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Molecular Structure and Baking Performance of Individual Glycolipid Classes from Lecithins

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The potential of individual glycolipid classes from lecithins (soybean, rapeseed, and sunflower) in breadmaking was determined in comparison to classical surfactants such as diacetyltartaric acid esters of mono- and diacylglycerides (DATEM), monoacylglycerides, sodium stearoyl-2-lactylate (SSL), and two synthetic glycolipids by means of rheological and baking tests on a microscale. A highly glycolipid-enriched sample containing the entire glycolipid moiety of the lecithin was obtained using an optimized batch procedure with silica gel. This sample was subsequently used to gain individual glycolipid classes through column chromatography on silica gel. The major glycolipid classes in the lecithins, digalactosyl diacylglycerides (**1**), sterol glucosides (**2**), acylated sterol glucosides (**3**), and cerebrosides (**4**), were identified and characterized. All isolated glycolipid classes displayed excellent baking performance. A better baking activity than that of the classical surfactants was displayed by **1**, **3**, and **4** and an equivalent baking activity by **2**. The same glycolipid classes, except **3**, of different lecithin origin showed only slight differences in their baking activities, due to different fatty acid compositions. Furthermore, the glycolipid classes influenced the crumb structure significantly by improving the crumb softness and grain. Interestingly, none of the glycolipid classes showed significant antistaling effect. A direct effect on the overall rheological behavior of the dough was only found for the commercial surfactants. However, the rheological effect seen on gluten isolated from surfactant-containing dough revealed that the surfactants could be divided into two main groups, one of them directly forming and stabilizing liquid film lamellae through adsorption to interfaces and the other indirectly increasing the surface activity of the endogenous lipids in the flour. The results suggest that in wheat dough, glycolipids seem to have an impact on the dough liquor rather than on the gluten−starch matrix.

KEYWORDS: Glycolipids; lecithin; breadmaking; polar lipids; acylated sterol glucosides; sterol glucosides; cerebrosides; digalactosyl diglycerides; microscale extension test; microscale baking test

INTRODUCTION

Glycolipids are compounds containing one or more monosaccharide moieties bound by a glycosidic linkage to a hydrophobic moiety. This IUPAC definition (*1*) implies that a huge variety of structures can be termed glycolipids. Different glycolipid classes exist having various possible backbone molecular structures such as acylglycerols, sphingoids, ceramides (*N*-acylsphingoids), or sterols. These main glycolipid classes are in no way homogeneous and show distinct variations in their structure, for example, the type and number of carbohydrate moieties or different fatty acid residues. Due to its amphiphilic behavior this group of lipids is surface active and has biological as well as technological importance. In the intact plant, glycolipids and phospholipids form the bilayers of all lipid membranes; in food, they can act as surfactants.

A lot of research has been done so far on elucidating the functionality of endogenous wheat flour lipids in breadmaking. There are detailed reviews (*2*−*7*) covering research on this topic. Despite being a minor constituent (2−4 wt %) of the whole wheat grain, the endogenous flour lipids significantly affect the baking performance of wheat flour (*2*). Many studies in the past have shown that the endogenous polar lipid content of wheat flour essentially improves bread volume as well as showing an antistaling effect (*8*−*10*). It was also reported that the improvements were mainly due to the endogenous glycolipid content, especially digalactosyl diacylglycerides (*11*−*13*). Studies by our group (*14*) and also by others (*7*, *11*, *15*) indicate that glycolipids are better improvers than phospholipids. Some authors (*16*) stated that glycolipids, whether natural or synthetic, are essential for the production of acceptable bread.

Lecithins are side-products of the refining process of edible plant oils (mainly soybean, rapeseed, sunflower) (*17*) and consist mainly of the polar lipid groups such as phospholipids and glycolipids, besides a remaining amount of nonpolar lipids and a nonlipid fraction. Due to their high content of polar lipids lecithins are very useful surfactants for food, and they can be modified in many ways for specific applications. Over the years a

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lot of attention has been given to the various phospholipid classes in lecithins, but the glycolipid classes have not been looked at any closer so far. The presence of glycolipids makes lecithins good candidates for the isolation of glycolipid-rich fractions, which might be highly active in breadmaking applications. However, information on the glycolipid content of lecithins is scarce, and almost no studies on the composition and baking potential of lecithin-based glycolipids have been carried out.

Therefore, the aim of this study was to develop a method to isolate glycolipid-enriched samples from lecithins of different origin as a starting point to obtain pure glycolipid classes and to evaluate the potential of these glycolipid classes in breadmaking in comparison to classical surfactants such as DATEM, monoacylglycerides, SSL, and two synthetic glycolipids.

MATERIALS AND METHODS

Wheat Flour. Wheat flour (cv. Tommi; Nordsaat, Langenstein, Germany), harvested in 2005, was obtained and characterized as described recently (*14*). Analytical characteristics of the flour were 15.2% moisture, 0.47% ash (dry mass), and 11.3% protein (dry mass).

Chemicals. The quality of all solvents was pro analysi (p.a.) or stated otherwise. Sodium sulfate, phosphorus pentoxide desiccant, boron trifluoride−methanol complex solution (13−15% BF_3 basis), heptadecanoic acid, and a Supelco 37 component fatty acid methyl ester mix were obtained from Sigma-Aldrich (Steinheim, Germany). Acetone (Suprasolv), chloroform, dichloromethane, diethyl ether, glacial acetic acid, ethanol, *n*-hexane (Suprasolv), silica gel G 60 (0.040−0.063 mm, 230 −400 mesh), methanol (Lichrosolv), sulfuric acid (95−98%), sodium chloride, high-performance thin layer chromatography (HPTLC) plates (20 × 10 cm) coated with silica gel G 60 with a concentrating zone (20 × 2.5 cm) on glass, tetrahydrofuran, 2-propanol, ammonia solution (25%), ascorbic acid, and sucrose were from Merck (Darmstadt, Germany). Chloroform was also obtained from Biosolve B.V. (Valkenswaard, The Netherlands) through ScienTest (Rheburg-Loccum, Germany). Methanol-d_4 + 0.03% tetramethylsilane (TMS), deuterium oxide, and chloroform-*d* + 0.03% TMS were obtained from Euriso-Top (Gif-sur-Yvette, France).

Surfactant Samples. Surfactant samples were the same as used in Selmair and Koehler (*14*).

Lecithins. Defatted soybean lecithin and crude sunflower lecithin were from Degussa Texturant Systems (Hamburg, Germany). Defatted rapeseed lecithin was from Lucas Meyer (Hamburg, Germany). Purified lecithin without the water-soluble nonlipid substances (carbohydrates, etc.) was isolated from the lecithin according to the modified procedure of Folch et al. (*18*) as described recently (*14*).

Batch Procedure. The procedure was carried out four times for soybean lecithin and two times each for rapeseed and sunflower lecithin. For each procedure the purified lecithin sample (40 g) was dissolved in chloroform (1500 mL), silica gel G 60 [200 g; pretreated according to Esterbauer (*19*), activity grade I] was added to the stirring solution [lipid sample/silica gel ratio 1:5 (w/w)], and the lipid−silica gel slurry was kept stirring for 15 min. Thereafter, the silica gel was left to sediment for 30 min, and the chloroform supernatant was filtered. The silica gel residue was re-extracted as described before. Chloroform that remained on the silica gel was then removed through evaporation under reduced pressure in a desiccator to get a dry residue. The recombined chloroform extracts were evaporated to dryness under reduced pressure, weighed, and redissolved in chloroform/methanol (0.5 g/mL; 2:1, v/v). In the next step the total glycolipid moiety was removed from the silica gel with the phospholipids remaining undissolved by extracting the silica gel with acetone/2-propanol (6 × 1 L; 75/25, v/v) at 0 °C (±1.0 °C) as described for the chloroform extraction. The combined glycolipid extracts were evaporated to dryness under reduced pressure and weighed, and the residue was redissolved in chloroform/methanol (0.5 g/mL; 2:1 v/v). The phospholipid moiety was extracted with different solvent mixtures [chloroform/methanol (2 × 1 L; 2:1 v/v); methanol (1 L); methanol/acetone (1 L; 3:1, v/v); acetone/methanol (1 L, 3:1, v/v); methanol/water (1 L, 9:1, v/v)] from the silica gel at room temperature. After recombination and evaporation, the phospholipids were redissolved in chloroform/methanol (0.5 g/mL; 2:1, v/v). All batch fractions were examined by thin layer chromatography (TLC).

Column Chromatography. Two glass columns (5 cm i.d.) were packed with silica gel G 60 [pretreated according to the method of Esterbauer (*19*), activity grade I], which had been preconditioned with chloroform, for the different chromatography methods used, differing in their height of the silica gel layer. For chromatography method I (glycolipid fractions) the height was 7.5 cm, and for chromatography method II (pure glycolipid classes) it was 22 cm. For each separation carried out the raw glycolipid mixtures obtained from the batch procedure (4 g) were dissolved in chloroform (5 mL) and applied onto the column.

Method I was carried out twice with the glycolipid mixture from soybean lecithin to gain glycolipid fractions. The column (height = 7.5 cm) was eluted with chloroform (5 L), providing nonpolar lipids, with chloroform/acetone mixtures (3 L of 90:10; 8 L of 70:30; 2 L of 50:50; 7 L of 30:70; 6 L of 0:100, v/v), and with chloroform/methanol mixtures to elute remaining substances. The eluate was collected in 500 mL or 1 L glass flasks, and fractions were evaporated to dryness under reduced pressure, weighed, and redissolved in 25 mL of chloroform/methanol (2:1, v/v). All fractions were examined by TLC. Fractions containing the same lipid class or lipid class mixtures were combined so that eight main fractions were obtained.

