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Baking Performance of Synthetic Glycolipids in Comparison to Commercial Surfactants

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To gain insight into structure−activity relationships of glycolipids in breadmaking monogalactosyl dilinoleylglycerol (**8**) and monogalactosyl monolinoleylglycerol (**6**) were synthesized. Then their functional properties in dough and breadmaking were compared to those of commercial surfactants such as lecithins (from soybean, rapeseed, and sunflower), diacetyltartaric acid esters of monoglycerides (DATEM), monoglycerides, and sodium stearoyl-2-lactylate. Chemical synthesis of the galactolipids consisted of a four-step reaction pathway, yielding amounts of 1−1.5 g suitable for the determination of the functional properties. Variation of the acylation time in the third step provided either the monoacyl (**6**) or the diacyl compound (**8**). The functional properties were determined by means of rheological and baking tests on a microscale (10 g of flour). The synthetic galactolipids both displayed an excellent baking performance, with **6** having by far the best baking activity of all examined surfactants. The baking activities of **8**, DATEM, and the monoglycerides were in the same range, whereas sodium stearoyl-2-lactylate was less active. Although the lecithins gained similar maxima in bread volume increases as the synthetic surfactants did, considerably higher concentrations were required to do so. An antistaling effect was found for only **6** and not for **8**. However, this effect was weaker than for sodium stearoyl-2-lactylate and the monoglycerides.

KEYWORDS: Glycolipids; synthesis; monogalactosyl diglyceride; monogalactosyl monoglyceride; wheat; microscale extension test; microscale baking test

INTRODUCTION

The ability of surfactants to improve the dough handling and the quality of baked bread and pastries depends on structure−function relationships of the active constituents. These are responsible for the direct effects that result from the application of surfactants, such as dough properties, volume, shape, texture, and taste. The direct effects of the surfactants are based on their ability to act as surface-active compounds and they occur only during dough mixing and baking. In addition, so-called indirect effects, such as the antistaling effect, have been described for some surfactants. In contrast to the direct effects the indirect effects remain effective until the bakery product is eaten, also beginning during dough mixing (*1*). The term "baking performance" includes the sum of the direct and indirect effects of surfactants. Various methods including sensory, rheological, and baking tests are required to measure the baking performance.

All surfactants, endogenous or synthetic, consist of molecular structures with hydrophilic and hydrophobic structural elements. This structural design can be described by the compounds' specific hydrophilic−lipophilic balance (HLB), which is expressed by the so-called HLB value that was introduced by Griffin (*2, 3*). HLB values are used to classify surfactants and to describe their functional properties by only one parameter. There are two approaches to obtain HLB values of surfactants: the analytical determination, for example, by measuring the partition between two liquid phases (*3*), and the calculation based on structural elements of the molecules (*2–5*). Regarding the calculation of HLB values, no useful method for different surfactants (ionic, nonionic) has been established yet.

Glycolipids and phospholipids dominate the endogenous polar lipid fraction of wheat flour (*6*). It has been established that in particular glycolipids act as surfactants and that they positively affect the volume, texture, and staling of bread (*7–9*). Therefore, this group of polar lipids can be regarded as a very interesting surfactant group for breadmaking. However, it is astonishing that over the years, a lot of attention has been given to various surfactants, but glycolipids have not been looked at any closer so far. The reason is that the isolation of glycolipids from plant sources is costly, and chemical synthesis is difficult.

The chemical synthesis of the naturally occurring glycoglycerolipids has been attempted by only a few researchers so far (*10, 11, 19*). Only Pomeranz and Wehrli (*10*) have studied the dose-dependent correlations between the baking activity and chemical structure of some individual glycolipids. They determined the effects of synthetic glycosylglycerides, varying in composition, on defatted wheat flour (petroleum ether extract) and untreated wheat flour enriched with soy flour. They found

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that octanoic acid was the optimum fatty acid chain length for monogalactosyl diglycerides. Their comparison of the effects of synthetic glycolipids indicated that both hydrophobic and hydrogen bonds are important for the effects in bread making. Their conclusion was that there must be an optimum hydrophilic−lipophilic balance at which single polar lipids or mixtures thereof increase bread volume the most. However, to the best of our knowledge, studies using individual lyso compounds of glycoglycerolipids have not been reported yet. Furthermore, no information on the influence of individual glycolipids on the retardation of bread staling is available; only the overall positive effect of the glycolipid part from oat lecithin was found (*12*).

Therefore, the aim of this study was to synthesize one glycolipid and the corresponding lyso-glycolipid in amounts suitable for the determination of the baking performance. The direct and indirect effects of the two synthetic compounds were then determined by means of microscale rheology and baking tests using 10 g of flour in comparison to classical surfactants such as lecithins from different plant sources, diacetyltartaric acid esters of monoglycerides (DATEM), sodium stearoyl-2-lactylate, and monoacylglycerides. Finally, HLB values of the glycolipids were calculated and correlated to the baking performance.

MATERIALS AND METHODS

Wheat Flour. Samples of the German wheat cultivar Tommi from the 2005 harvest (Nordsaat, Langenstein, Germany) were milled into flour with a Quadrumat Junior Mill (Brabender, Duisburg, Germany). The flour was sieved through a 0.2 mm diameter screen and allowed to rest for 3 weeks prior to use. The moisture and ash contents of the flour were determined according to ICC Standards 110 (*13*) and 104 (*14*), respectively. Nitrogen contents were determined by using the method of Dumas on a Leco FP328 nitrogen analyzer (Kirchheim, Germany). A conversion factor of 5.7 was used to calculate the protein content from the nitrogen content. Analytical characteristics of the flour were 15.4% moisture, 0.473% ash (dry mass), and 11.4% protein (dry mass).

Chemicals. The quality of all solvents was "pro analysi" (p.a.) or stated otherwise. All solvents used for the synthesis of monogalactosyl dilinoleylglycerol (**8**) and monogalactosyl monolinoleylglycerol (**6**) were dried and stored with molecular sieves (4 nm). Acetobromo-α-D-galactose (**1**), (*S*)-(−)-1,2-di-*O*-benzylglycerol (**2**), 2,6-dichlorobenzoylchloride, *N*,*N*′-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, hydrazine hydrate (98%), iodine (purified by resublimation), *cis*-9,*cis*-12-octadecadienoic acid (linoleic acid), 1-methylimidazole, sodium sulfate, palladium on activated charcoal (10%), silver carbonate, triethylamine, chloroform, aluminum oxide (activated neutral, Brockmann I), and phosphorus pentoxide desiccant were obtained from Sigma-Aldrich (Steinheim, Germany). Acetone (Suprasolv), chloroform, dichloromethane, diethyl ether, glacial acetic acid, ethanol, ethyl acetate, hexane (Suprasolv), silica gel G 60 (0.04−0.063 mm), methanol (Lichrosolv), hydrochloric acid (32%), sulfuric acid (95−98%), sodium chloride, thin layer chromatography (TLC) plates (20 × 20 cm) coated with silica gel F_{254} on aluminum, tetrahydrofuran (THF), 2-propanol, ammonia solution (25%), ascorbic acid, and sucrose were from Merck (Darmstadt, Germany). Chloroform was also obtained from Biosolve B.V. (Valkenswaard, Netherlands) through ScienTest (Rheburg-Loccum, Germany). Methanol-d_4 + 0.03% tetramethylsilane (TMS) and chloroform-d + 0.03% TMS were obtained from Euriso-top (Gif-sur-Yvette, France). The water used was deionized through a Millipore-O Milli-Q purification system.

