JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 1057–1063 **1057**

Effects of Endogenous Flour Lipids on the Quality of Semisweet Biscuits

ELENI PAPANTONIOU,[†] EUGENE W. HAMMOND,[§] AMALIA A. TSIAMI,[†] FIONA SCRIVEN,[§] MICHAEL H. GORDON,*[,†] AND J. DAVID SCHOFIELD[†]

School of Food Biosciences, The University of Reading, P.O. Box 226, Whiteknights, Reading RG6 6AP, United Kingdom, and Group Technical, United Biscuits (U.K.) Limited, Lane End Road, Sands, High Wycombe HP12 4JX, United Kingdom

Fractionation and reconstitution techniques were used to study the contribution of endogenous flour lipids to the quality of semisweet (Rich Tea-type) biscuits. Biscuit flour was defatted with chloroform and baked with bakery fat but without endogenous lipid addition. Semisweet biscuits baked from defatted flour were flatter, denser, and harder and showed collapse of gas cells during baking when compared with control biscuits. Defatted flour semisweet doughs exhibited a different rheological behavior from the control samples showing higher storage and loss moduli (G' and G'' values), that is, high viscoelasticity. Functionality was restored when total nonstarch flour lipids were added back to defatted flour. Both the polar and nonpolar lipid fractions had positive effects in restoring flour quality, but the polar lipid fraction was of greatest benefit. Both fractions were needed for complete restoration of both biscuit quality and dough rheological characteristics.

KEYWORDS: Lipids; biscuits; baking; rheological properties; microstructure

INTRODUCTION

Lipids are a minor component of wheat, comprising 2–4 wt % of the whole wheat grain (*1*). Despite this, small amounts of lipid can affect significantly the quality of bakery products. Several detailed reviews (*2*–*6*) have been published covering research on the nature of wheat lipids and their functionality in bread-making.

Flour lipids play a role in gas cell stabilization, and this has been shown to be very important in bread-making quality. When wheat flour is hydrated to form a dough, polar lipids may exhibit different types of phase behavior depending on factors such as the proportions of polar lipids and water and the structure of the polar lipid class (*7*). In a fermenting dough, the polar lipids may provide surface-active molecules at the gas–liquid interface to stabilize the dispersed gas phase. They have been found to be closely associated with the liquid phase of bread dough (*8*).

It was shown almost 40 years ago that removal of wheat flour lipids adversely affected the spread and top-grain of sugar-snap cookies (*9*), but very little research has been done in the intervening period to determine the mechanism by which they bring about their beneficial effects or to investigate the role of lipids in other biscuit types. Cookie quality is sensitive to the content and composition of the natural flour lipid. Opinion is divided about which lipid class is responsible for the functionality of the lipid fraction in sugar-snap cookies. Cole et al. (*9*) and Clements and Donelson (*10*) found that it was the polar lipid fraction that was responsible for the beneficial effects. Cole et al. (*9*) attributed these effects to the phospholipids, but Clements and Donelson (*10*) also recognized a role for digalactosyldiglycerides (DGDG). However, other workers (*11*) concluded that both the nonpolar and the polar lipids were needed to produce sugar-snap cookies of good quality. They also found that the beneficial effects of the flour lipid fractions were obtained whether the lipids were added to the flour or to the shortening used in the formulation.

Interestingly, Yamazaki and Donelson (*12*) and Clements (*13*) found that sugar-snap cookies baked with flour from which lipids had been extracted had an open structure internally with large gas cells being present, which suggested that the beneficial effects of flour lipids may be related to their ability to enhance gas cell stability in the baking cookie dough. In view of the low water content (11–12.5% w/w) (*14*) of such doughs, however, it seems unlikely that the lipids act through effects on the viscoelastic properties of interfacial films at gas–aqueous phase interfaces as appears to be the case in bread doughs with a much higher water content (~47% w/w) (*8*). It seems to be more likely that the effect of flour polar lipids in sugar-snap cookies is through an analogous effect at the interface between the gas phase and the liquid oil phase in the baking biscuit dough with the gas phase acting as the more polar phase and the liquid oil phase acting as the more hydrophobic phase.

To date, no experimental work has been published on the role of flour lipids in determining the quality characteristics of semisweet biscuits. Rich Tea biscuits are an example of

* Corresponding author [telephone +(44) 1183786723; fax +(44) 1189310080; e-mail M.H.Gordon@reading.ac.uk].
[†] The University of Reading.
[§] United Biscuits (U.K.) Ltd.

10.1021/jf0258196 CCC: $25.00 © 2003 American Chemical Society
Published on Web 01/08/2003

Table 1. Analytical Data for Cv. Consort Whole (Control) and Defatted Flour

	control flour	defatted flour
protein (%, N × 5.7)[a]	8.6	8.6
fat (%)	1.7	0.4
Farinograph		
water absorption[b] (%)	53.2	57.8
degree of softening	150	200
Extensograph		too sticky for analysis
extensibility (mm)	14.1	
max resistance (BU)	95	

[a] 13.1% moisture basis. [b] 600 BU.

semisweet dough biscuits. In this type of biscuit, the bakery fat and sugar levels used (12 and 14% w/w, respectively, in the final dough) are relatively low, resulting in some gluten network formation during the mixing of the dough (*15*). For short biscuit dough, such as that for shortcake biscuits, the levels of fat and sugar are higher (21 and 15% w/w, respectively). Some degree of gluten network development is necessary for the production of Rich Tea-type biscuits, and, in this sense semisweet biscuit doughs can be considered as being somewhat related to bread doughs. The objective of this study was to investigate the functional role of flour lipids in these biscuits.