Method II was carried out once with the glycolipid mixture from the batch procedure of each lecithin (soybean, rapeseed, and sunflower) to obtain four pure major glycolipid classes. The column (height = 22 cm) was first eluted with chloroform (5 L) and then with chloroform/acetone/methanol mixtures (3.5 L of 70:30:0; 4 L of 60:39:1; 4 L of 50:48:2; 5.5 L of 40:57:3; 6 L of 40:56:4, v/v/v). The eluate was collected in 250 mL fractions, which were evaporated to dryness under reduced pressure, weighed, and redissolved in 5 mL of chloroform/methanol (2:1, v/v). All fractions were examined by TLC, and fractions comprising the same pure lipid class were recombined.

Determination of Fatty Acid Composition. The sample (25 mg) was mixed with boron trifluoride methanol complex solution (2 mL) and heated to 60 °C for 2 h after the internal standard heptadecanoic acid (105 μL, 95.3 mg/mL in methanol/chloroform 2:1, v/v) had been added. After mixing with distilled water (3 mL), the solution was extracted with hexane (3 mL). The organic phase was dried with anhydrous sodium sulfate, and an aliquot of the solution (1 μL) was injected on-column and separated by gas−liquid chromatography on a 30 m × 0.25 mm i.d., 0.25 μm, Omega wax 250 fused silica capillary column (Supelco, Bellefonte, PA) with a 0.25 μm stationary phase and methyl silyl deactivated precolumn (3 m × 0.25 mm, Analyt, Mühlheim, Germany) on an Agilent 6890 Network gas chromatograph system with an Agilent 5973 Network Mass Selective Detector (Agilent, Waldbronn, Germany). Helium at a flow rate of 1.5 mL/min was used as carrier gas. The following temperature program was used: 50 °C for 2 min, then a programmed ramp of 10 °C/min to 180 °C, 180 °C for 10 min, 1 °C/min to 220 °C, and 220 °C for 10 min. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV in the scan range of *m*/*z* 50−550. For calibration a standard mixture of fatty acid methyl esters and the internal standard heptadecanoic acid methyl ester was analyzed.

Mass Spectrometry. Sample solutions in methanol were directly applied to a mass spectrometer (LCQ Classic, Thermo Fisher Scientific, Dreieich, Germany) running in the positive electrospray ionization mode (ESI^+) by means of a syringe, and full-scan spectra (*m*/*z* 100−2000) were recorded.

NMR Spectroscopy. The ^1H and two-dimensional NMR spectra (^1H, COSY (^1H ^1H, correlated spectroscopy), HMQC (^1H ^{13}C, heteronuclear multiple quantum coherence), HMBC (^1H ^{13}C, heteronuclear multiple bond correlation) were recorded on a Bruker AMX 400 Ultrashield spectrometer, and the ^{13}C NMR spectra were recorded on a Bruker AMX 360 spectrometer (Bruker BioSpin, Rheinstetten, Germany). Chemical shift values δ (in ppm) are given relative to the signal for internal TMS (δ = 0). The values for coupling constants *J* are given in hertz.

TLC. HPTLC silica gel G 60 plates (20 × 10 cm) and chloroform/methanol/25% aqueous ammonia/water (65:30:5:2.5, v/v/v/v) as mobile phase were used according to the method of Clayton (*20*). Compounds were visualized by spraying with 50% (w/w) sulfuric acid and charring for 10 min at 135 °C.

Microscale Baking Test. The microscale baking test with 10 g of flour was carried out as described recently (*14*) using the micro rapid mix test (MRMT) for dough preparation. Crumb firmness and staling were also determined as described recently (*14*). The ingredients based on the flour weight were NaCl, 2%; sucrose, 1%; fresh bakers' yeast, 7%; and ascorbic acid, 20 mg/kg. Individual glycolipid fractions and classes were added in a concentration range between 0.1 and 0.8% (based on flour weight). Glycolipid fractions (10, 20, 40, 60, and 80 mg) or individual glycolipid classes (40 or 60 mg) were dissolved in a suitable solvent (0.13, 0.25, 0.50, 0.75, and 1.0 mL; *n*-hexane or chloroform) and applied to the flour as described recently (*14*). Triplicate determinations were carried out.

Microscale Rheology. Extension tests with surfactant-containing dough and gluten ("Kieffer-curves") isolated from surfactant-containing doughs were carried out using a Texture Analyzer TA-XT2 (Stable Microsystems, Godalming, U.K.) equipped with a Kieffer Dough & Gluten Extensibility Rig (*14*).

Synthesis of Monogalactosyl Dilinoleylglycerol (MGDG) and Monogalactosyl Monolinoleylglycerol (MGMG). These glycolipids were synthesized as reference compounds as described recently (*14*).

RESULTS AND DISCUSSION

In comparison to the quantities needed for analytical characterization (1−10 mg) the examination of the technological properties of individual polar lipids and lipid mixtures requires substantially higher amounts (500−1000 mg). On the one hand, fractionation and isolation methods have to provide enough material for functional testing, and, on the other hand, fractionation has to be efficient and thorough enough to provide sufficiently pure compounds and compound classes.

Lipid Composition of Lecithins. TLC analysis was used to analyze the lipid composition of the lecithin samples and the various fractions thereof (**Figure 1**). HPTLC plates with a concentration zone were applied successfully, with clearly better results than with normal TLC plates. Individual lipid classes were identified by direct comparison with commercially available reference compounds and the characteristic coloring of their spots after charring. A distinction was possible between the sterol glycolipids showing a violet coloring, the other glycolipids with a variety of pale reddish blue shades, and the phospholipids with yellow brownish shades. Soybean, rapeseed, and sunflower lecithins all contained the same major glycolipid classes, these being digalactosyl diacylglycerides (**1**) (**Figure 2**) sterol glucosides (**2**) (**Figure 3**), acylated sterol glucosides (**3**) (**Figure 3**), and cerebrosides (**4**) (**Figure 4**), varying only in their quantities. Besides the glycolipids, many other compound classes were additionally identified (**Figure 1**) in comparison with the ones listed in the literature (*20*, *21*). Monogalactosyl diacylglycerols and monogalactosyl monoacylglycerols were not detectable via TLC in the lecithin samples.

Prefractionation of Lecithins. The first step in the fractionation was to remove nonlipid constituents by means of the Folch method (*18*), the favorable choice for this sample scale. An advantage of the Folch wash became obvious during the subsequent fractionation because Folch-washed material was separated more efficiently during the batch procedure as compared to nontreated lecithin. To separate the purified lecithin sample into nonpolar and polar lipid fractions as well as separating the polar lipid fraction into raw glycolipid and phospholipid fractions, a batch procedure based on the work of de Stefanis and Ponte (*22*) was developed. This method is based on the adsorption of the lipids by silica gel, followed by the selective extraction of lipid classes with suitable solvents or solvent mixtures. The batch procedure can be seen as a preparative scale solid-phase extraction (SPE) method. It is ideal for a relatively rough fractionation of complex lipid mixtures because it is quick and relatively large quantities of lipid sample can be processed with great efficiency in terms of solvent consumption. Modified versions of this procedure have been successfully used to fractionate wheat flour lipids (*4*, *12*, *13*). We optimized this method until we gained a

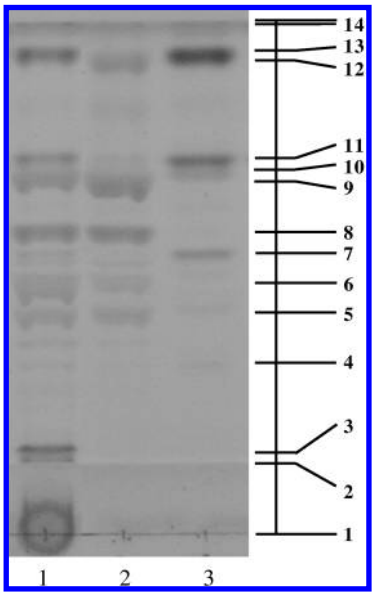

Figure 1. HPTLC separation of lipid mixtures from the batch procedure. Lanes: 1, soybean lecithin defatted; 2, phospholipid extract II (batch procedure I) from soybean lecithin; 3, glycolipid extract I (batch procedure I) from soybean lecithin. *y*-Axis: 1, application spot; 2, end of concentration zone; 3, nonlipid part (carbohydrates); 4, digalactosyl monoacylglycerides (tentative); 5, phosphatidic acid + lysophosphatidylcholine; 6, phosphatidylinositol + lysophosphatidylethanolamine + phosphatidylserine; 7, digalactosyl diacylglycerides; 8, phosphatidylcholine; 9, phosphatidylethanolamine + free fatty acids; 10, cerebrosides (monoglycosyl ceramides); 11, sterol glucosides; 12, *N*-acylphosphatidylethanolamine; 13, acylated sterol glucosides; 14, residual lipids (triacylglycerides, monoacylglycerides, diacylglycerides, sterol esters, free sterols).

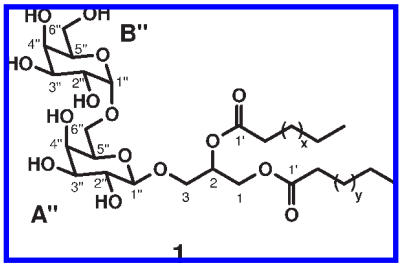

Figure 2. Structure of the glycolipid class digalactosyl diacylglycerides (**1**). x/y, alkyl or alkenyl.

