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Effects of free lipid enrichment on the quality factors of wheat flour

Qi Yu Lu, Shao Bing Zhang *, Dan Dan Meng, Xiao Yuan Guo and Yan Lin Yuan
School of Food Science and Technology, Henan University of Technology, 450001, China.
**e-mail: shaobingzhang@126.com*

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Abstract

The free lipids (FL) of two commercial flours (medium-strength flour, MSF; soft white flour, SWF) were extracted by petroleum ether (PE), respectively, followed by reconstitution of defatted flours with various levels of FL. The quality factors of reconstituted flours such as gluten, farinograph and pasting properties were investigated. The results showed that with the increase of FL amount in MSF, wet gluten and gluten index did not vary significantly. As for SWF, the extraordinarily high gluten content (43.9%) was obtained when 2% FL was added to defatted flour, indicating that the obvious aggregation of gluten protein was caused by lipid addition. The sodium dodecyl sulfate sedimentation volume (SDS-SV) of MSF was increased but that of SWF was decreased due to FL enrichment. Farinograph absorption and degree of softening (DS) of both flours decreased but stability time (ST) markedly increased with increased FL content. SEM photos captured the reinforcing properties of FL as obvious gluten films could be found and many starch granules were covered with gluten in the oil-containing dough. As flours were enriched by FL, the pasting parameters including holding strength, final viscosity, and peak time of both flours were decreased, peak viscosity of SWF also decreased, but breakdown and setback of both flours were significantly increased. Polar lipid (PL) contents exerted remarkable influences on gluten and farinograph characteristics of MSF. With the increase of PL relative proportion of FL, wet gluten and ST of MSF reduced but DS increased significantly. These results suggested that nonpolar lipids (NL) in flour helped improve the dough tolerance to mix.

Key words: Free lipids, wheat flour, gluten, farinograph, pasting, properties.

Introduction

Lipids comprise 1.4-2% of the flour weight. Differences in solubility provide a convenient and useful means of separating wheat flour lipids into two major categories, free and bound [1]. Free lipids (FL) are generally defined as those extracted with nonpolar solvents such as petroleum ether (PE), hexane or diethyl ether. FL can be fractionated into nonpolar lipids (NL) and polar lipids (PL) and the latter can be further classified into two subfractions - glycolipids and phospholipids - by silicic acid column chromatography. FL in wheat flour have been recognized as important factors affecting quality in breadmaking [2] and noodlesmaking [3-5]. A major method of studying the contribution of lipid content or variety to the properties of flour and its finished product was fractionation and reconstitution. FL were extracted and added to flour in varying amounts or they were interchanged between flours of differing quality. The choice of extracting solvent is important because minimizing the effects of solvent itself on flour is a key in fractionation and reconstitution studies. PE as FL extracting solvent has been used most extensively [6-10].

The influence of defatting on the rheological properties of wheat flour dough has been extensively reported. Narayanan and Hlynka [6] found an increase in the relaxation parameter of doughs from defatted flour and they speculated that the lipid fraction removed by defatting had a sort of protective action against the improver effect of oxygen in dough. Addo and Pomeranz [8] reported that PE-defatting increased alveograph parameters *P*, *W* and *DM* and decreased *L*. Georgopoulos *et al.* [11] studied the viscoelastic properties of dough and gluten after the flour lipids had been removed by different solvents. They found that removal of lipids from flour resulted in doughs with increasing storage modulus (G'). Recently, Sun *et al.* [12] compared the physicochemical properties of original and defatted flours. They showed that the lipid extraction produced much stronger gluten for the weak flour but slightly weaker gluten for the strong flour. It can be concluded from the above results that defatting generally enhances the strength of flour dough. This conclusion seems conflicting with the widely recognized knowledge that lipids are important component which are associated with gluten proteins and participate in the form of gluten matrix.

The objective of this study was to investigate the effects of FL content and their fractions on the major quality factors of flours using fractionation and reconstitution methods.

Materials and Methods

Materials: The wheat flours were a commercial medium-strength flour (MSF, 12.08% protein and 0.48% ash, 14% moisture basis, mb) and a commercial soft white flour (SWF, 9.95% protein and 0.51% ash, 14% mb). PE (boiling at 30-60°C) was analytical grade.