MATERIALS AND METHODS

Flour. The flour used for all of the biscuit-baking experiments and for the dough rheological measurements discussed in this paper was commercially milled from grain of the soft wheat Cv. Consort (Heygates Mills, Downham Market, U.K.). The flour was packed in bags in batches (1 kg) and stored under vacuum at −40 °C to prevent changes in lipid composition caused by lipases. The flour was analyzed for protein, Farinograph water absorption, extensibility, and maximum resistance according to AACC methods (*16*). The data obtained are given in **Table 1**. The protein, Farinograph, and Extensograph values indicate that the flour is a typical biscuit flour with low water absorption and low extensibility, which is required to ensure the biscuit dough does not recoil elastically after sheeting and cutting, which would produce an oval biscuit. It is interesting to note that the defatted flour absorbed more water than the control sample. The fat value of the control flour accounted for the total nonstarch and starch lipids. The nonstarch lipids were extracted (by chloroform) from the defatted flour, leaving the starch lipids, which were reported to have no technological importance (*3*, *17*, *18*). Sieved icing sugar was used in the biscuit formulation. The fat was PHR15 (Palm Hardened Rape) (PURA Foods, London, U.K.). This was made up from 85% refined/bleached/deodorized (rbd) palm oil and 15% hydrogenated rbd rapeseed oil, and it was incubated at 25 °C for at least 1 day prior to baking tests.

Defatting of Flour. Flour was defatted according to the procedure of MacRitchie and Gras (*17*) with the following modifications: (1) a glass rod was used for 3 min instead of an electric stirrer to stir the chloroform/flour slurry; (2) the solvent was evaporated at 30 °C rather than at 40 °C; (3) *tert*-butylated hydroquinone (TBHQ) was added in chloroform at 0.5% to protect lipids from subsequent oxidation; and (4) each batch extraction was for 1 kg of flour with three solvent washes each using 1.5 L of chloroform. Lipid was quantified by acid hydrolysis according to an AOAC method (*19*) (**Table 1**).

Analysis of Lipid Classes. Lipid extracts were analyzed using an HPLC procedure as described previously (*20*, *21*).

Separation of Polar and Nonpolar Lipid. The extracted flour lipids were separated into polar and nonpolar components by selective elution from silica gel according to the method of Ponte and DeStefanis (*22*) except a total of 12 elutions of the silica gel, rather than 4, were completed with each of the polar and nonpolar solvents.

Reconstitution of Defatted Flour with Extracted Flour Lipids. Flour lipids and their fractions were added back to defatted flour as solutions in chloroform. Flours were stirred constantly during the addition of lipid solutions for uniform distribution of the deposited material. Flours were air-dried to remove traces of chloroform. Treatment concentrations were calculated as fractions or multiples of the lipid components present in the original flour.

Baking Method. To economize on the amounts of lipid and lipid fractions required for reconstitution experiments, it was necessary to use a miniature baking test, which nevertheless uses a recipe and procedure representative of those for commercial biscuits. Ingredients were mixed in a modified Glutomatic gluten index washer (PERTEN Instruments AB, Huddinge, Sweden). The washing facility used for the standard gluten test procedures was disconnected. The sieve was replaced with an acetate disk cut to fit over the base of the mixing bowl, and a thermal plate-probe was fitted between the disk and the sieve base. Semisweet biscuits were made according to a "Rich Tea" formulation (*15*). Flour (12 g), sugar (2.8 g), and fat (2.4 g) were weighed directly into a mixing bowl and premixed for 1 min. Minor ingredients including sodium bicarbonate (0.06 g), sodium chloride (0.12 g), and sodium metabisulfite (SMS; 17 mg) were added as aqueous solutions. The mixing was restarted until the probe indicated a temperature of 42 °C. The dough was sheeted using an RTech Minilab sheeting line (Rtech Ltd., Wigan, Lancashire, U.K.) until the dough thickness was 0.60 mm. Dough pieces were cut using a square brass cutter, 32 mm × 32 mm. Dough piece weight (DPW) was measured with four dough pieces selected for baking. The biscuits were baked for 2.5 min in a Neff oven set at 205 °C.

Examination of the Biscuits and Data Treatment. Biscuits were left to cool to room temperature for at least 5 min before being measured. Biscuit dimensions of width, length, and height were measured using a micrometer. Length (L) was measured in the direction of sheeting and width (W) measured normal to the sheeting direction. Stack height (H) was calculated as the average of height following length and width for the miniature biscuits. The biscuits were weighed, and biscuit density (d) was calculated from the ratio of biscuit weight (BW) to biscuit volume (V) by using the equation

$$d = \mathrm{BW}/V$$

where $V = L \times W \times H$ and density is given in g/cm^3.

All samples were assessed in triplicate.

Textural Measurements. Biscuit hardness was determined with a penetration test using a Stable Micro Systems (Surrey, U.K.) instrument model TA-XT2i, 3 or 4 days after biscuit baking. The instrument was linked to a computer. The miniature biscuit was placed on the loading platform, and a stainless steel probe of 0.5 mm diameter was allowed to penetrate the biscuit; the maximum force for the penetration was recorded. The procedure was repeated four more times for each biscuit. The Texture Expert program version 1.15 (Stable Micro Systems) was used for data analysis. Force (in newtons) was plotted versus distance (in millimeters), and the area under the curve was calculated from the curves (expressed in N·mm). The results were averaged for each biscuit. The calculated area served as a measure of the hardness. The greater the value of the area, the harder the biscuit.