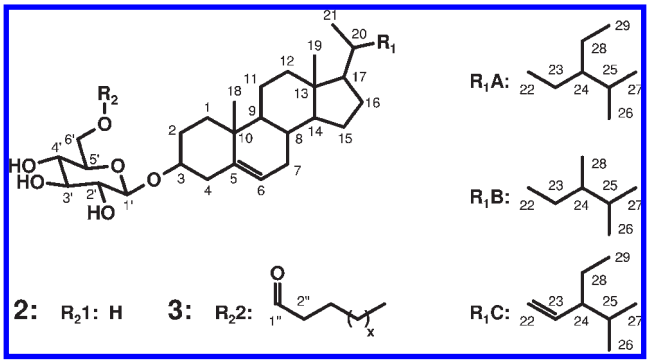

Figure 3. Structures of the glycolipid classes sterol glucosides (**2**) and acylated sterol glucosides (**3**). R_1, sterol moiety; R_2 (for **2**), various fatty acids; R_2 (for **3**), H; x, alkyl or alkenyl.

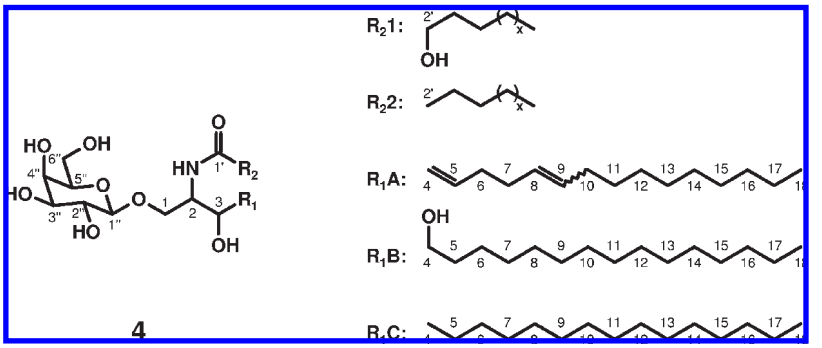

Figure 4. Structures of the glycolipid class cerebrosides (**4**). R_1A, 4,8-sphingadienine (d18:2−4t,8c/d18:2−4t,8t) or sphingosine (d18:1−4t); R_1B, phytosphingosine (t18:0/t20:0) or dehydrophytosphingosine (t18:1−8c/t); R_1C, dihydrosphingosine (d18:0/d20:0) or sphingosine (d18:1−8c/t); $R_2$1, 2-hydroxy-fatty acid; $R_2$2, saturated or unsaturated fatty acid (e.g., linoleic acid); x, alkyl or alkenyl.

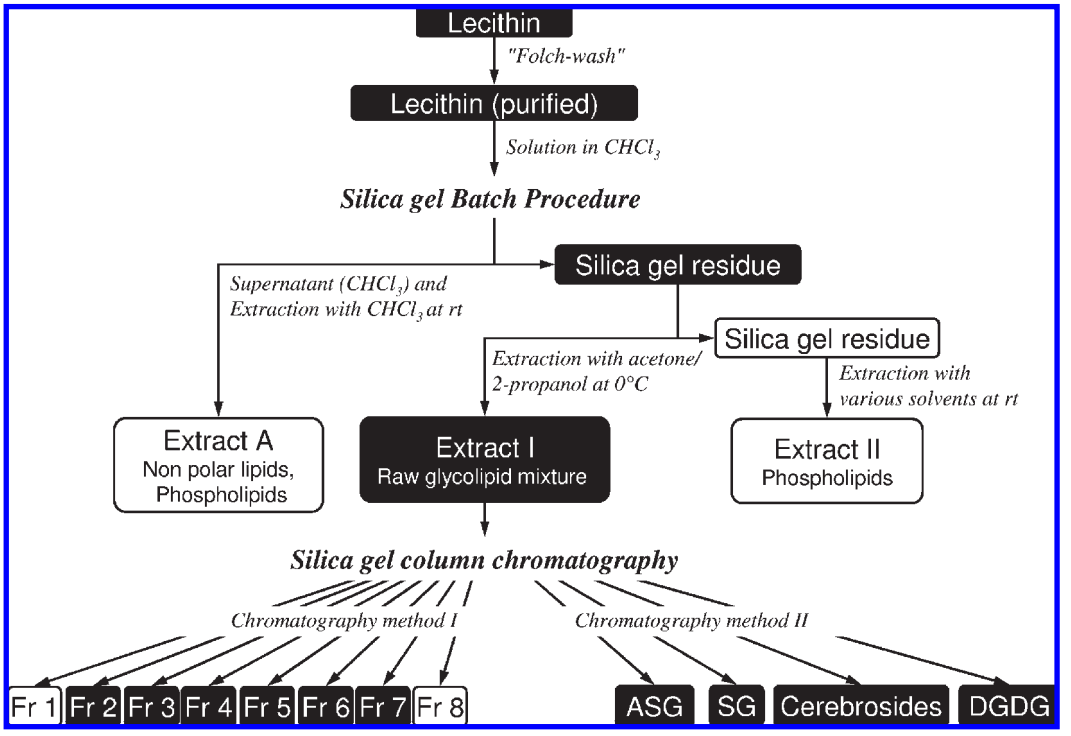

Figure 5. Flowchart for the isolation of glycolipids from lecithin showing the batch procedure and the column chromatography. Column chromatography method I: fraction 1, nonpolar lipids; fractions 2−7, main glycolipid fractions; fraction 8, phospholipids. Column chromatography method II: ASG, acylated sterol glucosides (**3**); SG, sterol glucosides (**2**); cerebrosides (**4**); DGDG, digalactosyl diacylglycerides (**1**); rt, room temperature.

good fractionation of the main lipid groups in the lecithin samples. The method was by far more effective and quicker than column chromatography for separating nonpolar and polar lipids or the phospholipid and the glycolipid groups. The batch procedure yielded a sample highly enriched with glycolipids, which contained almost the entire glycolipid part of the sample. A flowchart of the optimized batch procedure resulting in the raw glycolipid mixture is displayed in **Figure 5**. Defatted soybean lecithin, as the lecithin with the highest baking activity (*23*, *14*), was chosen as the starting material to develop and optimize the batch procedure. All other lecithins (soybean, rapeseed, and sunflower) were then successfully prefractionated using the optimized conditions. The efficiency of the batch procedure to separate lecithin into highly enriched glycolipid and phospholipid fractions is clearly visible on the TLC plate shown in **Figure 1** (soybean lecithin, defatted). The raw glycolipid fraction made up 13.0% (±0.3%) of the defatted soybean lecithin, 11.8% (±0.6%) of the defatted rapeseed lecithin, and 9.3% (±0.2%) of the crude sunflower lecithin.

Column Chromatography. Column chromatography was used to further fractionate the raw glycolipid mixtures from the batch procedure (**Figure 5**). In view of the amounts needed for the baking tests being in the range of 600−1000 mg per glycolipid class, column chromatography was the only plausible choice for the further fractionation. Silica gel 60 was used as stationary phase, which had already proven its great value while purifying the reference compounds (*14*) and fractionating entire lecithin samples (preliminary studies). Using a raw glycolipid mixture instead of the pure lecithin made column chromatography by far more effective, saving time and solvents. Two different chromatographic methods were developed to separate the raw glycolipid mixtures. Here the knowledge gained with the purification of the synthetic glycolipids via column chromatography (*14*) was a valuable basis. Method I with a low gel bed height provided fractions containing pure glycolipid classes or mixtures of different glycolipid classes. With method II and the higher gel bed height, the four major glycolipid classes were obtained in high purity.

Using method I the raw glycolipid mixture of defatted soybean lecithin was fractionated into eight main fractions, six of them being pure glycolipid fractions and two nonglycolipid fractions (**Figure 5**). The six main glycolipid fractions consisted either of a single compound class (fraction 1, glycolipid class **3**; fraction 3, glycolipid class **2**) or of a highly enriched compound class with further minor compound classes. The gravimetric analysis of these six main glycolipid fractions revealed an estimate for the quantitative glycolipid composition of defatted soybean lecithin. Fractions 1−6 represented 4.0, 0.5, 1.4, 1.1, 1.7, and 1.4% of the starting material, respectively. The first glycolipid fraction contained only glycolipid class **3**. The second one, being the exception and the smallest of all fractions, consisted of a mixture of compound classes, where none dominated quantitatively. Glycolipid class **2** made up the third fraction, whereas the fourth fraction consisted mainly of **4**, also known as monoglucosylceramides. The fifth fraction was highly enriched with **1** with a small remaining part of **4**. Fraction six was also dominated by one compound class, which has not been identified yet, but is most likely to be the digalactosyl monoacylglycerides. These six major glycolipid fractions form the true glycolipid part of the examined defatted soybean lecithin and made up 10% of the total lecithin, after the remaining non-glycolipid part had been removed from the raw glycolipid mixture by column chromatography. Usually the glycolipid part in defatted soybean lecithin is stated in the literature as being approximately 10% (*24*), with a relatively wide span of 6.5−11% (*25*).

The fractionation of the raw glycolipid mixtures from soybean, rapeseed, and sunflower lecithins through method II made it possible to obtain the four major glycolipid classes, which together accounted for approximately 85% of the total glycolipid part in the lecithins, with the remaining 15% being a huge variety of different classes, in sufficient quantities (180−1100 mg) for analytical characterization and technological testing.