Surfactant Sample. DATEM, sodium stearoyl-2-lactylate, and monoglycerides (Monomuls 90-25; monoacylglycerides containing 75% stearic and 25% palmitic acid; monoacylglyceride content 90%) were from Cognis (Illertissen, Germany).

Synthesis of 1,2-Dilinoleyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol (8). *1,2-O-Dibenzyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetylgalactopyranosyl)-sn-glycerol (***3***).* **2** (31.7 mmol) was dissolved in dichloromethane (310 mL) and stirred for 1 h after the addition of silver carbonate (65.5 mmol), iodine (16.6 mmol), and anhydrous sodium sulfate (80 g) under an argon atmosphere and protected from light. **1** (69.3 mmol) dissolved in dichloromethane (310 mL) at room temperature was added dropwise over a period of 1 h. The reaction mixture was stirred until no visible change of the reaction compounds could be observed by TLC (diethyl ether/hexane 5:1, v/v). The reaction mixture was purified twice by glass column chromatography. First, the reaction mixture residue was purified on a column of aluminum oxide (activated, neutral, Brockmann I), which was eluted with the isocratic mobile phase chloroform/methanol (150:1, v/v). The eluate was collected in test tubes, each containing 13 mL, with a fraction collector. All individual fractions were examined by TLC (diethyl ether/hexane 5:1, v/v). Then the fractions containing the highest concentration of the first-step product were recombined and evaporated to dryness. This intermediate residue was further purified on an activated silica gel column G60 (activity grade I), treated according to Esterbauer (*15*), which was eluted with the mobile phase diethyl ether/hexane (5:1, v/v). The eluate was collected, and the fractions were examined as detailed above. Only the fractions with the purest first-step product were recombined and evaporated to dryness. The yield was 64%.

*3-O-(β-D-2′,3′,4′,6′-Tetra-O-acetylgalactopyranosyl)-sn-glycerol (***4***).* **3** (11.1 mmol) was dissolved in ethyl acetate (100 mL), catalyst (3.7 g; 10% palladium on charcoal), and ethanol (0.3 mL), and, in initial experiments, glacial acetic acid (0.02 mL) was added. The reaction mixture was vigorously stirred in a hydrogen autoclave at room temperature under a hydrogen atmosphere of 0.5 MPa. The reaction was monitored by TLC (diethyl ether/methanol (8:1, v/v). After the reaction was completed, the catalyst was filtered off, washed three times with ethyl acetate (total volume = 150 mL), and the ethyl acetate fractions were combined, and evaporated to dryness. The average time for complete hydrogenolysis was 36 h per gram of educt. Residual catalyst particles were removed by filtration of a solution of the product in diethyl ether (30 mL) through a polytetrafluoroethylene (PTFE) membrane (0.45 μm). The yield was 55%.

*1,2-Dilinoleyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetylgalactopyranosyl)-sn-glycerol (***7***).* The reaction was carried out under an argon atmosphere. **4** (11.8 mmol) was dissolved in dichloromethane (100 mL). 1-Methylimidazole (141.6 mmol), 2,6-dichlorobenzoyl chloride (56.6 mmol), and linoleic acid (28.3 mmol) each dissolved in dichloromethane (35 mL), were added dropwise at the same time to the stirred solution of the educt. Stirring was continued until no visible change of the reaction products could be observed by TLC (diethyl ether/hexane 5.7:1, v/v). The reaction was completed after 3 h, and then water (94.4 mmol) and triethylamine (70.8 mmol) were added. The reaction mixture was stirred for another 10 min and then evaporated to dryness. The residue was purified on a silica gel column G 60, which was eluted with the isocratic mobile phase diethyl ether/hexane (3:1, v/v). The eluate was collected as described above, and all fractions were examined by TLC (diethyl ether/hexane 5.7:1, v/v). The fractions containing the purest third-step product were recombined and evaporated to dryness. The yield was 88%.

*1,2-Dilinoleyl-3-O-(β-D-galactopyranosyl)-sn-glycerol (***8***).* The reaction was carried out under an argon atmosphere. **7** (10.2 mmol) was dissolved in methanol (200 mL). Hydrazine hydrate (90.8 mmol) was added, and the mixture was stirred at 60 °C under reflux until no visible change of the reaction compounds could be observed by TLC (chloroform/methanol/acetone 8:1:1, v/v/v). To neutralize the hydrazine, a formic acid solution (85%, v/v; 3.7 mL) was added and stirred for another 10 min and then evaporated to dryness. The residue was purified on a silica gel column G 60, which was eluted with the isocratic mobile phase chloroform/methanol/acetone 48:1:1, v/v/v). The eluate was collected as described above, and all fractions were examined by TLC (chloroform/methanol/acetone 8:1:1, v/v/v). The fractions containing the purest end product (**8**) were recombined and evaporated to dryness. The yield was 17.6%.

Synthesis of 1-Monolinoleyl-2-hydroxy-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol (6/Lyso Compound). **3** and **4** were synthesized according to the procedures described for the synthesis of **8**.

*1-Monolinoleyl-2-hydroxy-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetylgalactopyranosyl)-sn-glycerol (***5***).* The reaction was carried out under an

argon atmosphere. To a solution of **4** (18.8 mmol) in dichloromethane (100 mL) were added dropwise 1-methylimidazole (112.8 mmol), 2,6-dichlorobenzoyl chloride (47 mmol), and linoleic acid (22.6 mmol), each dissolved in dichloromethane (35 mL), at the same time under stirring. Stirring was continued for 7 min (optimum for the formation of the lyso compound and minimum diacyl formation), and then water (75.2 mmol) and triethylamine (56.4 mmol) were added to stop the reaction. The reaction mixture was stirred for another 10 min and then evaporated to dryness. The residue was purified on a silica gel column G 60, which was eluted with the isocratic mobile phase diethyl ether/methanol (100:1, v/v). The eluate was collected as described above, and all fractions were examined by TLC (diethyl ether/hexane 5.7:1, v/v). The fractions containing the purest third-step product were recombined and then evaporated to dryness. The yield was 69%.

*1-Monolinoleyl-2-hydroxy-3-O-(β-D-galactopyranosyl)-sn-glycerol (**6**).* The reaction was carried out under an argon atmosphere. **5** (6.5 mmol) was dissolved in methanol (120 mL). Hydrazine hydrate (39 mmol) was added to the solution of **5**, and the mixture was stirred at 60 °C under reflux until no visible change of the reaction compounds could be observed by TLC (chloroform/methanol/acetone 8:1:1, v/v/v). To neutralize the hydrazine a formic acid solution (85%, v/v; 1.1 mL) was added and stirred for another 10 min and then evaporated to dryness. The residue was purified on a silica gel column G 60, which was eluted with the isocratic mobile phase chloroform/methanol/acetone (3:1:1, v/v/v). The eluate was collected as described above, and all fractions were examined by TLC (chloroform/methanol/acetone 8:1:1, v/v/v). The fractions containing the purest end product (**6**) were recombined and evaporated to dryness. The yield was 28.5%.