Statistical Analysis. All baking results were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for significant differences between the means of the biscuit properties. The least significant difference (LSD) was calculated to assess which differences were significant.

Small Deformation Rheological Measurements. Small deformation, oscillatory rheological measurements were carried out with the StressTech rheometer (Reologica Instruments AB, Lund, Sweden). Biscuit doughs were prepared as described above, and they were sheeted through the RTech sheeter to a height of 2.8 mm. A piece of dough was then cut off the sheet with a round cutter and placed between the two serrated compression parallel P25 plates (diameter = 25 mm) and compressed to a gap of 2.5 mm. The tests were carried out at 42 °C, which was above the melting point of the added bakery fat to avoid the effect of any solid fat on the rheology of the system. Frequency sweep tests were carried out over the frequency range of 10−0.10 Hz with a strain of 5×10^{-4} and a delay time of 1 s. A stress-sweep test was run to determine the linear viscoelastic region for the doughs prior to carrying out the frequency-sweep tests. All samples were assessed in triplicate.

Table 2. Effect of Total Unfractionated Flour Lipids on Semisweet Biscuit Properties

flour treatment[a]	length (mm)	width (mm)	height (mm)	wt (g)	vol (cm^3)	density (g/cm^3)	DPW[b] (g)	hardness (N·mm)
control	28.8a[c]	32.6abc	3.0d	0.99a	2.8d	0.35a	1.19a	3.31bc
chloroform treated	29.7a	32.7bc	2.7c	1.00ab	2.6c	0.38b	1.19a	2.99b
defatted	30.7b	32.4ab	1.7a	1.05abc	1.7a	0.62e	1.25ab	5.90e
50% rec	29.4a	32.5ab	2.7c	1.08c	2.5c	0.43c	1.27ab	4.19d
100% rec	29.5a	32.8b	2.7c	1.09c	2.6c	0.42c	1.30b	3.66cd
150% rec	29.1a	32.7b	2.6c	1.06c	2.5c	0.43c	1.27ab	3.23bc
200% rec	29.3a	32.8b	2.3b	1.05c	2.2b	0.48d	1.24ab	2.85b
300% rec	30.8b	32.4a	2.2b	1.06c	2.2b	0.48d	1.27ab	1.72a

[a] 50, 100, 150, 200, and 300% rec correspond to defatted flour reconstituted with 50, 100, 150, 200, and 300% of the natural level of flour lipids. [b] Dough piece weight. [c] Different letters (a–e) indicate significant differences between means ($P < 0.05$).

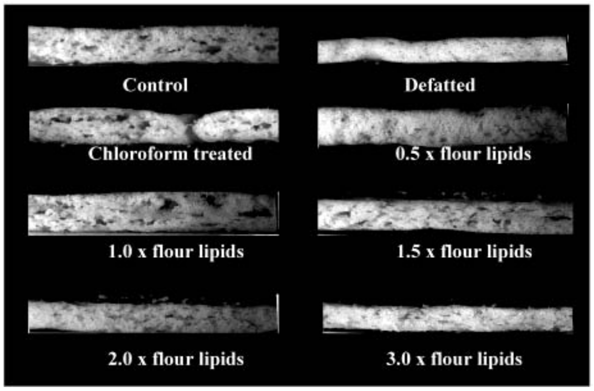

Figure 1. Cross sections of miniature semisweet biscuits: effect of total unfractionated flour lipids.

Microscopy. Scanning electron microscopy (SEM) was used to study the internal structure of the biscuits. Each biscuit was defatted three times with petroleum ether and once with diethyl ether. The defatted biscuit was left to dry overnight on a filter paper. Cross sections of the biscuits were mounted on specimen holders with a special glue (carbon paste) and sputter-coated with gold. The preparations were viewed and photographed in an ISI WB 6 scanning electron microscope (ISI Group, Manchester, U.K.) at an accelerating voltage of 5 kV and at ambient temperature. The digital imaging software used for the collection of micrographs was ISCAN (ISS Group, Manchester, U.K.).

RESULTS AND DISCUSSION

Effects of Total Unfractionated Flour Lipids on Semisweet Biscuit Characteristics. The miniature semisweet biscuits that were baked from defatted flour were very different from those baked from the control flour. Biscuit length (in the direction of sheeting) was significantly greater, indicating no significant contraction of the dough after sheeting and/or during baking, and biscuit height was dramatically lower than the height of the control biscuits (**Table 2**). The differences in the dimensions were reflected in a significant reduction in biscuit volume. Biscuit density was also affected, resulting in denser biscuits being baked from defatted flour. Cross sections of biscuits baked with defatted flour (**Figure 1**) showed that a collapse of their gas cells occurred during baking.

Adding back extracted flour lipids to defatted flours at increasing levels resulted in a recovery of biscuit characteristics so that they became comparable with those of the biscuits baked from the control flour. Optimum biscuit characteristics were achieved when lipids were added at the natural level present in the original flour from which they had been extracted. Addition of flour lipids at higher levels (twice or three times the natural level) did not improve biscuit characteristics but led to a decrease in biscuit height and an increase in biscuit density.