Analytical Characterization of Glycolipid Classes. The pure glycolipid classes from the three lecithin varieties, obtained through chromatography method II, were individually identified and characterized by means of TLC, one- and two-dimensional NMR spectroscopy, ESI-MS, and fatty acid analysis (GC-MS). Structural differences between the same glycolipid class derived from different lecithin varieties were mainly found for the fatty acid composition. A comparison of the different fatty acid compositions of all individual glycolipid classes can be found in **Table 1**. Major differences were found for the fatty acid composition not only between the different glycolipid classes but also between the same glycolipid class from different lecithins and the total fatty acid composition of the entire lecithin. The other structural elements of the individual glycolipid classes that were the same for the different lecithin types are exemplarily shown for defatted soybean lecithin (NMR and MS data).

*Digalactosyl Diacylglycerides (**1**).* The respective NMR data are given in direct comparison to the two synthetic glycolipids in **Table 2**. The ^{1}H and ^{13}C NMR signals were assigned according to ^{1}H, ^{1}H COSY, ^{1}H ^{13}C correlation experiments (HMQC and HMBC). The diacyl structure and the glycosylation of the glycerol moiety were established through characteristic resonances of the proton signals and through HMBC experiments. The HMQC and ^{13}C spectra showed that the saccharide portion consisted of two carbohydrate moieties, both identified as galactose through characteristic resonances of the proton signals. The coupling constant of H-1″ (A) ($J_{1,2}$ = 7.9 Hz) established that the galactopyranose sugar was linked to the glycerol moiety via a β-linkage. Finally, the HMBC spectrum revealed the crucial correlation between the two galactose moieties, and the coupling constant of H-1″ (B) ($J_{1,2}$ = 3.5 Hz) established an α-1,6-glycosidic linkage. Within the class of **1**, the individual components differed only with respect to the length of the acyl chains of the fatty acids and their degree of unsaturation. The fatty acid analysis carried out for each individual glycolipid class showed only slight differences in the fatty acid composition between the classes from soybean and rapeseed lecithin. Major differences in the composition between the class from sunflower lecithin and the other two lecithin varieties were found for linoleic and linolenic acid (**Table 1**). Analysis of the MS spectra showed the following possible fatty acid combinations: MS (ESI^+), C18:2/16:0 (MW 917), *m/z* 940 (100%, $[M + Na]^+$); C18:1/16:0 (MW 919), *m/z* 942 (79%, $[M + Na]^+$); C18:2/18:2 (MW 941), *m/z* 964 (48%, $[M + Na]^+$); C 18:2/18:1 (MW 943): *m/z* 966 (44%, $[M + Na]^+$); C18:1/18:1 (MW 945), *m/z* 968 (33%, $[M + Na]^+$); C18:2/16:0 (MW 917), *m/z* 1857 (10%, $[M + Na]^+$). Similar fatty acid pairings for **1** isolated from oats have been described in the literature (*26*). The determined chemical structures for this glycolipid class are shown in **Figure 2**.

*Sterol Glucosides (**2**) and Acylated Sterol Glucosides (**3**).* The respective NMR data are given in **Table 3**. The two compound classes differ solely in an additional fatty acid moiety (acylated sterol glucosides) connected to the C-6 position of the carbohydrate moiety. This connection, as well as the connection of the carbohydrate moiety to the sterol backbone, was shown with HMBC experiments. Additionally, they were detectable by means of characteristic resonances of the proton signals. The carbohydrate moiety was identified through characteristic resonances of the proton signals as a glucose moiety. The coupling constant of H-1′ ($J_{1,2}$ = 8.0 Hz), established that the glucopyranose moiety was linked to the sterol moiety via a β-linkage. Within the class of **3**, the individual components differed with respect to the length of the acyl chains of the fatty acid and its degree of unsaturation. Different fatty acid compositions for **3** have been reported. Lepage (*27*) found 33.7% C16:0, 0.9% C16:1, 7.0% C18:0, 8.8% C18:1, 47.4% C18:2, and 2.2% C18:3 in **3** isolated from soybean lecithin, whereas Milkova et al. (*28*) found 52.2% C16:0, 7.5% C18:0, 17.9% C18:1, and 22.4% C18:2. The results of our analyses of **3** from soybean lecithin (**Table 1**) were similar to those reported in ref (*27*). The fatty acid analysis carried out for each individual glycolipid class showed only slight

Table 1. Fatty Acid Composition of the Lecithins and the Four Major Pure Glycolipid Classes from the Lecithins As Obtained with Chromatography Method II[a]

	% of total peak area of fatty acids					
sample	C16:0	C18:0	C18:1	C18:2	C18:3	C16:0−2OH
soybean lecithin defatted	26.9	1.7	6.1	64.0	1.3	nd
SD ± %[b]	1.1	0.0	1.8	0.9	0.2	
ASG So	39.5	2.9	8.6	47.7	1.4	nd
cerebrosides So	9.3	0.7	1.8	19.3	2.6	66.3
DGDG So	15.1	1.7	7.3	58.8	17.1	nd
rapeseed lecithin defatted	17.4	0.5	57.3	23.6	1.3	nd
SD ± %	1.9	0.3	0.4	2.0	0.6	
ASG Ra	60.8	3.7	15.2	19.5	0.9	nd
cerebrosides Ra	9.0	0.8	15.4	38.4	8.6	27.8
DGDG Ra	9.0	1.0	8.8	59.0	22.2	nd
sunflower lecithin crude	11.0	1.2	11.5	76.4	nd	nd
SD ± %	1.6	0.1	0.8	0.9		
ASG Sn	43.8	3.2	8.3	44.6	nd	nd
cerebrosides Sn	12.4	1.4	3.6	43.9	0.8	37.9
DGDG Sn	5.8	0.7	4.3	88.9	0.2	nd

[a] ASG, acylated sterol glucosides (**3**); SG, sterol glucosides (**2**); DGDG, digalactosyl diacylglycerides (**1**); cerebrosides (**4**); So, soybean lecithin; Ra, rapeseed lecithin; Sn, sunflower lecithin; nd, not detected. [b] Standard deviation (n = 3 for lecithin samples; n = 1 for glycolipid classes; relative standard deviation for injection reproducibility was <8%).

Table 2. ^{1}H, HMQC, and ^{13}C Data of the Isolated Lipid Class Digalactosyl Diacylglycerides (**1**, DGDG) and the Synthetic Monogalactosyl Diacylglycerol (MGDG) and Monogalactosyl Monoacylglycerol (MGMG)

	chemical shift (ppm), multiplicity, coupling constant (Hz)								
carbon atom[a]	DGDG ($CDCl_3$)^{1}H	MGDG ($CDCl_3$)^{1}H	MGMG (methanol-d_4)^{1}H	DGDG ($CDCl_3$) HMQC	DGDG ($CDCl_3$)^{13}C	MGDG ($CDCl_3$) HMQC	MGDG ($CDCl_3$)^{13}C	MGMG (methanol-d_4)HMQC	MGMG (methanol-d_4)^{13}C
glycerol moiety									
1a	4.30, dd, 11.7, 6.6	4.21 dd, 11.7, 6.3	4.13, dd, 11.3, 5.8	67.5	67.8	63.2	61.9	65.7	65.6
1b	4.40, dd, 12.1, 3.1	4.40, dd, 11.7, 3.2	4.17, dd, 11.3, 4.6						
2	5.25, m	5.32, m	3.98, m	70.6	70.6	70.8	69.2	68.7	69.3
3a	3.72	3.75, dd, 11.0, 6.3	3.66, dd, 10.7, 4.3	63.3	63.3	69.0	67.4	71.0	71.6
3b	3.91, dd, 11.0, 5.5	3.92, dd, 11.0, 5.5	3.91, dd, 10.6, 5.4						
carbohydrate moiety A″									
1″	4.20, d, 7.0	4.29, d, 7.4	4.24, d, 7.4	104.3	104.3	104.7	103.0	104.5	104.3
2″	3.50	3.66, dd	3.56, dd	71.6	72.0	72.4	70.7	71.6	70.9
3″	3.49	3.60, dd	3.47, dd	73.5	74.9	74.1	72.5	74.0	73.9
4″	3.91	4.03, d, 2.7	3.85, d, 3.1	68.6	68.7	70.0	68.5	69.4	68.5
5″	3.68	3.56, t	3.53, t	73.4	73.8	75.0	73.6	75.9	75.7
6″a	3.63, dd, 11.7, 5.8	3.87, dd, 11.7, 3.1	3.72, dd, 11.3, 5.4	66.8	66.3	63.4	61.7	61.5	61.5
6″b	3.90	3.98, dd, 12.1, 5.9	3.78, dd, 11.3, 6.6						
carbohydrate moiety B″									
1″	4.90, d, 3.5			99.7	100.2				
2″	3.78, dd			69.4	69.9				
3″	3.71			70.5	70.3				
4″	3.94			70.1	70.1				
5″	3.83			71.3	71.4				
6″	3,72			62.0	63.0				
acyl moiety									
1′				174.7	173.9/174.1	174.7	172.7/172.5	174.8	174.4
2′	2.31, m	2.30, m	2.35, t	35.1	34.7	35.5	33.3	34.0	33.9
3′	1.60, m	1.62, m	1.62, m	25.7	26.0	25.2	23.8	24.9	25.0
4′−7′	1.32, m	1.25, m	1.31, m	29.9	29.4	29.6	28.6	29.2	29.6
8′	2.05, m	2.04, m	2.04, m	27.1	27.5	27.5	26.2	27.2	27.1
9′, 13′	5.34, m	5.32, m	5.35, m	130.3	130.5	130.6	129.0/129.2	129.9	129.9/129.9
11′	2.78, t	2.77, t	2.78, t	25.3	25.3	26.0	24.6	25.6	25.5
10′, 12′	5.34, m	5.31, m	5.35, m	128.4	128.3	128.9	126.9/127.1	128.0	128.0/128.1
14′	2.05, m	2.04, m	2.04, m	27.1	27.5	27.5	26.2	27.2	27.1
$CH_2-(\underline{CH_2})-CH_3$	1.32, m	1.25, m	1.31, m	29.9	29.4	29.6	28.6	29.2	29.6
$\underline{CH_2}-\overline{CH_2}-CH_3$	1.32, m	1.25, m	1.31, m	31.3	31.9	31.5	30.5	31.5	31.6
$\overline{CH_2}-\underline{CH_2}-CH_3$	1.32, m	1.25, m	1.31, m	23.0	23.0	23.0	21.6	22.5	22.6
CH_3	0.90, m	0.89, m	0.91, m	14.1	14.5	14.3	13.1	13.2	13.4