Analytical methods: Moisture, protein and ash were determined by AACC (2000) methods 44-15A, 46-13, and 08-01, respectively [13]. The gluten properties of flours were determined using a Glutomatic System (Perten Instruments AB, Huddinge, Sweden) according to standard methods [14]. Wet gluten was washed and separated from 10 g flour (14% mb) with 2% NaCl solution. The wet gluten was centrifuged using the gluten index sieve cassettes at 6,000 rpm for 1 min. After the amount of gluten that passed through the sieve was weighed, the weight of total wet gluten was measured. Gluten index was calculated as: gluten index = 100 × (wet gluten–gluten passed through sieve)/wet gluten. Sodium dodecyl sulfate sedimentation volume (SDS-SV) was assayed according to the National Standard of China GB/T 15685-1995.

Farinograph absorption, dough development time (DT), stability time (ST), and degree of softening (DS) were determined with the Brabender farinograph bowl and the constant flour weight (300 g) according to AACC method 54-21[13]. Flour pasting properties were determined using a Rapid Visco Analyzer (RVA, Model 3 D, Newport Scientific, Warriewood, NSW, Australia) as described by Konik *et al.* [15].

Scanning electron microscopy (SEM) of dough: The mixed doughs with different lipid content for SEM were prepared by the method of Watanabe *et al.*[16]. A small portion of dough was freezed in liquid nitrogen, followed by freeze-drying for two days. The dried doughs were fractured and mounted onto aluminium stubs with conductive carbon cement. The mounted samples were coated with gold under vacuum. The images were taken using SEM (Quanta-200, FEI Ltd., Eindhoven, The Netherlands) at an accelerating voltage of 10 kV. The micrographs were taken at 1500 × magnification.

Extraction and fractionation of lipids: The lipid extraction method was used as described by Panozzo *et al.* [17] with some modifications. Wheat flour (100 g, 14% mb) was extracted for 22 h with PE using a Soxhlet apparatus. The solvent-lipid mixture was condensed to certain degree and transferred into a covered flask. It was stored at -20°C until ready to use. The defatted flour was air-dried at room temperature until the solvent odors were no longer detected and was sifted through an 80-mesh sieve (200 µm openings). To obtain enough lipids and the defatted flour, the extracting procedure was replicated several times.

For fractionating lipids, PE was removed from the extracted lipids by a rotary evaporator under reduced pressure at 35°C until a little remained, and then the concentrate was transferred to a tared vial. The final traces of solvent were evaporated by gently blowing nitrogen gas over the lipid extract and the amount of lipids was weighed. About 2 g of lipids were fractionated by column chromatography (21mm × 400 mm) on silicic gel (50 g) into NL and PL by sequential elution with 300 mL chloroform and 300 mL methanol, respectively. The elution rate was 2.5 mL/min. The complete elution was ascertained by spot tests on thin-layer plate coated with silicic gel G. The solvent in the two fractions (NL and PL solutions) was evaporated on a rotary evaporator until a little remained, respectively. Then, the respective concentrates were dissolved in a fixed volume of PE and transferred into covered flasks in which air had been displaced with nitrogen. They were stored at -20°C until ready to use.

Reconstitution of defatted flours: Lipids were added to the defatted flours (MSF and SWF) by the method of Matsuo *et al.* [7]. The lipid concentration of collected oil-containing solutions was assayed before reconstitution. Various volumes of the oil-containing solution were thoroughly mixed into the defatted flours to obtain reconstituted flours differing in lipid content (1%, 2% and 3%, respectively). The solvent was allowed to evaporate at room temperature until odors were not detected. The reconstituted flours were sifted through an 80-mesh sieve (200 µm openings). The procedure of adding NL and/or PL to the defatted MSF was the same as above. The relative proportion of PL of FL in the reconstituted MSF (with the constant FL content of 1%) was 0, 30%, 60% and 100%, respectively.

Statistical analysis: Values represented are the means and standard deviations for at least two replicates. Analysis of variance (ANOVA) was performed using SPSS ver. 13.0 for Windows (SPSS Institute, Cary, N.C.). Significance of differences was defined at $p<0.05$ with Duncan's multiple range test (Duncan's test).

Results and Discussion

Effects of FL enrichment on flour gluten and farinograph properties: When the influences of FL content on flour quality were studied, all the samples should undergo the same solvent treatment process for deducting the solvent effects. In the present study, defatted flours were mixed with a fixed volume of PE without lipids as controls. Table 1 shows the effects of FL content on flour gluten and farinograph properties. With the increase of FL amount in MSF, wet gluten and gluten index did not vary significantly. As for SWF, the extraordinarily high gluten content (43.9%) was obtained when 2% FL was added to defatted flour, indicating that the obvious aggregation of gluten protein was caused by lipid addition. The SDS-SV of MSF was increased but that of SWF was decreased due to FL enrichment. The farinograph absorption of both flours decreased linearly with the increase of FL, which may

Table 1. The effects of FL enrichment on flour gluten and farinograph properties.