To ensure that none of the observed effects on biscuit properties were due to solvent effects on proteins (e.g., denaturation), the control flour was stirred for 3 min with chloroform and the chloroform was evaporated from the resulting slurry, leaving the flour with its own lipids. This flour, termed here "chloroform-treated", was also used in biscuit-making. The biscuits baked from this flour were very similar in their properties to those containing the control flour. This confirmed that the adverse effects of defatting on biscuit quality were due to the removal of lipids and not due to the solvent used to extract the lipids.

Statistical analysis of the data (**Table 2**) showed that there were, in fact, some significant differences with regard to some properties between the control biscuits and those prepared using reconstituted flour with the natural level of lipids. Differences were noted in some properties (height, weight, volume, density, and dough piece weight), but those differences were very small and the biscuits differed little in appearance. The variability in the data is due partly to the method used for making miniature semisweet biscuits. This method is very sensitive and requires standardizing with standard flour each time it is used. In particular, the sheeting stage is very sensitive to external factors because the very small gap of the second pair of rollers (0.60 mm) may change slightly, and this subsequently influences the dimensions and the weight of the final product.

Because a gluten network is at least partly developed in semisweet biscuit doughs during mixing, the differences in properties of biscuits prepared from defatted flour and full-fat flour may suggest that the flour lipids play an important role in stabilizing the three-dimensional architecture of the dough. However, the gas cell hypothesis proposed for bread (*8*) may not completely apply to the semisweet biscuit system due to the lower water content and the higher fat content of the biscuits. The physical state of the lipids in the biscuits is not liquid crystalline as it appears to be in bread dough.

Effect of Total Unfractionated Flour Lipids on Semisweet Biscuit Hardness. Biscuit hardness was measured for each different flour treatment as described under Materials and Methods, and the data are shown in **Table 2**. Biscuits baked with defatted flour were significantly harder than those baked with the control flour. By reconstituting the extracted lipids with the defatted flour at increasing concentrations, the biscuits became softer, and at the natural lipid levels they were as soft as the control samples. Further addition of lipids above the natural level resulted in even softer biscuits, with the softness increasing to the maximum level tested (3 times the natural lipid content). Rich Tea biscuits prepared with chloroform-treated flour had the same hardness values as the control samples, indicating that chloroform used as the lipid solvent does not introduce any deleterious effects on other flour constituents, such as proteins.

Table 3. Composition of Lipid Classes in Cv. Consort Wheat Flour and in the Respective Nonpolar and Polar Lipid Fractions

lipid class	abbrev	concn in flour[a] (g/kg)	% in lipid extract	nonpolar lipid fraction[a] (g/kg of flour)	% in lipid extract	polar lipid fraction[a] (g/kg of flour)	% in lipid extract
triglycerides	TG	7.14 ± 0.02	49.25 ± 0.12	6.19 ± 0.04	42.67 ± 0.25		
free fatty acids	FFA	0.66 ± 0.13	4.56 ± 0.89	1.12 ± 0.05	7.71 ± 0.33		
diglycerides	DG	1.09 ± 0.13	7.54 ± 0.88	1.10 ± 0.04	7.61 ± 0.27		
monoglycerides	MG	0.52 ± 0.01	3.56 ± 0.09	0.57 ± 0.02	3.90 ± 0.16	0.02 ± 0.00	0.14 ± 0.00
acylated sterylglycosides	ASG	0.14 ± 0.00	0.97 ± 0.03	0.14 ± 0.02	0.94 ± 0.16	0.01 ± 0.00	0.09 ± 0.01
monogalactosyldiglycerides	MGDG	1.46 ± 0.04	10.04 ± 0.31	0.25 ± 0.06	1.70 ± 0.38	1.30 ± 0.04	8.99 ± 0.28
monogalactosylmonoglycerides	MGMG					0.04 ± 0.01	0.26 ± 0.05
digalactosylmonoglycerides + digalactosyldiglycerides	DGMG + DGDG	2.59 ± 0.15	17.88 ± 1.02			2.26 ± 0.03	15.61 ± 0.19
unidentified peak	UP	0.08 ± 0.00	0.57 ± 0.01			0.23 ± 0.09	1.57 ± 0.65
phosphatidylglycerol	PG	0.19 ± 0.04[b]	1.29 ± 0.28[b]			0.11 ± 0.01	0.77 ± 0.04
phosphatidylethanolamine	PE					0.29 ± 0.03	1.99 ± 0.18
phosphatidylcholine	PC	0.57 ± 0.04	3.95 ± 0.30			0.81 ± 0.06	5.58 ± 0.44

[a] Mean and standard deviation is for three analyses of three replicate separations. [b] Includes PE.