[a] Hydrogen atoms attached to carbon atoms as designated in **Figure 4**.

differences between soybean and sunflower. Major differences in the fatty acid composition between the glycolipid classes from rapeseed lecithin and the glycolipids from soybean and sunflower lecithins were found for C16:0, C18:2, and C18:3 (**Table 1**). The sterol backbone of the two compound classes **3** and **2** was a mixture of various phytosterol moieties. Of the most common phytosterols campesterol, β-sitosterol, stigmasterol, and avenasterol, the first three have been shown to be present in **3** from soybean lecithin; however, considerable differences have been found in **3** and **2** from different sources. Lepage (*27*) reported 58.7% β-sitosterol, 18.9% stigmasterol, and 22.4% sterol A (not identified; probably campesterol) in **3**, and Milkova et al. (*28*) found 80% β-sitosterol, 18% campesterol, and 2% stigmasterol in **2** from Bulgarian soybean lecithin. The results of the NMR experiments of the isolated **3** and **2** compound classes from defatted soybean lecithin allowed the following conclusions with respect to the sterol composition. The NMR data were, as far as conclusive, similar for the three lecithin varieties. β-Sitosterol was the major sterol moiety. Campesterol, with one methylene group less, was difficult to distinguish from β-sitosterol. The additional carbon atom (**Table 3**, C-atom 29 in **Figure 5**) was found in the ^{13}C and HMBC spectra. The change in the chemical shifts of the neighboring atoms, caused by the missing methylene group, was observed only in the ^{13}C spectra of **3**. Stigmasterol, however, was clearly recognizable in the ^{1}H spectra due to the two characteristic H-atoms at the double bond (**Table 3**, proton at C-atom S22 and S23: 5.00 and 5.13 ppm), as well as the C-atom S24 (51.4 ppm) in the HMQC spectra of **3** and **2**. A quantitative ranking order was clearly visible with stigmasterol and campesterol, in second and third places, with clearly weaker signals in comparison to the overwhelming β-sitosterol. The analysis of the MS spectra of compound class **3** was very difficult due to the huge variety

Table 3. ^{1}H, HMQC, and ^{13}C Data of the Lipid Classes Acylated Sterol Glucosides (**3**, ASG) and Sterol Glucosides (**2**, SG)

	chemical shift (ppm), multiplicity, coupling constant (Hz)					
carbon atom[a]	ASG ($CDCl_3$)^{1}H	SG (DMSO-d_6)^{1}H	ASG HMQC	SG HMQC	ASG^{13}C	SG^{13}C
carbohydrate moiety						
1′	4.37, d, 7.8	4.34, d, 7.8	101.2	102.0	101.3	101.1
2′	3.35	3.14	73.6	74.1	73.5	73.6
3′	3.54	3.34	76.6	77.5	76.1	76.9
4′	3.36	3.29	70.2	71.1	70.3	70.6
5′	3.46	3.20	74.2	76.7	73.8	76.2
6′a	4.31	3.63, dd, 11.7, 5.9	63.3	62.3	63.5	61.9
6′b	4.37	3.75, dd, 11.3, 5.9				
acyl moiety						
1″			174.8		174.4	
2″	2.34		34.0		34.1	
3″	1.60		24.6		25.0	
4″−7″	1.28		29.3		29.6	
8″	2.10		26.8		27.3	
9″, 13″	5.31		130.1		130.0	
11″	2.77		25.4		25.7	
10″, 12″	5.31		128.9		128.1	
14″	2.10		26.8		27.3	
$CH_2-(CH_2)-CH_3$	1.28		29.3		29.6	
$CH_2-CH_2-CH_3$	1.28		31.3		31.5	
$CH_2-CH_2-CH_3$	1.28		22.5		22.6	
CH_3	0.92		13.9		14.1	
sterol moiety[b]						
1a/b	1.84/1.06	1.77/1.00	37.3	37.2	37.3	37.1
2a/b	1.93/1.55	1.83/1.51	29.5	29.6	29.8	29.5
3	3.51	3.48	79.6	78.6	79.7	78.6
4a/b	2.36/2.28	2.33/2.18	38.7	38.8	39.0	38.6
5					140.4	140.3
6	5.37	5.26, m	122.2	121.9	122.1	121.4
7a/b	2.01/1.42	1.88/1.43	32.2	31.5	32.0	31.5
8	1.49	1.48	32.2	31.5	32.0	31.5
9	0.92	0.83	50.1	49.9	50.2	49.9
10					36.7	36.5
11a/b	1.44/1.37	1.49/1.45	21.0	21.0	21.1	20.8
12a/b	2.01/1.17	1.93/1.09	39.6	39.4	39.8	39.3
13					42.4	42.1
14	0.98	0.91	56.6	56.4	56.8	56.5
15a/b	1.48/1.07	1.50/0.98	24.6	24.1	24.3	24.1
16a/b	1.74/1.24	1.74/1.23	27.5	27.6	28.3	28.0
17	1.09	1.05	56.0	55.7	56.2	55.7
18	0.67	0.68/0.57	11.7	11.6	11.8	11.7
19	1.00	0.93	19.2	19.2	19.4	19.2
20 (si/ca)	1.36	1.28	35.9	35.7	36.2	35.8
20 (st)					41.1	
21(si/ca)	0.93	0.87	18.5	18.6	18.8	18.7
21(st)					21.0	
22a/b (si/ca)	1.26/0.97	1.26/0.97	33.7	33.7	34.0	33.7
22 (st)	5.14, dd, 15.3, 8.6	5.13, dd, 15.2, 8.6	130.4		138.3	
23 (si)	1.17	1.08	25.9	25.8	26.2	25.8
23 (st)	5.00, dd, 14.5, 8.6	5.00, dd, 15.3, 8.6	130.4		132.0	
23 (ca)					26.0	
24 (si)	0.94	0.87	45.9	45.7	45.9	45.5
24 (st)	1.46	1.48	51.5	51.2	51.3	
24 (ca)					42.2	
25 (si)	1.31	1.24	29.3	29.5	29.4	29.0
25 (st)					32.0	
25 (ca)					29.3	
26 (si/ca)	0.84	0.78	19.4	19.4	19.8	19.7
26 (st)					21.3	
27 (si/ca)	0.82	0.76	18.8	19.0	19.1	19.0
27 (st)					19.4	
28a/b (si)	1.28/1.05	1.18/0.97	22.6	22.5	23.1	22.8
28 (st)					25.4	
28 (ca)					21.3	
29 (si)	0.85	0.81/0.74	12.9	12.2	11.9	11.9
29 (st)					12.2	

[a] Hydrogen atoms attached to carbon atoms as designated in **Figure 5**. [b] Sterol moiety: si, β-sitosterol; st, stigmasterol;ca, campesterol.

of possible fatty acid and sterol moiety combinations. The MS data confirmed the results of the NMR measurements. The major sterols present in **3** and **2** were β-sitosterol, campesterol, and stigmasterol. **2** and **3** were nearly exclusively found as $[2M + Na]^+$ ions. The monomeric Na adducts $[M + Na]^+$ were also visible, but considerably less intense. Three possible structures of **2** and **3** could not be identified by MS. MS (ESI^+): sterol glucoside with β-sitosterol (MW 577), *m*/*z* 1177 (100%, $[2M + Na]^+$); sterol glucoside with stigmasterol (MW 575), m/*z* 1173 (65%, $[2M + Na]^+$); unknown, *m*/*z* 1163 (65%); sterol glucoside with campesterol (MW 563), *m*/*z* 1149 (15%, $[2M + Na]^+$); unknown, *m*/*z* 1193 (10%); unknown, *m*/*z* 1207 (5%). In the MS spectra of **3** the combination of all possible fatty acids, found in the fatty acid analysis, and the three major sterol varieties as well as the three unidentified (known from **2**) were found. The chemical structures of **2** and **3** derived from the analytical data are shown in **Figure 3**.

*Cerebrosides (**4**)*. The NMR data of this glycolipid class are given in **Table 4**. This compound class showed an even bigger diversity than **3**, because the various fatty acids can be combined with a great variety of sphingoid moieties. Structural characteristics of some sphingoid moieties were identified with NMR experiments. A quantitative ranking based on the chemical shift intensity was established. The most dominating sphingoid backbone was the sphingadienine (d18:2−4t,8c or d18:2−4t,8t), a dihydroxy representative with two double bonds. The same structural characteristics were also present separately in the two sphingosine varieties (d18:1−4t or d18:1−8c/t), which were not distinguishable from the others. These were followed by the dehydrophytosphingosine (t18:1−8c/t) and the phytosphingosine (t18:0 or t20:0), representatives of the trihydroxy group. The group with the structural characteristics of the dihydrosphingosine (d18:0 or d20:0) were found with the least intensities. Through characteristic proton signals it was established that the carbohydrate moiety was glucose. The linkages of the acyl moiety and the glycosyl moiety to the sphingosine moiety were established through characteristic resonances of the proton signals and through HMBC experiments. The coupling constant of H-1″ ($J_{1,2}$ = 7.1 Hz) established that the glucopyranose carbohydrate moiety was linked to the sphingosine moiety via a β-linkage. The fatty acid analysis showed that this glycolipid class had a high percentage of 2-hydroxyhexadecanoic acid in all lecithin varieties. Further major differences were also found for all other fatty acids of this class between the three lecithin varieties. The determined structural formula of this compound class is displayed in **Figure 4**.