Purified Lecithin Preparation. Water-soluble nonlipid substances were removed from commercial lecithin samples (soybean, rapeseed) by a modified procedure according to the method reported by Folch et al. (*16*). Briefly, defatted lecithin (25 g) was dissolved in chloroform/methanol (2:1, v/v; 675 mL) and washed successively with water (135 mL) and twice with sodium chloride solution (0.5%, w/v; 135 mL, each). The resulting biphasic systems were separated and the upper water/methanol phases recombined separately. The lower organic phase, containing the total lipid content of the lecithin sample, was dried over anhydrous sodium sulfate, evaporated to dryness, weighed, and stored in a freezer at −24 °C.

Mass Spectrometry (MS). Sample solutions (in methanol) were directly applied to a mass spectrometer (LCQ Classic, Finnigan MAT, Weiterstadt, Germany) running in the electrospray ionization mode with positive polarity (ESI^+) by means of a syringe, and full-scan spectra were recorded. MS data for **8**: (MW 779) MS (ESI^+) *m/z* 1580 (100, $[2M + Na]^+$), 802 (14, $[M + Na]^+$), 780 (<5, $[M + H]^+$). MS data for **6**: (MW 516) MS (ESI^+) *m/z* 1055 (100, $[2M + Na]^+$), 539 (9, $[M + Na]^+$), 517 (<5, $[M + H]^+$).

NMR Spectroscopy. The ^1H and two-dimensional NMR spectra (^1H, COSY (^1H ^1H, correlated spectroscopy), HMQC (^1H ^{13}C, heteronuclear multiple quantum coherence), and 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 are given relative to the signal for internal TMS ($\delta = 0$). The values for coupling constants *J* are given in hertz.

Microscale Baking Test. The microscale baking test with 10 g of flour was carried out according to the method of Koehler and Grosch (*17*) with modifications displayed below. High-speed mixing (micro rapid mix test; MRMT) for dough preparation was used. The ingredients used were based on the flour weight: NaCl, 2%; sucrose, 1%; fresh bakers' yeast, 7%; ascorbic acid, 20 mg/kg; surfactant, 0.1−1.0%. Triplicate determinations were carried out.

Addition of the Surfactant. The surfactant (10, 20, 40, 60, and 80 mg) was dissolved in a suitable solvent (0.13, 0.25, 0.50, 0.75, and 1.0 mL; hexane, chloroform, or THF), added to the flour, and left to air-dry for 20 min (hexane, chloroform) prior to dough preparation. A homogeneous distribution of the surfactant in the flour and a better evaporation of the organic solvent were achieved through manually stirring the flour from time to time. The flour with the surfactant added in THF was placed in a desiccator and the THF evaporated under vacuum (20 kPa) for 1 h. Water-soluble surfactants were directly added as a suspension in the water used for dough mixing.

Dough Preparation. The water or watery surfactant suspension (6 mL) was cooled to 15 °C in the mixing bowl for 1 min. Flour (10 g; 8.6 g of dry mass), NaCl (0.2 g), sucrose (0.1 g), a solution of L-ascorbic acid (0.35 mL; 0.57 mg/mL), and yeast (0.7 g) were then added and mixed for 1 min at 1250 rpm.

Dough Handling and Baking. After removal of the dough from the mixer, it was allowed to rest for 20 min at 30 °C in a water-saturated atmosphere. The dough was then reshaped on a dough rounder for eight cycles. The resulting dough ball was passed through the rolls (roll-gap = 2 mm) of an AMPIA pasta machine (model 150 mm, Deluxe, Marcato, Italy) to form an oval dough piece. This was folded twice in half to form a triangle-shaped dough piece. This triangle-shaped dough was reshaped on the dough rounder for 20 cycles. Then the proofing (29 °C, 35 min) and baking (180 °C increasing to 250 °C, 10 min) were carried out on a fully automatic baking line, the bread thereby passing through a proofing chamber (with water-saturated atmosphere) and an oven on a conveyor belt. The oven was supplied with a continuous stream of water steam produced by a water-boiling device. The volume of the bread was determined by measuring the amount of water displaced by the wax-coated bread at room temperature (*17*).

Crumb Firmness and Crumb Staling. The top and bottom of the cooled bread (1 h after baking) were cut off, leaving the middle section with a height of exactly 1.5 cm. From the middle section of this bread slice a cylindrical part of the crumb was cut out with a 20 mm diameter cork cutter. This crumb cylinder was analyzed with a Texture Analyzer TA-XT2 (Stable Microsystems, Godalming, U.K.). The crumb cylinder was compressed by 0.7 cm twice using a plexiglass cylinder. Force−distance and force−time diagrams were recorded. Then the crumb cylinder was stored for 24 h at 22 °C in an airtight glass container, and the firmness was analyzed again.

Microscale Rheology. Microextension tests with dough and gluten from 10 g of flour were carried out as reported by Koehler and Grosch (*17*) with modifications displayed below. Surfactants were either added to the flour as a solution in an organic solvent prior to mixing or dissolved in the water used for mixing. For dough rheology, flour (10 g, 8.6 g of dry mass) and NaCl (0.2 g) were mixed in a microfarinograph (Farinograph E, Brabender, Duisburg, Germany) for 1 min at 60 rpm and 22 °C. Distilled water was added, and mixing was continued until a maximum consistency of 550 Brabender units (BU) was reached. The dough was removed, shaped to an ellipsoid form, and pressed into PTFE forms to give strands of 53 × 4 × 4 mm. After 40 min of resting in a desiccator at 22 °C in a water-saturated atmosphere, the strands were extended with a Texture Analyzer TA-XT2 (Stable Microsystems) until they disrupted. For gluten rheology flour was mixed for 2 min as described above. The dough was then washed for 10 min with a sodium chloride solution (0.4%, w/v; 540 mL) in a Glutomatic type 2200 (Perten Instruments, Huddinge, Sweden). The residue (gluten) was centrifuged in special PTFE devices for 10 min at 5000*g* and 22 °C, pressed into PTFE forms, and treated as described above.

Thin Layer Chromatography. Separations were carried out on plates coated with silica gel G 60 with F_{254} on aluminum foil. Compounds were first visualized using ultraviolet light (254 nm) and then by spraying with 50% (w/w) sulfuric acid and charring for 10 min at 135 °C.

RESULTS AND DISCUSSION

Synthesis of Monogalactosyl Dilinoleylglycerol (8) and Monogalactosyl Monolinoleylglycerol (6). Monogalactosyl dilinoleylglycerol (**8**) and monogalactosyl monolinoleylglycerol (**6**) were synthesized as reference and model compounds as illustrated in **Figure 1**. A modified four-step synthetic strategy for both compounds combined the previously published procedures of Mannock et al. (*11*) and Gaffney and Reese (*18*).