Table 4. Effect of Flour Lipid Fractions on Semisweet Biscuit Properties

flour treatment[a]	length (mm)	width (mm)	height (mm)	wt (g)	vol (cm^3)	density (g/cm^3)	DPW[b] (g/cm^3)	hardness (N·mm)
control	29.4b[c]	32.9b	2.8d	1.01bc	2.7c	0.37b	1.21bc	1.41a
defatted	30.4c	32.1a	1.8a	1.00bc	1.8a	0.58e	1.21bc	4.90d
pol100	29.0b	32.8b	3.1e	1.02bc	2.9cd	0.35bc	1.22c	1.34a
pol200	29.0b	32.8b	2.3b	0.96ab	2.2b	0.44d	1.16abc	1.67a
npol100	28.8ab	32.5b	2.4bc	1.03c	2.2b	0.46d	1.22c	3.90c
npol200	28.6ab	32.5b	2.6c	0.97abc	2.4b	0.41c	1.15ab	2.88b
pol100:npol100	28.0a	32.8b	3.3f	0.97abc	3.1d	0.32a	1.15ab	1.21a
pol200:npol200	28.5ab	32.9b	2.5bc	0.92a	2.3b	0.40bc	1.11a	1.340a

[a] po100, pol200: defatted flour reconstituted with natural and 2 times natural levels of polar flour lipids. npol100, npol200: defatted flour reconstituted with natural and 2 times natural levels of nonpolar flour lipids. pol100:npol100, pol200:npol200: defatted flour reconstituted with natural and 2 times natural levels of polar and nonpolar flour lipids, respectively. [b] Dough piece weight. [c] Different letters (a–d) indicate significant differences between means ($P < 0.05$).

Effects of Flour Lipid Fractions on Semisweet Biscuit Characteristics. Having established that total unfractionated flour lipids have a significant effect on semisweet biscuit properties, it was of interest to identify which lipid classes are responsible for this functionality.

The compositions of the lipid fractions were determined by HPLC (**Table 3**). The chloroform flour lipid extract contained a great number of lipid classes ranging in polarity from nonpolar (e.g., triglycerides) to very polar (phosphatidylcholines). The nonpolar lipid classes—65% of the lipid extract (triglycerides, free fatty acids, diglycerides, monoglycerides, and acylated sterylglycosides)—accounted for the majority of the extracted lipid mass from the flour. The polar lipids classes, which comprised 35% of the lipid extract, were the galactolipids and phospholipids, with galactolipids being more abundant. The fractionation procedure yielded very pure fractions, as there was only a small contamination of the nonpolar lipid fraction with the more polar monogalactosyldiglycerides (MGDG) and of the polar lipid fraction with the less polar monoglycerides.

Table 4 shows the effects of the polar and nonpolar lipid fractions on biscuit dimensions, volume, weight, density, and dough piece weight, and **Figure 2** shows the appearance and the internal structure of those biscuits.

The dimensions of length and width for the biscuits made with flour reconstituted with polar lipids alone at their natural level (pol100) were not significantly different (**Table 4**) from those of the control biscuits, but the height was significantly greater. The biscuit weight and dough piece weight were also similar to those of the control. Therefore, reconstitution of the flour with the polar lipid fraction resulted in biscuits having density and volume similar to those of the control samples. It is clear from these data that polar lipids have a beneficial effect in restoring semisweet biscuit characteristics.

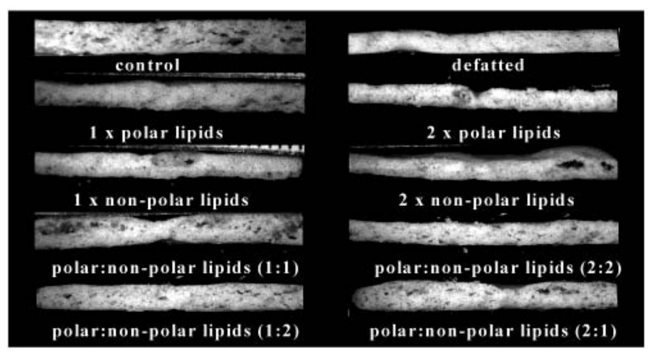

Figure 2. Cross sections of miniature semisweet biscuits: effect of flour lipid fractions.

When polar lipids alone at twice the natural level (pol200) were added to defatted flour, the dimensions of width and length were not significantly different from those of the control, but the biscuits were flatter. The biscuit weight was also somewhat lower (although not significantly so). These two factors led to significantly denser biscuits. This suggests that the addition of a higher than normal amount of polar lipids to the semisweet biscuit system did not further improve the biscuit quality, but, on the contrary, it resulted in somewhat poorer quality biscuits. Those biscuits were also significantly different in most of their properties from those made from flour reconstituted with the natural level of polar lipids (pol100).

The addition of nonpolar lipids alone at their natural level (npol100) did not improve biscuit quality. The biscuits were significantly flatter than the control samples. The biscuit weight was as great as in the control samples, and the biscuits were denser. Moreover, when nonpolar lipids alone were added at twice their natural level (npol200), this improved slightly the biscuits' characteristics compared with those biscuits baked from defatted flour reconstituted with nonpolar lipids at the natural level (pol100), but again the quality was not as good as for the control biscuits.

When both fractions were added together at their natural level (pol100:npol100), this resulted in restoration of the biscuit characteristics. Biscuit height was significantly greater than that of the control, and the biscuits were less dense. However, the addition of fractionated flour lipids together at twice the natural level (pol200:npol200) produced biscuits that were significantly flatter and that had lower volumes than the control biscuits. Biscuit weight and dough piece weight were also very much lower than for the control samples and for the samples prepared with the reconstituted flour with polar and nonpolar lipids added back at twice the natural level. This indicated that the doughs prepared with enhanced lipid levels trapped more air during mixing (hence, lower dough and biscuit weight) but also released more air during baking, producing denser biscuits.