Baking Performance of Glycolipids. A microscale baking test with 10 g of flour using a straight dough procedure was used to study the technological properties of the glycolipid classes. Additives were dissolved in *n*-hexane or chloroform and added to the flour, and the organic solvent was evaporated under ambient conditions. This application method was shown to have no significant influence on the baking result (*14*). Additionally, it was shown that the synthetic glycolipid monogalactosyl monolinoleylglycerol (MGMG) showed essentially the same baking results whether added as a chloroform solution or as a watery suspension (**Table 5**). This indicates that the baking results found for the glycolipids with this application method (organic solvent) are the same when applied as a watery suspension. The glycolipid fractions (method I) were applied in concentrations of 0.1−0.8% according to their amount available. The pure glycolipid classes (method II) were applied in the concentration that had been found to gain the maximum bread volume increase of the corresponding glycolipid fraction (method I). The two synthetic glycolipids were applied in concentrations of 0.1−0.8% (based on flour weight). All results were compared to reference breads obtained by using classical surfactants such as lecithins, DATEM, monoacylglycerides, SSL, and the two synthetic glycolipids as noted in Selmair and Koehler (*14*). In addition to the bread volume, the influence of the additive on the crumb firmness was evaluated. Individual sensory tests showed no influence of the glycolipids on the aroma of the freshly baked breads. To compensate for dough losses during bread production, the bread volumes were based on a dough weight of 10 g as determined before the second fermentation. The increases in bread volume with additive were expressed in relation to the respective mean value of the control breads in the individual test series, thus taking into account possible climatic or technical fluctuations.

Effect on Bread Volume (Glycolipid Fractions Derived from Method I). Microscale baking test data are shown in **Table 5**. The nonpolar lipid fraction clearly had a negative effect on the baking performance of the flour with increasing concentration (0.1−0.6%). Not only was the loaf volume negatively affected but also dough handling, bread shape, and crumb structure were as well. In contrast to this, the first glycolipid fraction (**3**) showed by far the best bread volume increase of all fractions and surfactants at low concentrations of 0.1−0.2%. A 0.4% DATEM concentration would be required to get approximately the same volume increase (**Table 5**). The second glycolipid fraction (monogalactosyl diacylglycerides + minor components) showed only little baking activity up to a level of 0.4%. Higher concentrations were not investigated because of the limited amount of material. The baking performance in the examined concentration range was comparable to that of the synthetic reference compound monogalactosyl dilinoleylglycerol. The third glycolipid fraction (**2**) was only slightly soluble in *n*-hexane. Therefore, chloroform was used for solubilization. Considerably higher amounts of **2** compared to **3** were required to get a bread volume comparable to that of **3** and DATEM (**Table 5**). The fourth glycolipid fraction (**4**) was soluble in chloroform and gave an optimal bread volume at a concentration of 0.4%, with the baking activity in the concentration range of 0.1−0.4% being one of the best. The fifth glycolipid fraction, mainly containing **1** and a small amount of **4**, was soluble in hexane. It displayed a clearly lower baking activity in the concentration range of 0.1−0.2% than **4** and **3**. However, the volume increases gained from the additive amount of 0.4% upward rose continuously to the highest maximal volume increase of 61.3% at 0.6% measured in all our test series. Similar values in this concentration range had only been found with the synthetic reference glycolipid monogalactosyl monolinoleylglycerol (**Table 5**). This showed that glyceroglycolipids with two carbohydrate moieties have to contain two fatty acid residues connected to the glycerol moiety to attain the same baking activity as glyceroglycolipids with one carbohydrate moiety and one fatty acid. This demonstrates the importance of the right ratio between hydrophilic and lipophilic elements in the molecular structure of each individual surfactant for its baking potential. The sixth glycolipid fraction yielded a maximal volume increase of 26.5% at the 0.4% level. It contained unknown glycolipids, possibly digalactosyl monoacylglycerides, as the major compound class. The baking activity in this concentration range in comparison to the fraction with **1** was clearly lower, showing the less positive effect of only one fatty acid residue in a galactolipid with a disaccharide unit.

Effect on Bread Volume (Glycolipid Fractions Derived from Method II). Microscale baking test data are shown in **Table 6**. All glycolipid fractions from soybean lecithin (method I) had shown a positive baking potential (**Table 5**). Therefore, the four major glycolipid classes from three different lecithin varieties (soybean, rapeseed, and sunflower) were isolated by method II,

Table 4. ^{1}H, HMQC, and ^{13}C Data of the Lipid Class Cerebrosides (**4**)

	chemical shift (ppm), multiplicity, coupling constant (Hz)					
carbon atom[a]	cerebroside A ^{1}H (DMSO-d_6)	cerebroside A HMQC (DMSO-d_6)	cerebroside B ^{1}H (DMSO-d_6)	cerebroside B HMQC (DMSO-d_6)	cerebroside C ^{1}H (DMSO-d_6)	cerebroside C HMQC (DMSO-d_6)
sphingosine moiety[b]						
1a	3.51, dd, 10.2, 3.5	69.6	3.65	69.7	3.79	69.7
1b	3.91, dd, 10.2, 5.5		3.79			
2	3.79	53.6	4.07	50.7	3.44	54.1
3	3.97, dd, 12.5, 6.5	71.3	3.36	75.0	3.14	75.0
4	5.41	132.7	3.33	71.6	1.49, 1.38	32.5
5	5.57	131.7	1.49, 1.38	32.5	1.15−1.27	26.0−32.0
6	2.00	32.7	1.15−1.27	26.0−32.0		
7	1.96	27.4				
8	5.37	130.4				
9	5.28	130.7				
10	1.91	32.7				
11−15	1.15−1.27	26.0−32.0				
16	1.15−1.27	32.2	1.15−1.27	32.2	1.15−1.27	32.2
17	1.15−1.29	23.0	1.15−1.29	23.0	1.15−1.29	23.0
18	0.84 t	14.6	0.84 t	14.6	0.84 t	14.6
NH	7.37, d		7.52, d		7.47, d	
carbohydrate moiety						
1″	4.08, d, 7.1	104.4	4.08, d, 7.1	104.4	4.08, d, 7.1	104.4
2″	2.95	74.2	2.95	74.2	2.95	74.2
3″	3.14	76.9	3.14	76.9	3.14	76.9
4″	3.06	70.8	3.06	70.8	3.06	70.8
5″	3.08	77.6	3.08	77.6	3.08	77.6
6″a	3.45	61.9	3.45	61.9	3.45	61.9
6″b	3.68, dd, 11.7, 5.9		3.68, dd, 11.7, 5.9		3.68, dd, 11.7, 5.9	
acyl moiety	acyl moiety 1	acyl moiety 1	acyl moiety 2	acyl moiety 2	acyl moiety 3	acyl moiety 3
1′		174.6		174.1		174.1
2′	3.78 dd	71.8	2.21	34.2	2.21	34.2
3′	1.49, 1.38	35.7	1.48	25.8	1.48	25.8
4′	1.29	25.4	1.15−1.27	26.0−32.0	1.15−1.27	28.0−32.0
5′−7′	1.15−1.27	26.0−32.0				
8′					1.95	27.4
9′, 13′					5.28	130.7
11′					2.70	25.3
10′, 12′					5.28	130.4
14′					1.95	27.4
$CH_2-(\underline{CH_2})-CH_3$					1.15−1.27	2.0.−32.0
$\underline{CH_2}-CH_2-CH_3$	1.15−1.27	32.2	1.32 m	32.2	1.32 m	32.2
$CH_2-\underline{CH_2}-CH_3$	1.15−1.29	23.0	1.32 m	23.0	1.32 m	23.0
CH_3	0.84 t	14.6	0.90 t	14.6	0.90 t	14.6

[a] Hydrogen atoms attached to carbon atoms as designated in **Figure 4**. [b] Sphingosine moiety: the identified cerebroside structures can overlap with similar structures given in brackets. Cerebroside A, 4,8-sphingadienine (d18:2−4t,8c/d18:2−4t,8t) [sphingosine (d18:1−4t)]; cerebroside B, phytosphingosine (t18:0/t20:0) [dehydrophytosphingosine (t18:1−8c/t)]; cerebroside C, dihydrosphingosine (d18:0/d20:0) [sphingosine (d18:1−8c/t)]; acyl moiety: 1, 2-hydroxy-fatty acids; 2, saturated fatty acids; 3, unsaturated fatty acids (e.g., linoleic acid). The combinations of the sphingosine moiety and the acyl moiety are interchangeable.

characterized and subjected to individual baking tests. The results gained hereby confirmed the excellent baking potential of the individual glycolipid classes in comparison to the classical surfactants. The maximal bread volume increases for the individual glycolipid classes **3**, **2**, and **4** from soybean lecithin were slightly higher than for the corresponding fractions (method I), due to higher purity. Only the glycolipid class **1** showed a slightly lower volume increase in comparison to the fifth fraction (method I, **Table 5**), probably caused by the cerebroside impurity in the fraction possibly resulting from a synergistic effect between **1** and **4**. There were no significant differences in the baking activities between the individual glycolipid classes isolated from the different lecithin varieties for the glycolipid classes **2**, **4**, and **1**, although there were slight differences visible between the glycolipid classes from different lecithin varieties with the glycolipid classes **4** and **1** (**Tables 5** and **6**). For the glycolipid class **3**, however, there was a significant difference, clearly higher, between the class isolated from rapeseed and the two others. These differences in baking activity between the same glycolipid class from different origins were correlated to their differences in fatty acid composition. Increasing proportions of saturated fatty acids or 2-hydroxy fatty acids (**4**) improved the baking performance. Comparison of the results gained for the two synthetic glycolipids with those published recently (*14*) showed that the microscale baking test used displayed an excellent long-term repeatability. No significant variations were detectable.