Glycosylation. The first reaction step was the condensation of the appropriately protected glycerol molecule with the glycosyl donor. This could be achieved via either the orthoester pathway (*19*) or the Koenigs−Knorr reaction pathway (*20*). According to

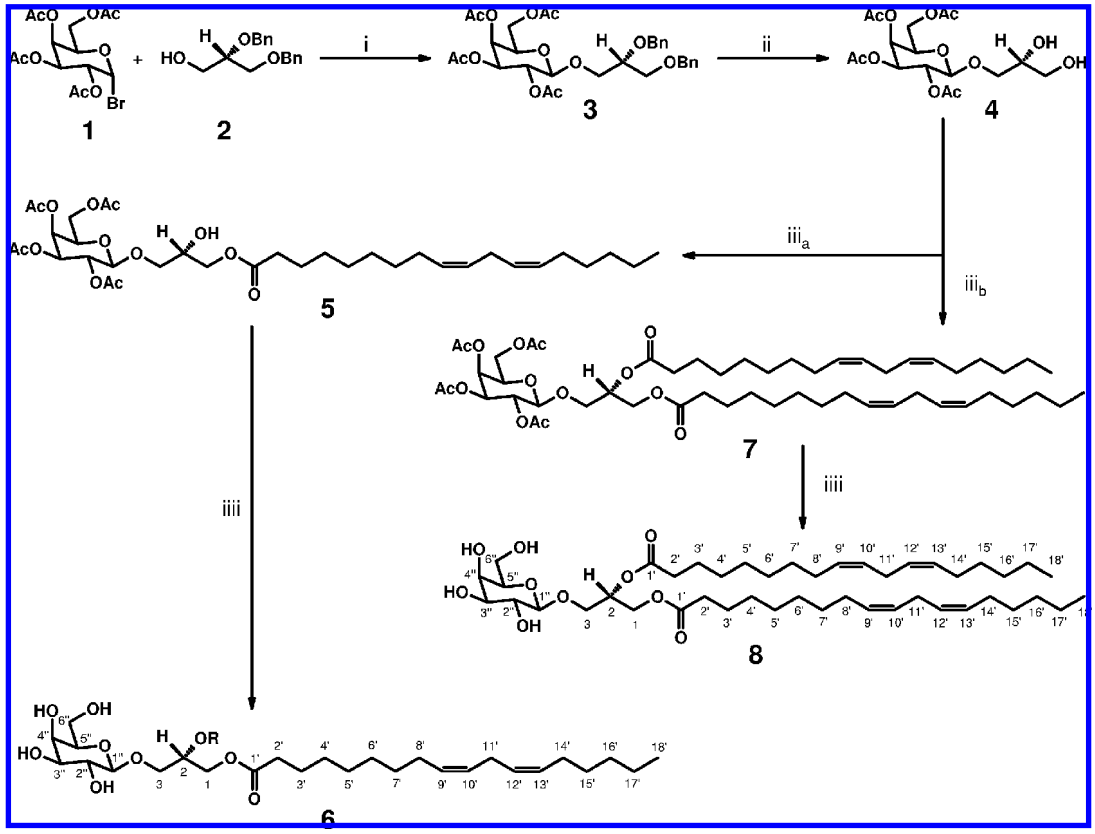

Figure 1. Reaction pathway for 1-monolinoleyl-2-hydroxy-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol (**6**) and 1,2-dilinoleyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol (**8**): i, glycosylation; ii, catalytic hydrogenation; iii, acylation; iiii, deacetylation; (**1**) acetobromo-α-D-galactose; (**2**) (*S*)-(−)-1,2-di-*O*-benzylglycerol; (**3**) 1,2-*O*-dibenzyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetylgalactopyranosyl)-*sn*-glycerol; (**4**) 3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetylgalactopyranosyl)-*sn*-glycerol; (**5**) 1-monolinoleyl-2-hydroxy-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetylgalactopyranosyl)-*sn*-glycerol; (**7**) 1,2-dilinoleyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetylgalactopyranosyl)-*sn*-glycerol.

Mannock et al. (*11*) the orthoester pathway is by far the more difficult because the exclusion of water and protons has to be adhered very rigorously. In contrast, the condensation of **1** with the hydroxyl group of the appropriately protected glycerol molecule under Koenigs−Knorr conditions is easier. Under these conditions protecting groups for the two hydroxyl groups (*sn*-1 and *sn*-2) are required, which are stable enough in the first reaction step, but which then can be selectively removed in the presence of the ester group in the second reaction step (**Figure 1**). According to Mannock et al. (*11*) the *sn*-1 and *sn*-2 positions of the glycerol molecule can be protected best by using the benzyl ether group. Unlike several years ago the compound **2** is now commercially available. This made the Koenigs−Knorr pathway the favorable choice. The choice of catalyst for the Koenigs−Knorr reaction is also of great importance. Mannock et al. (*11*) found that silver carbonate was clearly more appropriate to gain the 1,2-*trans*-(β)-galactopyranosidic linkage with the glycerol moiety than silver oxide. For the glycosylation of steroid alcohols with glycosyl halides other catalysts such as the insoluble silver salts of hydroxy carbonic acids (e.g., 4-hydroxyvaleric acid) or dicarbonic acids (e.g., maleic acid) with diethyl ether as the solvent have been described (*21*). Wulff and Roehle (*22*) found that in particular the disilver maleinate gained better yields of the glycosides than silver oxide and silver carbonate. In our experiments disilver maleinate in combination with diethyl ether or a diethyl ether/dichloromethane mixture as the solvent did not perform better than silver carbonate and silver oxide in combination with dichloromethane. Unlike disilver maleinate silver carbonate was commercially available and was, therefore, used to catalyze the first step of the synthesis (**Figure 1**). The structure of **3** was confirmed by ESI^+ mass spectrometry and NMR experiments.

Debenzylation. The purified first-step reaction product was debenzylated by catalytic hydrogenation using palladium on activated charcoal as catalyst (**Figure 1**). Pretreatment of an ethanolic suspension of the catalyst with hydrogen or the addition of catalytic amounts of glacial acetic acid according to Mannock et al. (*11*) did not have any noticeable influence on the course of the reaction. Therefore, the catalyst was used without pretreatment, and the hydrogenation was carried out in ethyl acetate containing 0.3% (v/v) ethanol. The second reaction step product (**4**) was formed in high yield, showing that the benzyl ether protecting groups had been successfully removed in the *sn*-1 and *sn*-2 positions of the glycerol without affecting the galactosidic linkage. The structure of **4** was confirmed by ESI^+ mass spectrometry and NMR experiments.

Acylation. In preliminary tests we compared the methods published by Gaffney and Reese (*18*), Mannock et al. (*11*), and a patent from Schaefer (*23*). The two reaction procedures of Gaffney/Reese (*18*) and Mannock et al. (*11*) took place at room temperature in dichloromethane as the solvent, whereas the reaction in the patent of Schaefer (*23*) was carried out in the molten state at 70 °C. The yields found were the highest with the method from Gaffney and Reese (*18*), followed by the patent (*23*), and the lowest yields were obtained with the method of Mannock et al. (*11*). A further great advantage of the method from Gaffney and Reese (*18*) in comparison to the others was that the free acid could be used and that there was no need to synthesize an activated acyl derivative, for example, the anhydride in an additional reaction step. Therefore, the esterification of the *sn*-1 and *sn*-2 positions of the second-step reaction product with linoleic acid [18:2 (*cis*-9,12)] was carried out according to the method of Gaffney and Reese (*18*). Both the