Thus, both the polar and the nonpolar lipid fractions had an effect on semisweet biscuit quality. The polar lipids added alone at their natural level were found to be more beneficial in restoring biscuit quality. The appearance and the height of these biscuits were very similar to those of the control, and, because a gluten network is developed during the dough mixing, it is possible that the polar lipids may have an effect on gas cell stabilization during baking. However, a 2-fold excess of polar lipids was detrimental because biscuit height fell.

On the other hand, the nonpolar lipids alone at the natural level had a less beneficial effect than that of the polar lipids. A partial restoration of biscuit quality was observed when the nonpolar lipids alone were added at twice the natural level. The nonpolar flour lipid fraction comprised mainly triglycerides but also smaller amounts of diglycerides, free fatty acids, some monoglycerides together with a slight contamination by the more polar MGDG. The triglyceride-rich bakery fat does not compensate for the absence of flour lipids in defatted biscuits, so it may be that diglycerides or monoglycerides, which are known to be surface active, have some beneficial effect in the system. Alternatively, the small enrichment of the flour system with MGDG may be responsible for the beneficial role observed for biscuits made from defatted flour reconstituted with twice the amount of nonpolar lipids naturally present.

The addition of both fractions at their natural level was required for full restoration of biscuit quality. This indicates an additive effect of the fractions in biscuit-making. A deleterious effect was observed, however, when they were added together at twice their natural levels.

Effect of Flour Lipid Fractions on Semisweet Biscuit Hardness. The effect of flour lipid fractions on semisweet biscuit hardness is shown in **Table 4**. The addition of polar lipids either at the natural (pol100) or at twice (pol200) the natural level was beneficial in restoring the textural characteristics of these biscuits (not significantly different from control samples). Nonpolar lipids alone added at their natural level (npol100) gave harder biscuits than the control samples, and the biscuits remained harder than the control even with twice the natural level of nonpolar lipids (npol200). Reconstitution of flour with both the polar and nonpolar lipid fractions at the natural level (pol100:npol100) or twice the natural level (pol200:npol200) completely restored the hardness of the biscuits to that of the control samples.

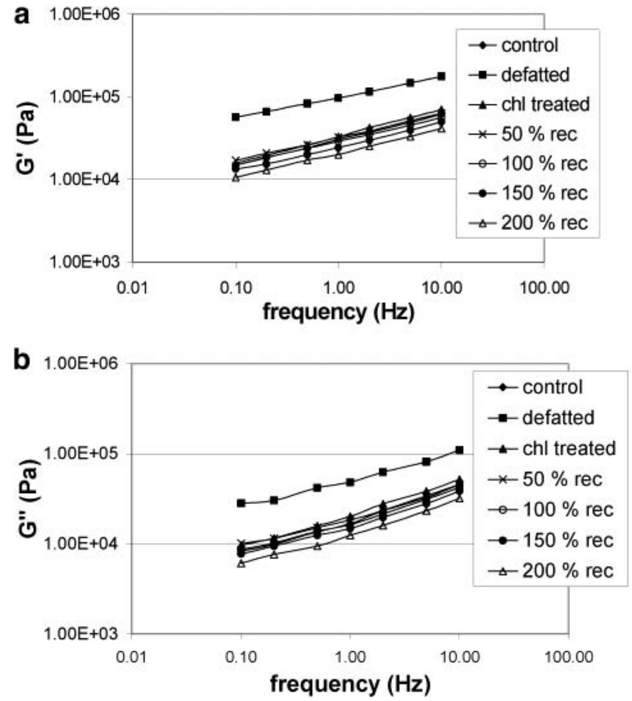

Figure 3. Frequency-sweep curve for (a) G' and (b) G'' for semisweet biscuit doughs; strain, 0.05%; temperature, 42 °C. (50–200% rec refer to doughs prepared from flour samples comprising defatted flour reconstituted with lipids at 50–200% of their natural level.)

Effect of Total Unfractionated and Lipid Fractions on Semisweet Dough Rheology. Parts a and b of **Figure 3** present the effects of flour lipids on the storage and loss moduli (G' and G'' values) of semisweet dough. The flour lipids were added in increasing amounts (0.5, 1.0, 1.5, and 2.0 times the natural level) to the flour. Defatting had an important effect on dough rheology with both G' and G'' increasing significantly compared with the values for the doughs prepared from control flour or from flour reconstituted with flour lipids. This may suggest a higher degree of interaction between the gluten proteins in biscuit dough made from defatted flour, resulting in strengthening of its structure. Reconstitution of flour with total lipids in increasing amounts led to full restoration of rheological properties. There was no significant difference between the G' and G'' values of the control and the reconstituted flours apart from the sample in which flour lipids were added back at half the natural level. Those results indicate that the effects of flour lipids start during the mixing of the flour with the other ingredients. When chloroform-treated flour (chl-treated) was used, there was no significant difference in the rheological behavior of those doughs compared with dough made from the control flour, indicating that chloroform treatment of flour did not affect the rheological properties of the resulting dough. These results follow the same trend as the baking experiments. Although rheological studies cannot be used alone to predict the outcome of baking, they are important because they determine the behavior of dough pieces during mechanical handling, such as dividing, rounding, and molding. The dough rheology may also affect the quality of the finished product (*23*).