Table 5. Microscale Baking Test with 10 g of Flour (cv. Tommi) with Addition of Fractions Obtained by Column Chromatography (Method I) of the Raw Glycolipid Mixture from Defatted Soybean Lecithin as well as Commercial and Synthetic Surfactants: Change of Bread Volume As Affected by Concentration of Lipid[a]

		change of bread volume (BV, %) and standard deviation (SD, ± %) at concentration of									
		0.1%[b]		0.2%[b]		0.4%[b]		0.6%[b]		0.8%[b]	
additive	solution in	BV	SD	BV	SD	BV	SD	BV	SD	BV	SD
fraction 1: nonpolar lipids fraction	hexane	−3.3	1.5	−8.3	1.1	−13.1	1.7	−15.3	0.2	−[c]	−
fraction 2: ASG	hexane	14.7	1.0	28.4	1.0	35.2	1.2	34.8	0.8	34.5	0.8
fraction 3: MGDG + minor components	hexane	1.5	1.8	1.1	2.2	18.4	0.8	−	−	−	−
fraction 4: SG	chloroform	0.4	1.4	0.5	0.9	20.2	0.8	31.3	1.1	32.7	1.5
fraction 5: cerebrosides	chloroform	1.0	1.9	13.9	1.0	36.7	1.1	36.4	1.1	−	−
fraction 6: DGDG + cerebrosides	hexane	1.6	1.7	5.9	0.5	48.1	1.5	61.3	1.0	53.2	1.5
fraction 7: DGMG + minor components	hexane	−5.1	0.8	4.2	0.8	25.6	0.7	−	−	−	−
DATEM	chloroform	−0.7	1.2	4.4	1.1	31.5	1.4	38.9	1.4	36.2	1.1
SSL	water	−	−	−0.3	1.2	18.4	1.4	20.7	1.8	17.6	0.8
monoacylglycerides	tetrahydrofuran	−	−	8.5	0.3	39.1	0.7	45.7	0.7	40.6	0.5
MGDG (synthetic)	hexane	−2.9	0.9	−2.7	1.8	22.1	2.0	37.0	1.2	34.5	1.1
MGMG (synthetic)	chloroform	3.1	0.4	9.2	1.9	41.4	2.4	53.2	2.3	51.8	1.1
MGMG (synthetic)	water	6.2	1.6	8.9	2.6	37.5	2.4	49.0	0.9	−	−

[a] ASG, acylated sterol glucosides (**3**); MGDG, monogalactosyl diacylglycerides; SG, sterol glucosides (**2**); cerebrosides (**4**); DGDG, digalactosyl diacylglycerides (**1**); DGMG, digalactosyl monoacylglycerides; DATEM, diacetyltartaric esters of mono- and diacylglycerides; SSL, sodium stearoyl-2-lactylate; MG, monoacylglycerides; MGMG, monogalactosyl monolinoleylglycerol. [b] Concentration of additive based on 10 g of flour (= 100%). [c] −, not tested.

Table 6. Microscale Baking Test with 10 g of Flour (cv. Tommi) with Addition of Glycolipid Classes Obtained by Column Chromatography (Method II) from Different Lecithin Varieties: Change of Bread Volume As Affected by Concentration of Lipid

	change of bread volume (BV, %) and standard deviation (SD, ± %) at concentration of							
	0.4%[a] acylated sterol glucosides		0.6%[a] sterol glucosides		0.4%[a] cerebrosides		0.6%[a] digalactosyl diacylglycerides	
additive	BV	SD	BV	SD	BV	SD	BV	SD
soybean lecithin, defatted	40.3	0.5	37.7	1.6	42.2	1.9	55.8	1.9
rapeseed lecithin, defatted	48.5	0.4	37.0	0.3	41.5	1.7	54.9	1.0
sunflower lecithin, crude	43.2	1.7	37.5	1.7	40.3	1.4	53.8	1.1
solution in	hexane		chloroform		chloroform		hexane	

[a] Concentration of additive based on 10 g of flour (= 100%).

Table 7. Hydrophilic−Lipophilic Balance (HLB) Values of Polar Lipids Calculated According to the Method of Griffin (*30*) As Affected by the Fatty Acid Residue

		HLB values for acyl residue						
lipid class	variant	none	C16:0	C18:0	C18:1	C18:2	C18:3	C16:0−2OH
DGDG			10.5	9.9	9.9	10.0	10.0	
cerebroside	R_1A[b]		8.0	7.7	7.7	7.7	7.8	8.6
cerebroside	R_1B[b]		8.6	8.3	8.3	8.3	8.4	9.2
cerebroside	R_1C[b]		7.9	7.6	7.7	7.7	7.7	8.6
SG	β-sitosterol	6.2						
SG	campesterol	6.4						
SG	stigmasterol	6.2						
ASG	β-sitosterol		5.1	4.9	4.9	4.9	4.9	
ASG	campesterol		5.1	5.0	5.0	5.0	5.0	
ASG	stigmasterol		5.1	4.9	4.9	4.9	4.9	

[a] DGDG, digalactosyl diacylglycerides (**1**); SG, sterol glucosides (**2**); ASG, acylated sterol glucosides (**3**); cerebrosides (**4**). [b] Structures given in **Figure 4**.

HLB Value and Baking Activity. As described recently (*14*), the HLB values for the individual glycolipid classes in lecithins were calculated by using the methods proposed by Griffin (*30*) and Davies (*31*). Again, the values gained by the Griffin method were more suitable to predict the baking performance of glycolipids than those obtained by using the Davies method (data not shown). The values gained for the glycoglycerolipids (**1**, monogalactosyl dilinoleylglycerol, monogalactosyl monolinoleylglycerol) and the glycosphingolipids (monoglycosylceramides) by the Griffin method showed the typical values expected for classical O/W surfactants (**Table 7**), with the optimal HLB value being between 8 and 12 in correlation with the baking activity. The HLB values calculated for the glycolipid classes **2** and **3** would depict them as W/O surfactants (**Table 7**). Their hydrophilic/lipophilic ratio showed quite a different value from that of the other glycolipid classes. These values were not in correlation with their baking activities. This demonstrates the fact that there is no optimal hydrophilic liphophilic ratio or HLB value that predicts the highest baking activity for all of the different glycolipid classes. The right ratio between hydrophilic and lipophilic elements in the molecular structure of glycolipids is dependent on the role it plays in the dough, hence, with which constituents of the dough it interacts and where their functional site in the dough is located. This will be subject to further research. On the one hand, the glycolipid classes **1** and **4** represent the classical O/W surfactant, and, on the other hand, the glycolipid classes **2** and **3** have a different functionality but similar or better baking activities than **4**. O/W surfactants typically are located at the liquid−gas bubble interphase in dough. Other surfactants can be located at other sites in the dough, such as the starch−, protein−, or lipid−water interphase, with different surfactant properties being necessary.

Table 8. Crumb Firmness of Breads As Affected by Type and Concentration of Lipid[a]

	crumb firmness (N)						
additive	0.0%[b]	0.1%[b]	0.2%[b]	0.4%[b]	0.6%[b]	0.8%[b]	1.0%[b]
			Lecithins				
soybean defatted	0.8		1.0	0.7	0.4	0.3	0.6
soybean crude	0.8		0.7	0.7	0.5	0.4	0.3
			Reference Compounds				
monoacylglycerides	1.1	0.8	0.8	0.6	0.8	0.6	
SSL	0.8	1.1	0.9	0.7	0.8	0.7	
DATEM	0.8	0.9	1.0	0.6	0.6	0.4	
MGDG (synthetic)	1.0	1.2	1.1	0.9	0.7	0.5	
MGMG (synthetic)	1.1	1.0	0.8	0.6	0.5	0.4	
			Glycolipid Fractions				
fraction 1: nonpolar lipids fraction	0.6	0.8	0.5	0.7	0.9		
fraction 2: ASG	1.2	0.7	0.4	0.4	0.4	0.4	/
fraction 3: MGDG + minor components	1.2	1.1	1.2	0.6	/	/	/
fraction 4: SG	1.2	1.2	0.9	0.7	0.7	/	/
fraction 5: cerebrosides	1.3	1.1	0.9	0.7	0.5	/	/
fraction 6: DGDG + cerebrosides	1.3	0.9	0.6	0.3	0.3	0.5	/

[a] Glycolipid fractions isolated by chromatography method I. Number of experiments $n = 3$; standard deviation $\pm$ 0−0.2 N; relative standard deviation 0−22%. DATEM, diacetyltartaric esters of mono- and diacylglycerides; SSL, sodium stearoyl-2-lactylate; MGDG, monogalactosyl diacylglycerol; MGMG, monogalactosyl monoacylglycerol; ASG, acylated sterol glucosides (**3**); SG, sterol glucosides (**2**); cerebrosides (**4**); DGDG, digalactosyl diacylglycerides (**1**). [b] Concentration of additive based on 10 g of flour (= 100%).

