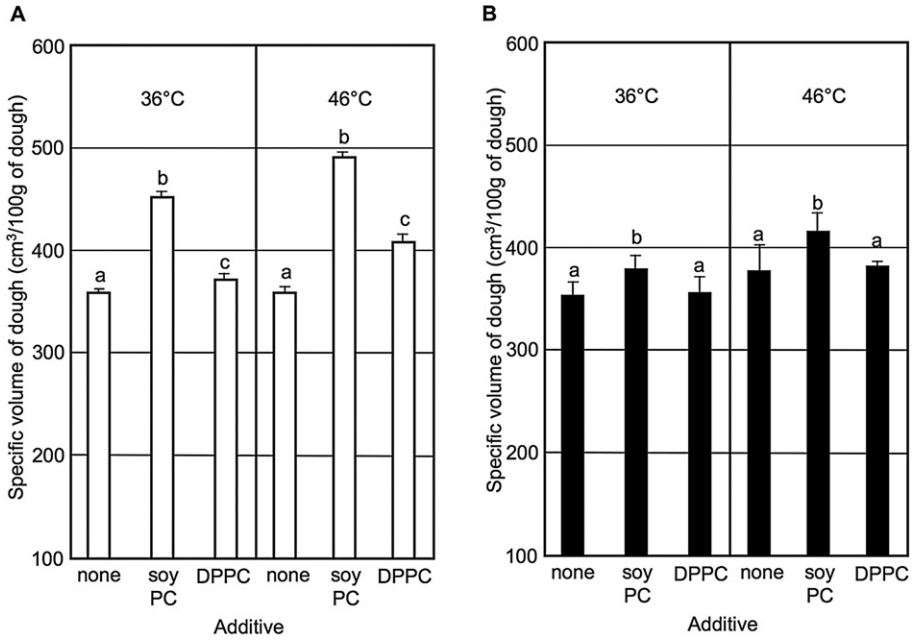

Fig. 5. Relationship between the fermentation temperature and the effect of DPPC on the specific volume of dough. Dough was prepared from native (A) or delipidated flour (B) without or with soy PC or DPPC added at 2 wt% of flour. Error bars indicate standard deviation for three experiments. Different letters (a, b, c...) indicate significant differences among the doughs ($p < 0.05$).

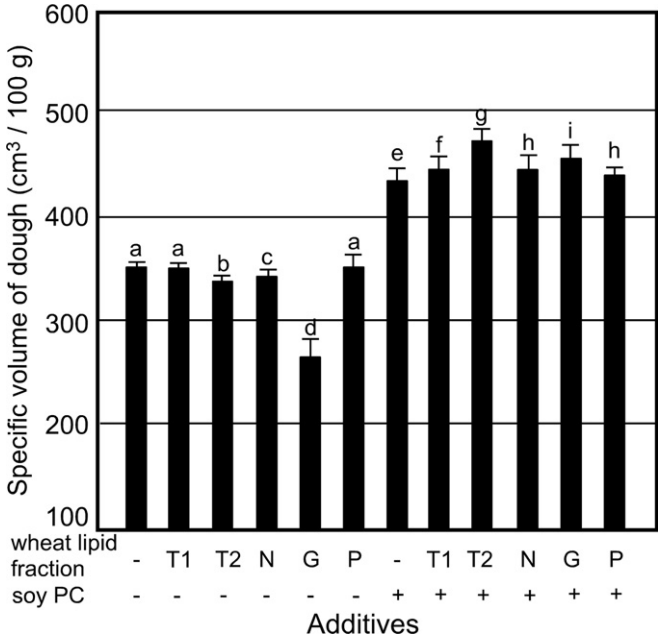

Fig. 6. The synergistic effect of soy PC and the wheat flour lipid fractions separated by silicic acid gel column chromatography on the specific volume of dough prepared from delipidated flour. Soy PC and total lipids of wheat flour were added at 1 (T1) or 2 wt% (T2) of flour. The non-polar lipids (N), glycolipids (G), and phospholipids fraction (P) of wheat flour lipids were added at 1.12, 0.66, and 0.22 wt% of flour, respectively. The specific volume of the dough was determined after second fermentation for 40 min at 36 °C. Error bars indicate standard deviation for three doughs. Values followed by the different letters are significantly different ($p < 0.05$).

dough was considered to depend on the foaming properties and stability of the free aqueous phase in dough (Wilde, 2003). The foam stability of the aqueous phase is influenced by wheat proteins (He & Hoseney, 1992) and lipids (MacRitchie, 1983). It has been reported that polar lipids positively affected the foaming stability, but that non-polar lipids functioned were inhibitory (MacRitchie & Gras, 1973). The mechanisms underlying the foam stability caused by wheat proteins and polar lipids appear to be different, and they may be antagonistic (Sarker, Wilde, & Clark, 1998; Wilde, 1996). Polar lipids of wheat flour at a low concentration may inhibit the foaming ability of wheat proteins (MacRitchie & Gras, 1973). However, in a higher concentration, the foaming ability of polar lipids itself may cause an increase of the loaf volume. The concentration of glycolipids used in this experiment is thought to be inhibitory. Hence, it cannot be excluded that the addition of PC to this system lead to the formation of a glycolipid-PC complex, thereby decreasing the concentration of free glycolipids. This glycolipid-PC complex is assumed to stabilize the foams.

As other possible mechanism for increase in the gas retaining ability of dough, it assumed that interaction of glycolipid–PC complex with gluten and starch granules may influence the physical environment and hence functionality of dough. Polar lipids bind to them via both polar and hydrophobic interactions (Wehrli & Pomeranzs, 1970). The binding of polar lipids may cause on conformational changes of wheat proteins and change formations of inner and intermolecular covalent and non-covalent bonds during mixing. In addition, Pomeranz and Chung (1978) proposed that polar lipids could enable them to strengthen the gluten by effectively bridging protein and starch components. These effects are likely to improve the dough functionality.

4. Conclusions

The increase in the dough volume caused by PC was shown to depend on the cooperation of PC and wheat flour lipids. The gas-retaining ability may be enhanced by the interaction of PC and glycolipids, which may stabilize the foam.

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