Sample	Wet gluten (%)	Gluten index	SDS-SV (mL)	Farinograph characteristics			
				Absorption (%)	DT (min)	ST (min)	DS (FU)
PE-defatted MSF, reconstituted with							
0% FL	32.7±0.4a	80±0ab	47.2±1.3b	64.2±0.0a	4.7±0.2b	4.8±0.2d	85±1ab
1% FL	32.6±0.2a	82±1a	56.0±0.1a	61.8±0.4b	4.2±0.0c	6.0±0.1c	79±5b
2% FL	32.1±0.7a	82±0a	56.1±1.5a	60.6±0.6c	5.3±0.1a	7.7±0.3b	64±6c
3% FL	32.5±0.4a	80±0ab	55.4±0.7a	59.5±0.1d	5.3±0.1a	8.7±0.3a	47±4d
PE-defatted SWF, reconstituted with							
0% FL	22.2±0.1bc	78±3c	42.5±0.2a	60.1±0.1a	1.8±0.1ab	5.9±0.3c	79±3a
1% FL	22.6±0.2b	83±1b	40.2±0.1c	57.1±0.4b	1.4±0.2b	7.0±0.1b	66±3b
2% FL	43. 9±0.1a	78±0c	38.7±0.4d	54.9±0.3d	1.5±0.1b	8.5±0.4a	50±4c
3% FL	21.9±0.0c	83±2b	38.5±0.6d	53.7±0.1e	1.7±0.0ab	8.5±0.2a	51±3c

Values for a given flour within the same column followed by the same letter are not statistically different ($p < 0.05$). Abbreviations: FL, free lipids; PE, petroleum ether; MSF, medium-strength flour; SWF, soft wheat flour; SDS-SV, sodium dodecyl sulfate sedimentation volume; DT, development time; ST, stability time; DS, degree of softening; FU, farinograph units.

be due to the hydrophobic action of lipids. Likewise, excess lipids could effectively prevent water absorption of cooked noodles [5]. With the increase of FL, DT of MSF decreased to a minimum and then rose again to a high value, while that of SWF remained almost the same. ST of both flours increased and their DS was reduced significantly as FL was increased, which suggested that FL in flour helped improve the dough strength.

As mentioned in the introduction section, many researchers have found that lipid removal could increase the viscoelastic properties of dough. Their results were obtained by comparison of original and defatted flours. According to the results summarized in Table 1, it could be concluded that enrichment of reconstituted flours with FL generally significantly increased their dough strength. This discrepancy might be explained as follows: the strength increase of defatted dough compared with original dough was mainly attributed to solvent effects rather than the decrease of lipid content. If the solvent effects are deduced and only the effects of lipid content are considered as was done in this study, the experimental results will not be the same. It has been suggested that total FL content had a significant positive correlation with flour mixing time required for breadmaking [18]. Watanabe *et al.* [16] found that added oil (maize oil) was absorbed in the gluten structure, causing the aggregation of the gluten, which gave rise to more elastic behavior.

The microstructure of doughs made by MSF with different FL contents was examined using SEM (Fig. 1). Fig. 1A shows that few starch granules were embedded in the defatted dough and aggregates of gluten were hardly found. Fig. 1B, C captured the reinforcing properties of FL as obvious gluten films could be found and many starch granules were covered with gluten in the oil-containing dough. This result was consistent with SEM photos of the oil-containing gluten-starch dough [16]. The oil-containing dough had a higher number of gluten films, which may provide higher tolerance to mix.

Effects of FL enrichment on flour pasting properties: Lipids associated with isolated cereal starch granules occur on the surface as well as inside the granule. Surface lipids are mainly triglycerides, and to a lesser extent free fatty acids, glycolipids and phospholipids, which can be extracted with diethylether. Complexation of starch with endogenous or added lipids may occur during gelatinization, or when starch and lipids are heated together in the RVA [19]. The effect of exogenous lipids on viscosity of starch pastes has been widely investigated [20-23]. However, there are few studies focusing on the effects of endogenous FL on flour pasting properties. The effects of FL content on flour RVA parameters are shown in Table 2. As the FL content was increased, peak viscosity of MSF did not change significantly whereas that of SWF was markedly decreased. Holding strength, final viscosity and peak time of both flours were markedly decreased but their breakdown and setback were significantly increased by addition of FL.