Effect on Crumb Firmness. Another direct effect of surfactants in breadmaking is known to be on the softness of the bread crumb. The respective data are given in **Table 8**. To eliminate the influence of the volume increase caused by the surfactant on the crumb firmness, a volume factor [bread volume$_{\text{additive amount}}$/ bread volume $_{\text{control}}$ (data taken from the microscale baking test)] was used for each additive level. Because of the rather high relative standard deviation (up to 22%), tendencies rather than significant changes were observed. The crumb firmness curves, all but one (nonpolar lipid fraction), displayed a more or less distinct downward sloping tendency over the examined additive concentration range (data not shown). This effect expressed clearly that the surfactants had a positive effect not only on the bread volume but also on the crumb softness.

Antistaling Effect. Some polar lipids are known to slow aging of the bread crumb, hence acting as antistaling agents. This effect was measured by comparing the firmness of the fresh and the stored (24 h) bread crumb. The relative increase of the firmness of the stored as compared to the fresh bread crumb was evaluated. SSL and monoacylglycerides were the only two samples, which inhibited the staling of the crumb significantly (data not shown). For all glycolipid fractions (method I) neither a significant nor a noticeable antistaling effect was detected. This indicates that the necessary molecular structure for a surfactant to show an antistaling effect was not present in a high enough concentration in the different glycolipid classes. That glycolipids are capable of exhibiting an antistaling effect was shown for the synthetic glycolipid monogalactosyl monolinoleylglycerol, however, considerably less effective than SSL (*14*).

Microscale Extension Tests with Dough and Gluten. Surfactants can interact with various flour constituents, for example, proteins, starch, and endogenous lipids, in different ways and therefore cause many alterations in the gluten and dough network. The microextension tests conducted with the dough (data not shown) and the pure glycolipid classes (method II) did not show a significant effect on the overall rheological behavior of the dough. However, the reference compounds DATEM, monoacylglycerides, and SSL showed a significant decrease in extensibility in comparison to the control dough. DATEM and SSL, being anionic surfactants, are able to aggregate with the gluten proteins due to ionic interactions and hence result in an increase of dough firmness. On the other hand, monoacylglycerides are known to form inclusion complexes with the starch, hence also directly interacting with a major flour component. These results clearly indicate that the glycolipids do not interact directly with a major flour component during dough mixing and are therefore most probably located in the dough liquor.

The data obtained from tests carried out with gluten gained from doughs containing pure glycolipid classes (method II) as additives are shown in **Table 9**. The lecithins showed no significantly different values for the three parameters from the control gluten. For the reference compounds (DATEM, monoacylglycerides, and SSL) no significant differences were found for the resistance to extension, whereas the extensibility of the gluten was significantly increased. This was also the case for the glycolipid classes **1** from soybean lecithin and **4** from soybean, rapeseed, and sunflower lecithins. The highest extensibility value was found for the pure glycolipid class **1** from soybean lecithin. The only surfactants with a significant increase in resistance to extension were the pure glycolipid classes **3** and **2** from soybean lecithin. **1** was the only surfactant that showed a significant decrease in resistance to extension, this being in correlation with the high increase in extensibility. A clear distinction of the surfactants into two groups was possible. The two glycolipid classes **3** and **2** formed the first group. They directly influenced the resistance to extension of the gluten, without showing an influence on the extensibility. The second group including the glycolipid classes **1** and **4** as well as the reference compounds DATEM, monoacylglycerides. and SSL only affected the extensibility but not the resistance to extension. These changes in the rheological properties of the gluten are the result of the surfactants interacting with the flour proteins when present in the dough during dough washing. During this process they solubilize and remove some of the proteins from the gluten network. This solubilization of individual proteins from the gluten network into the water phase (washing water), resulting in different rheological changes in the gluten for the two groups of surfactants, is most probably related to the ability of the surfactants, due to their chemical

Table 9. Microscale Extension Test with Gluten from 10 g of Flour (Wheat cv. Tommi): Maximum Resistance to Extension (RE), Extensibility (EX), and Extension Energy As Affected by the Type and Concentration of Polar Lipid in Comparison to Control Gluten from Flour without Additive

additive	concentration[a] (%)	solvent	no. of doughs	no. of strands	wet gluten (g)	±SD (g)	RE (N)	±SD (N)	EX (mm)	±SD (mm)	energy (N·mm)	±SD (N·mm)
control gluten			29	87	3.56	0.12	0.63	0.03	148.5	8.2	44.5	3.8
					Lecithins							
Ra defatted	0.6	water	2	6	3.63	0.03	0.61	0.02	151.1	6.9	42.3	3.2
Ra defatted	0.6	chloroform	2	6	3.51	0.14	0.64	0.04	141.8	8.6	43.2	7.1
So defatted	0.6	water	2	6	3.55	0.13	0.64	0.00	143.0	5.7	41.2	3.1
So defatted	0.6	chloroform	2	6	3.66	0.08	0.66	0.03	150.2	5.0	45.6	2.7
					Reference Compounds							
MG	0.6	chloroform	2	6	3.63	0.01	0.65	0.01	160.3	9.4	53.0	8.5
DATEM	0.6	water	2	6	3.63	0.07	0.62	0.04	165.7	3.8	49.1	2.6
DATEM	0.6	chloroform	2	6	3.59	0.04	0.60	0.01	161.1	2.0	47.5	3.3
SSL	0.6	water	2	6	3.60	0.04	0.61	0.06	163.6	8.6	49.1	5.8
SSL	0.6	chloroform	2	6	3.61	0.05	0.57	0.02	164.4	9.3	48.5	6.5
					Glycolipid Classes							
ASG So	0.4	hexane	2	6	3.44	0.06	0.77	0.04	146.7	7.7	53.9	5.7
SG So	0.6	chloroform	1	3	3.38		0.69	0.06	145.3	1.0	45.6	4.1
DGDG So	0.6	chloroform	2	6	3.46	0.14	0.53	0.02	172.0	2.8	45.5	3.3
cerebrosides So	0.4	chloroform	2	6	3.59	0.08	0.61	0.05	156.6	9.6	44.1	5.8
cerebrosides Sn	0.4	chloroform	2	6	3.52	0.04	0.63	0.04	156.7	9.8	46.0	2.5
cerebrosides Ra	0.4	chloroform	1	3	3.36		0.58	0.03	152.6	2.2	44.7	1.0

[a] ± SD, standard deviation (2−3 strands of gluten); So, soybean lecithin; Ra, rapeseed lecithin; Sn, sunflower lecithin; MG, monoacylglycerides; SSL, sodium stearoyl-2-lactylate; ASG, acylated sterol glucosides (**3**); SG, sterol glucosides (**2**); DGDG, digalactosyl diacylglycerides (**1**); cerebrosides (**4**). [b] Concentration of additive based on 10 g of flour (= 100%).

structure and corresponding hydrophilic−liphophilic balance, to solubilize different protein groups from the gluten network. This would explain the different influence on the rheological behavior of the gluten found for ASG and SG in comparison to the other surfactants. However, these results indicate that the different effects the individual surfactants had on the rheological behavior of the gluten, through solubilizing different protein groups, in these margins are not directly responsible for the baking activity of the surfactant, because the baking activities could not be directly correlated with the different rheological effects found and additionally no effects on the overall dough rheological properties, this being the case if a change in the rheological properties of the dough liquor had occurred, had been detected. However, the results did indicate that these two groups exhibited most probably different modes of action in the dough.

Hypotheses on the Dough and Bread Improving Effects of Glycolipids. This work confirmed the excellent properties of glycolipids in breadmaking. For the glycolipid classes **2** and **3** this has been shown for the first time. The comparison of the calculated HLB values and the baking activities of the surfactants tested showed that there was no optimal hydrophilic/liphophilic ratio or HLB value that would allow predicting the functionality of the different glycolipid classes. The improving effect of a given glycolipid depends on the role it plays in dough, hence with which constituents of the dough it interacts and where its functional site in the dough is located. The HLB values could only be correlated with technological properties for typical O/W surfactants such as the glycolipid fractions **1** and **4** or the synthetic galactolipids.

Therefore, it can be stated that the high baking activities of the different glycolipid classes and the two synthetic galactolipids as well as of the other surfactants might be explained by modes of action based on the formation of liquid films at the dough liquor/gas cell interface. Possible modes of action are the direct influence of the surfactants on the liquid film lamellae and gas cell interfaces through direct adsorption and an indirect influence on the liquid film lamellae by changing the phase behavior of the endogenous lipids present in the dough liquor, resulting in an increase of surface activity. The indications, such as HLB value, bread volume, and rheological data, as found during this work, suggest that **1** and **4** as well as the synthetic galactolipids with HLB values of 8−12 take the mode of action of directly forming and stabilizing liquid film lamellae through adsorption to interfaces, especially when the gas cells expand during proofing and oven-spring, as proposed by Gan et al. (*29*) and Sroan et al. (*33*) as the secondary stabilizing mechanism in the dual film theory. This suggests the presence of liquid lamellae, providing an independent mechanism of gas cell stabilization. As shown recently, the effects of different surface active components may be explained by the type of monolayer that they form (*32*, *33*).

However, the good baking activity of the less polar glycolipid classes **2** and **3** (HLB value ca. 5), especially the excellent activity of **3** in the lower concentration range, cannot be explained with this mode of directly stabilizing the liquid film lamellae. Here another mode of action could be the answer, for example, the indirect stabilization of the dough liquor/gas cell interface through this type of surfactant. These less polar glycolipid classes have a positive influence on the phase behavior of the endogenous lipids present in the dough liquor in that they lead to an increase in surface activity of the endogenous lipids and hence a better availability and accumulation at the liquid film lamellae/gas cell interface, thus increasing gas cell stabilization and, consequently, bread volume.

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