Parts a and b of **Figure 4** present the effects of polar and nonpolar flour lipid fractions on G' and G'' of semisweet doughs. Addition of nonpolar lipids to the defatted flour at their natural level (npol100) resulted in partial restoration of the rheological

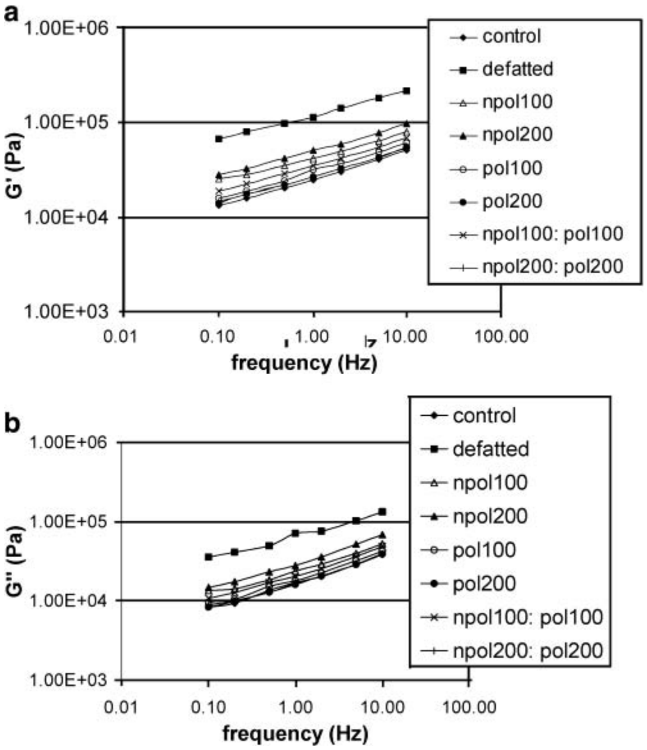

Figure 4. Effect of flour lipid fractions on (a) G' and (b) G'' of semisweet biscuit doughs; strain, 0.05%; temperature, 42 °C.

behavior of semisweet doughs. Higher levels of addition of nonpolar lipids (at twice the natural level, npol200) had no further effect on the rheological behavior.

On the other hand, polar lipids alone at the natural level (pol100) and at twice the natural level (pol200) had a more pronounced beneficial effect on the rheology of those doughs. As in the baking experiments, however, full restoration of the dough rheological properties was obtained when both fractionated polar and nonpolar lipid fractions at their natural levels (npol100:pol100) were added to defatted flour. Doughs prepared with polar and nonpolar lipids at twice their natural levels (npol200:pol200) did not result in further reductions in the G' and G'' values, but they were similar to the control dough.

Effect of Flour Lipids on Semisweet Biscuit Microstructure. Scanning electron micrographs of the biscuits are shown in **Figure 5**. These pictures show that the control semisweet biscuits and samples reconstituted with the natural level of flour lipids are similar in their microstructures, exhibiting a similar degree of aeration and air cells of similar size (<400 μm). On the other hand, biscuits prepared with flour from which lipids have been removed exhibited much smaller (<300 μm) air cells and were denser than both the control and the reconstituted biscuits.

The effects of endogenous flour lipids and their fractions on the quality of semisweet biscuits were quite similar to those observed in the past for American sugar-snap cookies. Defatting of flour resulted in sugar-snap cookies with smaller diameters and heights, and the quality of those cookies was restored when total flour lipids or polar and nonpolar lipid fractions were reconstituted at their natural levels to the defatted flour (*9*−*12*). The effects of flour lipids or lipid fractions on cookie hardness, microstructure, and dough rheology were not reported.

Biscuit volume decreased for semisweet biscuits made with defatted flour, thus indicating a gas cell collapse. The fact that addition of polar lipids to defatted flour was very important in restoring biscuit quality, whereas bread loaf volume did not decrease when defatted flour was used for its production (*17*), suggests important differences between the two systems. The effect in biscuits may be explained on the basis of gas cell stabilization by the surface active flour lipids. In bread doughs, which have a free aqueous phase, polar lipids are likely to have a beneficial effect on gas cell stability through their ability to alter the properties of the interface between the gas phase and the dough aqueous phase. For biscuit doughs, in which the water levels are very low, the interface at which polar lipids are likely to have an effect is that between the more polar gas phase and the more hydrophobic fat or oil phase. Further work is needed to understand the mechanism behind the functional effect of endogenous flour lipids on biscuit quality.

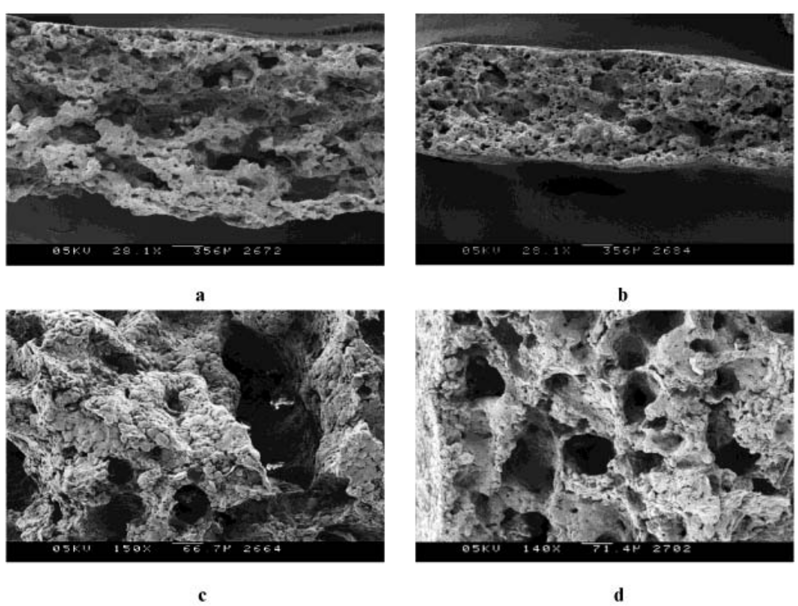

Figure 5. Scanning electron micrographs of miniature semisweet biscuits with control flour (a, c) and defatted flour (b, d). Biscuits were observed at ambient temperature and at 5 kV.