Ozcan and Jackson [22] reported that amylose-fatty acid complexes had decreased viscosities when pasted compared to their native starch or amylose counterparts. Liang *et al.* [21] pointed out that a greater final viscosity and total setback were characteristics for rice starch mixed with fatty acids and other exogenous lipids, and breakdown was mainly affected and increased by up to 39 RVU for fatty acids in starches. The changes of these RVA parameters

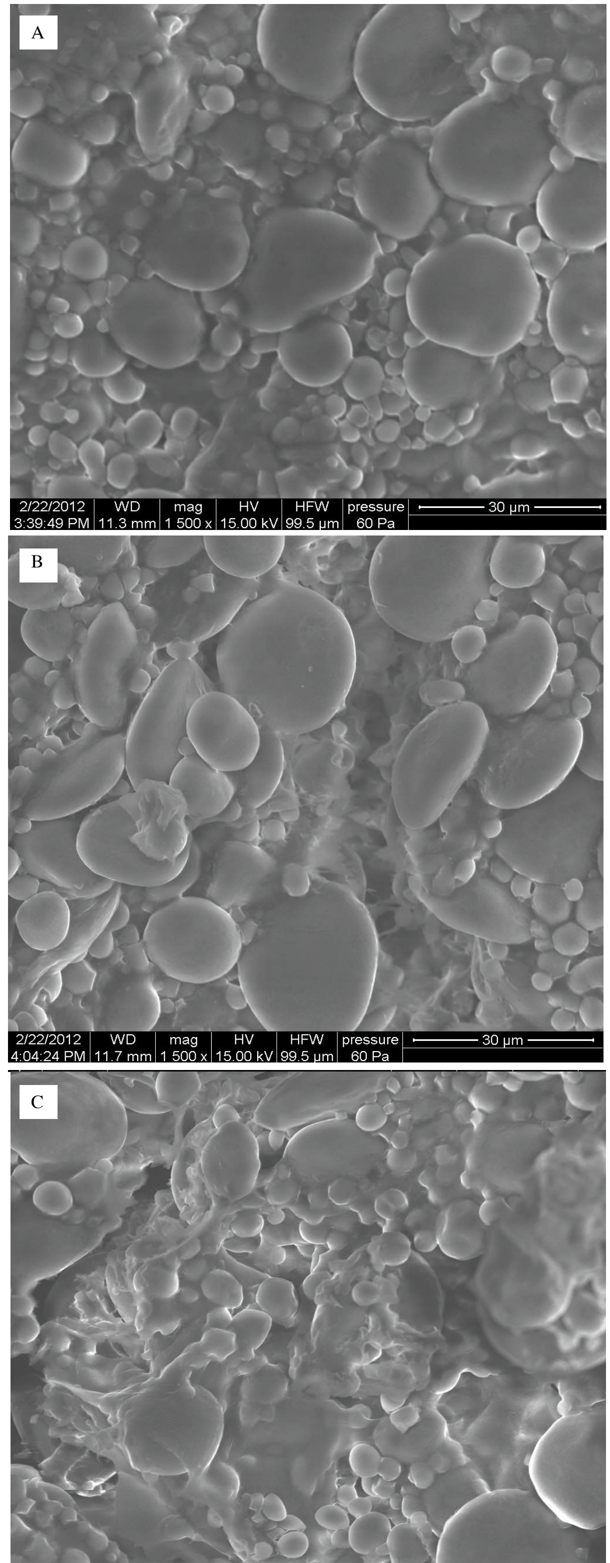

Figure 1. A: Dough structure observed with SEM in the defatted medium-strength flour (MSF) dough. B: Dough structure observed with SEM in the MSF dough containing 1% free lipids. C: Dough structure observed with SEM in the MSF dough containing 3% free lipids.

Table 2. The effects of FL enrichment on flour pasting properties.