Table 1. ^{1}H NMR Data of Intermediates and End Products of the Synthesis of Monogalactosyl Dilinoleylglycerol (**8**) and Monogalactosyl Monolinoleylglycerol (**6**)

	chemical shift (ppm), multiplicity, coupling constant (Hz)							
	^{1}H ($CDCl_3$)					^{1}H (methanol-d_4)		
carbon atom (steps 1 and 2)[a]		step 1	step 2	step 3 (**8**)	step 3 (**6**)	step 4 (**8**)	step 4 (**6**)	carbon atom (steps 3 and 4)[a]
glycerol moiety	1a	3.61, m	3.62, dd, 11.2, 4.7	4.16, m	3.97, m	4.21 dd, 11.7, 6.3	4.13, dd, 11.3, 5.8	1a glycerol moiety
	1b		3.69, dd, 11.2, 4.7	4.31, dd, 11.9, 3.6	4.01, m	4.40, dd, 11.7, 3.2	4.17, dd, 11.3, 4.6	1b
	2	3.76, dd, 9.6, 4.5	3.85, m	5.18, m	3.90, m	5.32, m	3.98, m	2
	3a	3.71, dd, 9.9, 5.4	3.78, dd, 12.8, 6.5	3.68, dd, 10.9, 5.7	3.55, dd, 10.3, 5.3	3.75, dd, 11.0, 6.3	3.66, dd, 10.7, 4.3	3a
	3b	3.98, dd, 9.9, 4.2	3.85, m	3.95, dd, 10.9, 4.9	3.76, dd, 10.3, 5.6	3.92, dd, 11.0, 5.5	3.91, dd, 10.6, 5.4	3b
carbohydrate moiety	1″	4.51, d, 7.9	4.51, d, 7.9	4.49, d, 7.9	4.48, d, 7.9	4.29, d, 7.4	4.24, d, 7.5	1″ carbohydrate moiety
	2″	5.20, dd, 10.8, 7.9	5.19, dd, 10.8, 7.9	5.18, m	5.07, dd, 10.4, 7.9	3.66, dd, 9.5, 7.5	3.56, dd, 9.8, 7.5	2″
	3″	5.00, dd, 10.4, 3.4	5.03, dd, 10.4, 3.4	5.00, dd, 10.5, 3.4	4.95, dd, 10.4, 3.4	3.60, dd, 9.5, 3.3	3.47, dd, 9.7, 3.3	3″
	4″	5.40, d, 3.4	5.40, d, 3.4	5.41, m	5.30, m	4.03, d, 2.7	3.85, d, 3.1	4″
	5″	3.86, t, 6.7	3.94, t, 6.2	3.90, t, 6.6	3.90, m	3.56, t, 4.6	3.53, m	5″
	6″a	4.15, dd, 6.7, 2.4	4.15 dd, 7.0, 1.6	4.12, m	4.07, m	3.87, dd, 11.7, 3.1	3.72, dd, 11.3, 5.4	6″a
	6″b					3.98, dd, 12.1, 5.9	3.78, dd, 11.3, 6.6	6″b
benzyl ether moieties	pos *sn*-1: a/b	4.56, d, 4.4						1′ acyl moiety
	pos *sn*-2: a/b	4.67, s		2.30, t, 8.4	2.25, t, 7.5	2.30, t, 7.5	2.35, t, 7.5	2′
aromate (*sn*-1 and *sn*-2)		7.33, m		1.62, m	1.56, m	1.62, m	1.62 m	3′
				1.26, m	1.23, m	1.25, m	1.31 m	4′−7′
				2.04, m	1.95, m	2.04, m	2.04 m	8′
				5.31, m	5.24, m	5.32, m	5.35 m	9′, 13′
				2.77, t, 6.6	2.68, t, 6.4	2.77, t, 7.2	2.78, t, 6.2	11′
				5.31, m	5.24, m	5.31, m	5.35 m	10′, 12′
				2.04, m	1.95, m	2.04, m	2.04 m	14′
				1.26, m	1.23, m	1.25, m	1.31 m	CH_2-($\underline{CH_2}$)-CH_3
				1.26, m	1.23, m	1.25, m	1.31 m	$\underline{CH_2}$-CH_2−CH_3
				1.26, m	1.23, m	1.25, m	1.31 m	$\overline{CH_2}$-$\underline{CH_2}$-CH_3
				0.89, m	0.79, m	0.89, m	0.91 m	CH_3
acetate moieties	1	1.98, s	2.00, s	2.00, s	1.90, s			
	2	2.01, s	2.07, s	2.07, m	1.89, m			
	3	2.06, s	2.09, s	2.08, m	2.02, m			
	4	2.17, s	2.18, s	2.17, s	2.10, s			

[a] Hydrogen atoms attached to carbon atoms designated in **Figure 1**.

monoacyl (*sn*-1; lyso) and diacyl (*sn*-1 and *sn*-2) compounds were accessible via this reaction pathway (**Figure 1**). For gaining the lyso compound we found that we could successfully utilize the fact that in the first phase of the reaction only the *sn*-1 position was acylated. Therefore, the reaction had to be stopped at the right moment to gain the maximum yield of the lyso (*sn*-1) compound. The optimum reaction time for this was between 5 and 7 min. The maximum yield of the diacyl compound was obtained after a reaction time of 3 h. The compounds **5** and **7** were purified by column chromatography, and the structures were confirmed by ESI^+ mass spectrometry and NMR experiments.

Deacetylation. The removal of the acetate protecting groups of the hydroxyl groups on the sugar moiety had to be carried out selectively, without affecting the fatty acid esters with the glycerol moiety. Mannock et al. (*11*) had suggested hydrazonolysis as the method of choice (**Figure 1**). In the literature yields of 40−90% are reported (*19, 20*), hereby using either an aqueous ethanol solution (85%, v/v) or methanol as the solvent and various temperatures and reaction times according to the carbon chain length of the fatty acid. Preliminary tests showed that methanol performed much better than aqueous ethanol (85%, v/v). Therefore, methanol was used as solvent for deacetylation. However, in all experiments lower yields (18−29%) than reported in the literature (*19, 20*) were obtained. After both compounds **8** and **6** had been purified by column chromatography, their structures were confirmed by ESI^+ mass spectrometry and NMR experiments. In total, 1.4 g (1.8 mmol) of **8** and 1.1 g (2.2 mmol) of **6** were synthesized.

Characterization of 8 and 6. The structures of the intermediate and final products were confirmed by ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments. The NMR data are presented in **Tables 1** and **2**. Clearly noticeable are the different effects caused by the benzyl ether, hydroxyl, and fatty acid moieties on the hydrogen atoms in the *sn*-1 and *sn*-2 positions of the glycerol moiety. The same can be seen with the effects caused by the acetate protection groups on the hydrogen atoms in the carbohydrate moiety. The ester linkages (C-1 and C-2) and the glycosidic linkage (C-3) with the glycerol moiety were established with HMBC experiments and characteristic hydrogen signals. The β-configuration of the glycosidic linkage was established with the coupling constant of the doublet from the hydrogen atom at the C-1″ position in the carbohydrate moiety ($J_{1,2} = 7.4$ Hz). A small amount of the 2-isomer of **6** (C-1-OH: ^{1}H = 5.05 ppm and ^{13}C = 73.8 ppm), due to acyl migration through acid contact

Table 2. HMQC and ^{13}C NMR Data of the Synthetic Galactolipids Monogalactosyl Dilinoleylglycerol (**8**) and Monogalactosyl Monolinoleylglycerol (**6**)

		chemical shift (ppm)			
		8		**6**	
carbon atom[a]		HMQC ($CDCl_3$)	^{13}C ($CDCl_3$)	HMQC (methanol-d_4)	^{13}C (methanol-d_4)
glycerol moiety	1	63.2	61.9	65.7	65.6
	2	70.8	69.2	68.7	69.3
	3	69.0	67.4	71.0	71.6
carbohydrate moiety	1″	104.7	103.0	104.5	104.3
	2″	72.4	70.7	71.6	70.9
	3″	74.1	72.5	74.0	73.9
	4″	70.0	68.5	69.4	68.5
	5″	75.0	73.6	75.9	75.7
	6″	63.4	61.7	61.5	61.5
acyl moiety	1′	174.7	172.7/172.5	174.8	174.4
	2′	35.5	33.3	34.0	33.9
	3′	25.2	23.8	24.9	25.0
	4′−7′	29.6	28.6	29.2	29.6
	8′	27.5	26.2	27.2	27.1
	9′, 13′	130.6	129.0/129.2	129.9	129.9/129.9
	11′	26.0	24.6	25.6	25.5
	10′, 12′	128.9	126.9/127.1	128.0	128.0/128.1
	14′	27.5	26.2	27.2	27.1
CH_2-($\underline{CH_2}$)-CH_3		29.6	28.6	29.2	29.6
$\underline{CH_2}$-$\overline{CH_2}$−CH_3		31.5	30.5	31.5	31.6
$\overline{CH_2}$-$\underline{CH_2}$-CH_3		23.0	21.6	22.5	22.6
$\overline{CH_3}$		14.3	13.1	13.2	13.4