ACKNOWLEDGMENT

We thank United Biscuits Ltd. Group Research and Development, U.K., for providing facilities.

LITERATURE CITED

(1) Morrison, W. R. Acyl lipids in cereals. In *Lipids in Cereal Technology*; Barnes, P. J., Ed.; Academic Press: New York, 1983; pp 11−32.

(2) Mecham, D. K. Lipids. In *Wheat Chemistry and Technology*, 2nd ed.; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1971; Vol. 3, pp 393−451.

(3) Morrison, W. R. Lipids in wheat and their importance in wheat products. In *Recent Advances in the Biochemistry of Cereals*; Laidman, D. L., Jones Wyn, R. G., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1979; p 313.

(4) Morrison, W. R. Recent progress on the chemistry and functionality of flour lipids. In *Wheat End-Use Properties: Wheat and Flour Characterisation for Specific End-Uses*; Salovaara, H., Ed.; University of Helsinki: Lahti, Finland, 1989; pp 131−149.

(5) Chung, O. K. Lipid−protein interactions in wheat flour dough, gluten and protein fractions. *Cereal Foods World* **1986**, *31*, 242−256.

(6) Pomeranz, Y. Composition and functionality of wheat flour components. In *Wheat Chemistry and Technology*, 3rd ed.; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; Vol. 2, p 285.

(7) Larsson, K. Physical state of lipids and their technical effects in baking. In *Lipids in Cereal Technology*; Barnes, P. J., Ed.; Academic Press: New York, 1983; pp 237−251.

(8) Gan, Z.; Ellis, P. R.; Schofield, J. D. Gas cell stabilization and gas retention in wheat bread dough. *J. Cereal Sci.* **1995**, *21*, 215−230.

(9) Cole, E. W.; Mecham, D. K.; Pence, J. W. Effect of flour lipids and some lipid derivatives on cookie-baking characteristics of lipid-free flours. *Cereal Chem.* **1960**, *37*, 109−120.

(10) Clements, R. L.; Donelson, J. R. Functionality of specific flour lipids in cookies. *Cereal Chem.* **1981**, *58*, 204−206.

(11) Kissell, L. T.; Pomeranz, Y.; Yamazaki, W. T. Effect of flour lipids on cookie quality. *Cereal Chem.* **1971**, *548*, 655−662.

(12) Yamazaki, W. T.; Donelson, J. R. Effects of interactions among flour lipids, other flour fractions and water on cookie quality. *Cereal Chem.* **1976**, *53*, 998−1005.

(13) Clements, R. L. Note on the effect of removal of free flour lipids on the internal structure on cookies as observed by a resin-embedding method. *Cereal Chem.* **1980**, *57*, 445−446.

(14) Finney, K. F.; Morris, V. H.; Yamazaki, W. T. Micro versus macro cookie baking procedures for evaluating the cookie quality of wheat varieties. *Cereal Chem*. **1950**, *27*, 42−49.

(15) Wade, P. Introduction to biscuit-making. In *Biscuits, Cookies and Crackers Vol. 1: The Principles of the Craft*; Wade, P., Ed.; Blackie Academic and Professional: London, U.K., 1995; Vol. 1, pp 1−17.

(16) American Association of Cereal Chemists. *Approved Methods*; AACC: St. Paul, MN, 1983; Methods 54-40, 54-10, 54-21.

(17) MacRitchie, F.; Gras, P. W. The role of flour lipids in baking. *Cereal Chem.* **1973**, *50*, 292−302.

(18) Morrison, W. R. Cereal lipids. *Adv. Cereal Sci. Technol.* **1978**, *2*, 221−348.

(19) *Association of Official Analytical Chemists*, 14th ed.; AOAC: Arlington, VA, 1984; Method 922-06.

(20) Conforti, F. D.; Harris, C. H.; Rinehart, J. T. High-performance liquid chromatographic analysis of wheat flour lipids using an evaporative light scattering detector. *J. Chromatogr.* **1993**, *645*, 83−88.

(21) Papantoniou, E.; Hammond, E. W.; Gordon, M. H.; Scriven, F.; Schofield, J. D. Isolation of polar flour lipids classes with preparative high performance liquid chromatography. *Cereal Chem.* **2001**, *78*, 663−665.

(22) Ponte, J. G.; DeStefanis, V. A. Note on the separation and baking properties of polar and nonpolar wheat flour lipids. *Cereal Chem.* **1969**, *46*, 325−329.

(23) Bloksma, A. H.; Bushuk, W. Rheology and chemistry of dough. In *Wheat: Chemistry and Technology*, 3rd ed.; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; Vol. 2, pp 131−217.

Received for review July 15, 2002. Revised manuscript received November 27, 2002. Accepted December 2, 2002. We acknowledge financial support from United Biscuits Ltd. Group Research and Development, U.K., and from the State Scholarships Foundation of Greece.

JF0258196