Sample	Peak viscosity (cP)	Hold strength (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Peak time (min)
PE-defatted MSF, reconstituted with						
0% FL	2461±10a	1746±47a	716±57c	2697±5a	951±52d	6.45±0.10a
1% FL	2438±35a	1433±53b	1006±18b	2664±40a	1232±13c	6.12±0.09bc
2% FL	2471±30a	1281±19c	1191±49a	2582±13b	1301±6b	5.95±0.04cd
3% FL	2464±13a	1194±31c	1270±18a	2570±21b	1376±11a	5.89±0.05d
PE-defatted SWF, reconstituted with						
0% FL	3198±9a	2416±19a	782±28d	3400±1a	985±21d	6.65±0.00a
1% FL	3123±21b	1761±1c	1362±21b	3066±4c	1305±3bc	6.22±0.05b
2% FL	3102±25b	1581±52d	1521±77a	2981±22d	1400±30a	6.09±0.05c
3% FL	3008±5c	1567±6d	1441±11ab	2912±15e	1345±9ab	6.02±0.05c

Values for a given flour within the same column followed by the same letter are not statistically different ($p < 0.05$). Abbreviations: FL, free lipids; PE, petroleum ether; MSF, medium-strength flour; SWF, soft wheat flour.

Table 3. The effects of PL relative content on gluten and farinograph properties of reconstituted MSF (with the constant FL content of 1%).

Polar lipid relative content (%)	Wet gluten (%)	Gluten index	SDS-SV (mL)	Farinograph characteristics			
				Absorption (%)	DT (min)	ST (min)	DS (FU)
0	32.3±0.4a	89±2b	60.4±0.7a	64.0±0.1b	6.5±0.3a	10.0±0.3a	12±2c
30	29.0±0.7b	95±0a	60.2±0.4a	63.4±0.0d	6.0±0.2a	9.1±0.6b	22±2b
60	28.4±0.2b	91±0a	60.1±1.3a	63.7±0.1c	5.5±0.6a	8.9±0.3c	20±2b
100	28.0±0.8b	78±2c	58.1±0.3b	65.3±0.1a	6.2±0.8a	6.7±0.3d	27±2a

Values for a given flour within the same column followed by the same letter are not statistically different ($p < 0.05$). Abbreviations: PL, polar lipids; MSF, medium-strength flour; FL, free lipids; SDS-SV, sodium dodecyl sulfate sedimentation volume; DT, development time; ST, stability time; DS, degree of softening; FU, farinograph units.

except final viscosity caused by exogenous lipid addition were in agreement with the present results. The peak viscosity and peak time are indicative of the water-binding capacity of the starch and the ease with which the starch granules are disintegrated. Complexation with FL may reduce the solubility of starch in water, hence resulting in the decrease of peak viscosity, peak time and holding strength. The increased setback of starch in the presence of lipids has been explained by the formation of gels with widely spaced junction zones due to the reduced availability of amylose to form networked aggregates [24].

Effects of PL content on flour gluten and farinograph properties: Table 3 shows the effects of PL content on gluten and farinograph properties of MSF. As PL was completely replaced with NL, there was a significant increase in wet gluten of MSF; when NL was completely replaced with PL, gluten index and SDS-SV of MSF significantly decreased but farinograph absorption increased. With the increase of PL relative proportion of FL, DT of MSF did not vary significantly while ST was reduced and DS increased significantly. These results suggested that NL in flour helped improve the dough tolerance to mix.

Addo and Pomeranz [8] also reported that nonpolar lipids from the original flour were more effective in restoring alveograph characteristics of the defatted flour than were polar wheat flour lipids. There is now substantial evidence that PL fraction in FL of flour play a significant role in the baking quality of flour [10, 18, 25, 26] and cooked noodle texture [5]. Hence, the effects of the lipid fractions on dough mixing properties differed from their effects on bread and noodle-making properties. An experimental study using acetic acid based extraction suggested that nonpolar lipids are either associated with the glutenin polymeric network through hydrophobic interactions or entrapped within the gluten matrix; glycolipids are likely to be associated with glutenins whilst phospholipids preferentially interact with gliadins and lipid binding proteins [27]. It could be proposed that with the increase of NL content, more NL as important binder among glutenin facilitate the former and development of gluten network which finally leads to the strengthening of dough mixing properties.

Conclusions

To two commercial flours (MSF and SWF), the changes of gluten properties with lipid addition were different. The farinograph absorption and DS of both flours decreased but ST markedly increased with increased FL content, which indicated the reinforcing properties of FL. As flours were enriched by FL, the RVA parameters including holding strength, final viscosity, and peak time of both flours were decreased, peak viscosity of SWF also decreased, but breakdown and setback of both flours were significantly increased. PL content exerted remarkable influences on farinograph characteristics of MSF. With the increase of PL relative proportion of FL, DT of MSF did not vary significantly while ST reduced and DS increased significantly.

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