[a] Carbon atoms designated in **Figure 1**.

in the last reaction step or during column chromatography on silica gel (*24*), could not be avoided (<1%). The ESI^+ mass spectra of **8** and **6** (see Materials and Methods) were in accordance with the NMR results. The glycolipid model compounds had been successfully synthesized.

Functional Properties of 8 and 6. As galactolipids have been reported to be highly active in breadmaking (*6*–*9*) the synthetic compounds were used as additives in microscale baking tests and microscale rheological tests based on 10 g of wheat flour. Although the relative standard deviation is higher, small-scale methods have been shown to be highly correlated with standard-scale methods based on 1000 g of flour (*25*), but they require only a very low amount of additive. Thus, the amounts of synthetic **8** (1.4 g) and **6** (1.1 g) were sufficient for all rheological and baking tests. Commercial synthetic surfactants such as DATEM, sodium stearoyl-2-lactylate, and monoglycerides as well as crude, defatted, and purified lecithins were used as reference surfactants to evaluate the potential of galactolipids in bread making.

Effect of 8 and 6 on the Baking Performance of Wheat Flour. To get a homogeneous distribution of the surfactants in the dough, they were either added in the water (watery suspension) used for mixing or dissolved in suitable solvents (hexane, chloroform, THF), which were then removed by evaporation under ambient conditions (hexane, chloroform) or under reduced pressure (THF). Preliminary studies had shown that the solvents did not have an influence on the baking result. There were no significant differences between the control breads baked with and without the solvent method (pure solvent without surfactant). The values for the relative change in bread volume, with the control breads set to a volume change of ±0%, are shown in **Table 3**. To determine the optimum amount of the surfactants to gain the maximum increase of the bread volume, concentrations of 0.1−1.0% were used for the baking tests. Higher amounts are unrealistic in regard to the concentrations used in the baking industry. Besides measuring the bread volume, the influence of the additive on the crumb firmness was additionally recorded. To take the dough losses during the bread production into account the volumes measured were based on the dough weight of 10 g (before the second proofing session). Control experiments (with and without solvent method) were carried out for each individual test series to take into account possible environmental or technical fluctuations. The intraday variation of the bread volumes was below 2.7%, and the interday variation calculated from the control bread volumes was 23% with loaf volumes ranging from 23.1 to 28.5 mL per 10 g of dough. For better comparison, the values for the baking performance are expressed as volume change in percent based on the control breads.

Effect on Bread Volume. The crude lecithins showed the poorest baking performance of all surfactants with a maximum volume increase of 34% at a concentration of 0.8−1.0% (**Table 3**). As already described by Helmerich and Koehler (*26*) the direct comparison of the three crude lecithins revealed that the baking activity of sunflower lecithin was clearly inferior to that of soybean and rapeseed lecithin. The defatted and purified lecithins behaved similarly and were more active (maximum volume increase = 35.5%) than the crude lecithins. Most of them showed the maximum activity at a concentration of 0.8%, with the purified samples being more active at 0.4%. Thus, it can be assumed that the extraction of the nonlipid part by Folch wash positively affects the baking performance at low concentrations.

The commercial synthetic surfactants DATEM, sodium stearoyl-2-lactylate, and monoglycerides were chosen as reference samples. DATEM was applied as a watery suspension and as a solution in chloroform. There were no significant differences between the two application forms. They both reached their maximum volume increase at a concentration of 0.6% (**Table 3**). However, quite a similar volume increase was already reached at the additive amount of 0.4%, and this level of increase stayed more or less the same up to the maximum additive

Table 3. Microscale Baking Test with 10 g of Flour (Wheat Cv. Tommi): Change of Bread Volume As Affected by the Type and Concentration of Surfactant in Comparison to Control Bread without Additive

		change of bread volume (BV, %) and relative standard deviation (RSD, %) at a concentration[a] of											
		0.1%		0.2%		0.4%		0.6%		0.8%		1.0%	
additive		BV	RSD[b]	BV	RSD	BV	RSD	BV	RSD	BV	RSD	BV	RSD
lecithin from	soybean, purified[c,e]	−[d]	−	−3.0	0.6	23.2	0.1	28.3	0.6	33.1	0.1	30.3	1.9
	soybean, defatted[e]	−	−	−4.4	0.3	10.9	1.0	25.5	1.1	34.5	0.8	30.3	1.1
	rapeseed, purified[c,e]	−	−	−6.4	1.6	23.6	2.2	27.2	2.4	33.4	0.9	35.6	1.7
	rapeseed, defatted[e]	−	−	−7.6	1.3	14.3	2.5	24.0	2.4	34.7	1.8	30.2	2.3
	soybean, crude[e]	−	−	−5.0	1.4	1.1	0.8	3.2	1.6	24.6	1.3	32.8	0.9
	rapeseed, crude[e]	−	−	−0.1	0.9	−1.3	0.8	9.0	1.5	19.6	1.6	16.2	1.6
	sunflower, crude[e]	−	−	−0.9	1.4	−0.5	1.5	2.7	1.2	9.2	0.7	19.9	2.7
diacetyltartaric esters of monoglycerides (DATEM)[f]		−0.7	1.2	4.4	1.1	31.5	1.4	38.9	1.4	36.2	1.1	35.7	1.0
diacetyltartaric esters of monoglycerides (DATEM)[e]		−	−	6.4	1.8	32.5	1.5	36.7	1.7	37.1	0.9	−[d]	−[d]
monoglycerides[h]		−	−	8.5	0.3	39.1	0.7	45.7	0.7	40.6	0.5	32.1	0.5
sodium stearoyl-2-lactylate[e]		−	−	−0.3	1.2	18.4	1.4	20.7	1.8	17.6	0.8	30.0	0.8
monogalactosyl diglyceride (**8**)[g]		−3.4	0.6	−1.1	0.9	19.2	0.6	35.1	1.1	35.0	1.4	−	−
monogalactosyl monoglyceride (**6**)[f]		2.4	0.3	8.7	1.5	45.6	1.5	54.4	1.1	51.8	1.1	−	−

[a] Concentration of additive based on 10 g of flour (= 100%). [b] Relative standard deviation; number of experiments $n = 3$. [c] Defatted lecithin purified by Folch wash. [d] Concentration not tested. [e] Added as a watery suspension. [f] Added as a solution in chloroform. [g] Added as a solution in hexane. [h] Added as a solution in THF.

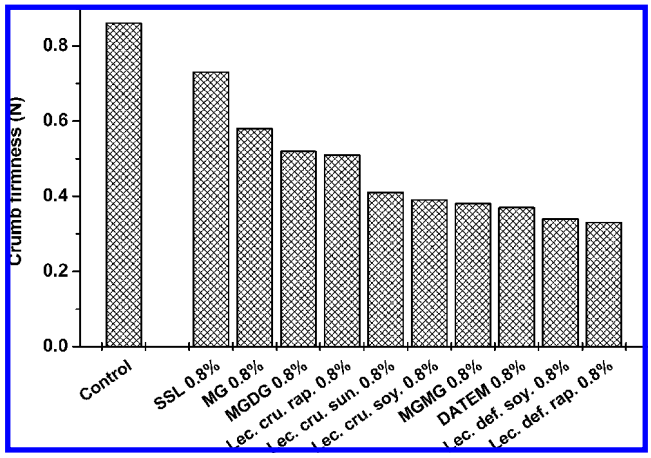

Figure 2. Data on crumb firmness of breads with various additives at a concentration of 0.8% based on flour (10 g = 100%). DATEM, diacetyltartaric acid esters of monoglycerides; Lec, lecithin; cru., crude; def., defatted; rap., rapeseed; soy., soybean; sun., sunflower; MG, monoglycerides; MGDG, monogalactosyl dilinoleylglycerol; MGMG, monogalactosyl monolinoleylglycerol; SSL, sodium stearoyl-2-lactylate. Number of experiments $n = 3$ (relative standard deviation below ±20%).

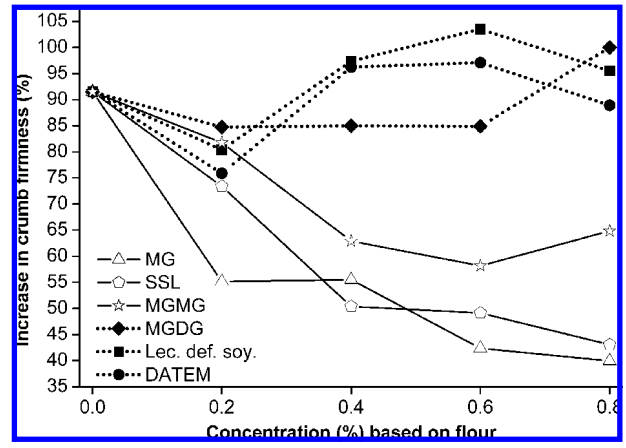

Figure 3. Increase in crumb firmness after a storage period of 23 h as affected by the type and concentration of surfactant in comparison to control breads. Amount of additive based on 10 g of flour (=100%). DATEM, diacetyltartaric acid esters of monoglycerides; Lec, lecithin; def., defatted; soy., soybean; MG, monoglycerides; MGDG, monogalactosyl dilinoleylglycerol; MGMG, monogalactosyl monolinoleylglycerol; SSL, sodium stearoyl-2-lactylate. Number of experiments $n = 3$ (relative standard deviation = ±2−10%).

amount of 1.0%. This sort of plateau phase in this additive amount range is known (*17*) and is quite in contrast to the lecithins, showing a clear maximum. The application of the monoglycerides was a little bit more challenging. The direct addition of the powdery monoglycerides resulted in slightly negative volume increases in the additive amount range of 0.4−1.0%. The application of the monoglycerides as a solution in THF revealed a surprising result. The bread volume increase was even higher than the increase caused by DATEM, showing the same plateau phase phenomena. This surprising difference in the volume increases between the two application methods demonstrated the enormous importance of the complete dispersal of the surfactant in the flour before dough mixing in order to unfold its entire baking activity. Sodium stearoyl-2-lactylate was applied as a watery suspension. The baking activity did not reach by far the level of DATEM (chloroform or water) or the monoglycerides (THF). However, similar to DATEM and the monoglycerides, it showed a plateau phase phenomenon. The baking activity of DATEM (chloroform or water) and the monoglycerides (THF) in comparison to the lecithins was clearly better, especially at lower levels of addition. Sodium stearoyl-2-lactylate (water), however, was between the lecithins (defatted/water) and the lecithins (crude/water) with its baking activity. These results are not in accordance with those of Schuster (*1*), who reported that glycerol monostearate (applied on a calcium phosphate support) had gained a lower bread volume increase in comparison to DATEM and sodium stearoyl-2-lactylate, with DATEM displaying the highest increase. This demonstrates that the true baking performance of a surfactant is revealed only when the dispersal of the surfactant in the flour is optimum.

The two synthetic glycolipids were applied in different organic solvents because of their different solubility behaviors. **8** was dissolved in hexane and **6** in chloroform. The volume increase data are given in **Table 3**. They both displayed quite different baking activities. The first positive bread volume increase was already registered with **6** at an additive amount of 0.1%, in contrast to **8**, for which the first positive volume

Table 4. 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 of Surfactant at a Concentration of 0.6% in Comparison to Control Gluten without Additive

additive (concn = 0.6%[a])		RE (N)	RSD[b] (%)	EX (mm)	RSD (%)	energy (N × mm)	RSD (%)
control	(no additive)	1.00	4.0	137.7	1.7	61.0	8.0
lecithin from	soybean, purified[c,d]	1.06	0.9	151.9	3.4	68.6	5.5
	soybean, defatted[d]	1.07	3.7	151.1	2.2	74.6	6.2
	rapeseed, purified[c,d]	1.00	4.0	149.0	4.0	63.6	10.2
	rapeseed, defatted[d]	0.92	3.2	144.1	3.1	61.6	6.7
diacetyltartaric esters of monoglycerides (DATEM)[e]		1.22	2.5	152.9	3.5	87.5	3.5
monoglycerides[g]		1.14	3.5	145.7	2.7	73.6	6.6
monogalactosyl diglyceride (**8**)[f]		0.95	3.2	142.4	4.2	59.0	6.0
monogalactosyl monoglyceride (**6**)[e]		1.11	5.4	181.1	1.8	87.3	5.0

[a] Concentration of additive based on 10 g of flour (= 100%). [b] Relative standard deviation; number of experiments $n = 2$. [c] Defatted Lecithin purified by Folch wash. [d] Added as a watery suspension. [e] Added as a solution in chloroform. [f] Added as a solution in hexane. [g] Added as a solution in THF.

Table 5. Hydrophilic−Lipophilic Balance (HLB) Values of Monogalactosyl Diglycerides (**8**), Monogalactosyl Monoglycerides (**6**), Monoglycerides and Sodium Stearoyl-2-lactylate (SSL) Calculated According to Davies (*4*) and Griffin (*2, 3*) As Affected by the Fatty Acid Residue (Reference Data from the Literature Are Given for Comparison)

	HLB value of				
fatty acid	**8**	**6**	monoglycerides	SSL	calcd according to
8:0	12.2	15.0			Davies
	12.2	14.8			Griffin
16:0	4.6	11.2	4.7		Davies
	8.5	11.4	7.3		Griffin
18:0	2.7	10.3	3.7	23.3	Davies
	7.9	10.8	6.7	9.4	Griffin
			2.8−3.8 (*29*)	18−21 (*29*)	from the literature

increase was found for the additive amount of 0.4%. Both reached their individual maxima in volume increase at the additive amount of 0.6% with **8** and **6**, showing volume increases of 35.1 and 54.4%, respectively. These experiments showed that glycoglycerolipids with one carbohydrate moiety (here galactose) and linoleic acid as the fatty acid monoacyl compound (lyso compound) possess a far better hydrophilic lipophilic ratio in comparison with the diacyl compound. **6** caused the highest volume increase at all concentrations and displayed the best baking activity of all examined surfactants. Therefore, it was the surfactant with the best hydrophilic lipophilic ratio. The baking activity of **8** was between the activities of DATEM, the monoglycerides, and the best lecithins (**Table 3**).

Effect on Crumb Firmness. The downward force required to gain a deformation of 0.7 cm of a standardized piece of bread crumb was used as a measure for the crumb firmness. Some of the results are shown in **Figure 2**. To eliminate the effect of crumb softening due to the increase of the bread volume after the addition of surfactants, a volume factor (F = bread volume$_{\text{with additive}}$/bread volume$_{\text{control}}$), calculated from the data of the microscale baking tests for each additive and concentration, was used. The relatively high standard deviation (up to 20%) is typical for this method. Therefore, the detailed analysis of the data was not feasible. However, general tendencies can be recognized when looking at the highest concentration range of 0.8% (**Figure 2**). The visible tendency was that the bread crumbs in all test series over the whole additive concentration range became softer with higher concentrations of additive. As in the baking tests **6** was more effective than **8**.

Antistaling Effect of Surfactants. Some polar lipids are known to inhibit the growth of amylose and amylopectin crystals during storage after gelatinization, and some of them slow starch retrogradation. Thus, the so-called staling or aging of the crumb is slowed. To measure this effect the firmness of the fresh (1 h after baking) and stored (24 h) bread crumb was compared. The bread crumb cylinder used for the measurement of the crumb firmness of fresh bread was stored in a small glass cylinder with a ground lid, to prevent the crumb from drying while it was stored. After 24 h, the crumb firmness was measured again. For better comparison, the results were expressed as increase of the crumb firmness in percent after 24 h in comparison to the firmness of the fresh crumb. An antistaling effect becomes evident when the increase of the bread crumb firmness after the addition of a surfactant is lower than the increase measured for the control. The results of these experiments are shown in **Figure 3**. For all lecithins and DATEM no significant antistaling effect was present. As expected, sodium stearoyl-2-lactylate and the monoglycerides were the only two samples, which retarded the staling of the crumb due to the formation of amylose and amylopectin complexes (*27, 28*) with saturated fatty acids (16:0, 18:0) present in the samples. From the two synthetic glycolipids only **6** showed a weak antistaling effect, but it was considerably less effective than sodium stearoyl-2-lactylate and the monoglycerides because linoleic acid present in this compound is not as capable of forming complexes with amylose and amylopectin due to its bent shape. This is also valid for compounds having two fatty acid residues such as **8**, which had no detectable effect at all. These results show that the antistaling effect of surfactants being an indirect effect in breadmaking does not have to be in correlation with the direct effects, such as bread volume increase.

Microextension Tests. Polar lipids can interact with proteins in various ways and therefore cause many changes in the gluten network of wheat dough. However, the extension tests with dough containing surfactants did not exhibit any recordable differences in dough stability and extensibility. Therefore, all further extension tests were carried out with gluten. In **Table 4** the data of the extension tests of gluten from dough containing 0.6% of surfactant are shown. The resistance to extension and the extensibility of the examined gluten with the various additives exhibited a significant difference to the control in the least amount of cases. The values obtained from the area under the extension curves in a typical extensigram (extensibility energy) usually give quite a good indication toward the possible baking activity of the sample. The higher the value for the

extensibility energy is, the higher the bread volume increase will be. The commercial surfactant DATEM and the synthetic glycolipid **6**, followed by the monoglycerides and soybean lecithin (defatted), displayed significant increases in comparison to the control. The results generated by the microextension tests were in relatively good correlation with the results gained by the microscale baking tests. The microextension tests were of great value to verify the results obtained from the microscale baking tests. Therefore, the combination of both made an accurate evaluation of the individual baking activity of the tested surfactants possible.

HLB Values of Galactolipids. Griffin (*2, 3*) introduced the HLB concept as an index ranging from 0 to 20 that conveyed the affinity of a nonionic surfactant to oil or water. Derived from Griffin's original equation, the individual HLB value of a surfactant is calculated on the basis of the ratio between the molecular weight of the hydrophilic part of the molecule (M_h) and the molecular weight of the entire molecule (M):

$$HLB_G = 20(M_h/M) \quad (A)$$

The HLB value of a surfactant may be used as a guide to its most appropriate application. For instance, the following applications have been suggested: w/o surfactant (HLB of 3−6), wetting agent (HLB of 7−9), o/w surfactant (HLB of 8−18), detergent (HLB of 13−15), and solubilizer (HLB of 15−18) (*29*). However, the Griffin system cannot be used for indexing ionic surfactants. This drawback was eliminated by the proposed system of Davies (*4*), in which numerical values were assigned to the individual functional groups of surfactants:

$$HLB_D = \sum(\text{hydrophilic group numbers}) - \sum(\text{lipophilic group numbers}) + 7 \quad (B)$$

To correlate the functional properties of the synthetic galactolipids with structural features, the HLB values were calculated according to eqs A and B (**Table 5**). According to the Davies method no group number for the glycosidic linkage to the sugar moiety was available. Therefore, the group number of a free ester was assigned to this type of linkage. The method proposed by McGowan (*5*) is similar to that of Davies, and the values gained hereby were in the same range.

Pomeranz and Wehrli (*10*) had found that octanoic acid was the optimum fatty acid chain length for **8**. The calculated HLB value for this molecule was 12.2, obtained by both the Davies and Griffin methods. This is in accordance with the optimum emulsion stability at a HLB value of 12.0 for o/w emulsions (*30*). For **6** palmitic acid would be the fatty acid providing HLB values nearest to an optimum emulsion stability (HLB = 11.2−11.4), followed closely by our synthetic glycolipid **6** with linoleic acid (HLB = 10.3−10.9). This is in accordance with the high baking performance of this compound (**Table 3**). Unlike for **6** the HLB values for **8** calculated by the Davies and Griffin methods did not match for fatty acids different from octanoic acid such as palmitic, stearic, oleic, and linoleic acid. Here the HLB value provided by the Davies method was always clearly smaller (**Table 5**). When the HLB values calculated for our synthesized glycolipids (both with linoleic acid) were compared with their results gained by the baking tests (**Table 3**), the HLB values calculated with the Griffin method showed clearly the better correlation than the Davies method and demonstrated that HLB values can be used to predict the baking activity of glycoglycerolipids. Comparison of the two methods had shown that the Davies method exhibited weaknesses in calculating the HLB values for galactolipids accurately. In general, there is still no universal calculation method devised to accurately calculate HLB values of different surfactants (ionic, nonionic).

Concluding Remarks. This study confirmed that galactolipids are highly active as flour improvers, in particular **6**, which performed substantially better than any other surfactant in this study. Chemical synthesis enabled the detailed investigation of the individual effects caused by the number of fatty acids in monogalactosylglycerides. For monoacyl galactolipids such as **6**, not only is the baking activity higher as compared to diacyl galactolipids such as **8**, but there is also a positive effect on the retardation of bread staling. However, even the less active diacyl galactolipid **8** was able to compete with several commercial surfactants that are used in breadmaking. The HLB concept according to Griffin (*2, 3*) can be used to predict functionalities even if inaccuracy has to be tolerated to a certain extent. HLB values ranging from 8 to 12 seem to be typical for surfactants that are highly active in baking.

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