

## FINAL REPORT

## Evaluation of Monoacylglycerols Derived from Butteroil as Emulsifiers in Foods

Personnel: Kirk L. Parkin, associate professor, Kathleen Wiederholt, research specialist, (7/1/93-10/21/94) replaced by Purwiyatno Hariyadi and Shu-Jung Kuo, research assistants, Department of Food Science; Marit Reierstad, Honorary Fellow (graduate student), Agricultural University of Norway

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### Objectives

1. Prepare monoacylglycerol (MAG)-rich fractions from butteroil at levels suitable for functionality and applications evaluation.
2. Develop procedures to partially purify and/or fractionate MAG-rich fractions prepared from butteroil for functionality and applications testing.
3. Evaluate the physicochemical properties and related functionality of MAG-rich fractions prepared from butteroil as emulsifying agents.
4. Evaluate the effectiveness of selected MAG-rich fractions prepared from butteroil in selected food systems.

### Summary

Objective 1: Two basic reaction configurations were evaluated for several lipase sources for their ability to produce MAG (and DAG) blends derived from butteroil fatty acids. Glycerolysis reactions used butteroil and glycerol as substrates, whereas esterification reactions used glycerol and fatty acids (FA) derived from butteroil (by saponification) as substrates. In the latter case, the fatty acid composition of the starting substrate was diminished in short chain-length fatty acids because of partial losses during saponification.

Of the enzymes evaluated, both soluble and immobilized *Rhizomucor miehei* lipases (Palatase and Lipozyme, respectively) were capable of yielding 85-90% [MAG + DAG] with >50% MAG in as little as 10 hr reaction time, in esterification reactions. Pancreatic lipase, the other food-grade enzyme evaluated in this manner, could yield only 30-50% [MAG + DAG], primarily as DAG, in 48 hr reaction times. Non-food-grade enzymes from *Pseudomonas* sp (type AK and PS-30), and *Candida rugosa* yielded 60-70% [MAG + DAG] in as little as 4-10 hr reaction time, but the principal product was DAG.

In glycerolysis reactions, only the soluble *R. miehei* lipase was capable of yielding >70% [MAG + DAG], requiring 40-80 hr reaction time to do so, with the fungal preparations (Genus/species unknown) "Lipase Preparat" and "Lipolact K1" being less effective. Pre-gastric lipases in this reaction configuration were not effective. Previous work in this laboratory established that the *Pseudomonas* lipases (Amano, type AK and PS-30) were the most effective in yielding [MAG + DAG] from butteroil in glycerolysis reactions. In a recent trial, Novozyme 435 lipase (a *Candida antarctica* lipase cloned into *Aspergillus oryzae*, potentially a food-grade preparation) showed promise in glycerolysis reactions, yielding up to 50% MAG after 24-48 hr reaction time.

Objective 2: Starting with about 100 g of butteroil, an MAG-rich (>90%) fraction of about 40 g was prepared using various lipases. The >90% pure MAG fraction was obtained from the final product mixture by repeated precipitation with 4-5 volumes hexane, followed by filtration. Amano type PS-30 (*Pseudomonas cepacia*) and Novozym 435 (*Candida antarctica* B lipase produced by a cloned *Aspergillus oryzae* strain) lipases were effective in this manner. Two food-grade lipases, Novo's Lipozyme IM and Palatase M (both from *Rhizomucor miehei*) are also effective, but only if butteroil was first hydrolyzed and the free fatty acids (FA) then combined with

glycerol and catalyst in an esterification reaction configuration.

Both PS-30- and Palatase M-derived MAG preparations were evaluated fully by separating the product mixture into triacylglycerol (TAG), FA, DAG and MAG components by chromatography using silicic acid and aminopropyl-bonded solid phase cartridges. Both enzyme-derived MAG preparations became enriched in myristic, palmitic and stearic acids, and diminished in shorter chain-length and unsaturated fatty acids, compared to native butteroil (indicated by "product mixture") (Figures 1 and 2). Palatase-mediated processes tended to concentrate C4-6 FA in the DAG/FA fractions, C8-12 FA in the TAG/FA fractions, and C18:1-2 FA in the TAG/DAG fractions. The PS-30-mediated processes tended to concentrate C4-6 FA in the TAG/DAG fractions, C8-12 in the FA fraction, and C18:1-2 in the TAG/DAG/FA fractions. Thus, preparing MAG from butteroil in this fashion also resulted in an effective fractionation of fatty acids into different pools. High- mid- and low-melting butteroil fractions (HMF, MMF and LMF, respectively) were also used as starting materials for preparing MAG fractions. Relative to MAG prepared from native butteroil, MAG from LMF was enriched in C8-12, 18:1 FA and diminished in C14-16 FA; MAG from MMF was enriched in C8-12, 18:1 FA and diminished in C14-16 and C4-6 FA; MAG from HMF was diminished in C4-6 FA and enriched in C8-12 FA.

Objective 3: Hexane-precipitated MAG (66-74% purity) from the enzyme-modified butteroil preparations were compared with a commercial MAG (>90%) preparation for physicochemical analyses. The commercial MAG preparation was effective at reducing surface tension at an oil-water interface (at levels of MAG addition of 0.05-0.5% of the oil phase) to 15-5 mN m<sup>-1</sup>. (The commercial MAG product was 70, 17 and 11% in 18:1, 18:0 and 16:0, respectively). MAG prepared from the high- and mid-melting butteroil fractions were as functionally capable as the commercial emulsifier, whereas MAG prepared from low-melting fraction and native butteroil were slightly less effective at reducing surface tension.

Solid fat content of the MAG-rich fractions indicated that they had behavior intermediate to the

commercial MAG preparation and native butteroil (Figure 3). MAG-rich fractions from HMF of butteroil had greater solidity than MAG prepared from LMF. However, the most important feature was the relatively constant % solids content for the butteroil-derived MAG preparations over the range of 0-35°C, indicating a substantial plastic range. This behavior would be promising for the development of fat-based table spreads. At a 0.5% level of addition to native butteroil, MAG-rich fractions prepared with Palatase tended to slightly increase solidity over the range of 0-15°C, whereas MAG-rich fractions prepared with PS-30 tended to slightly suppress solidity over the same temperature range. Between 20-40°C, there was little effect of MAG addition on melting behavior of butteroil.

Emulsion stabilizing ability was tested in 6, 12 and 20% water-in-oil model systems using 0.5% MAG (based on oil phase). Stability was greatest in 6% water systems, and there was little difference in stabilizing effect among the butteroil-derived and commercial MAG preparations. All MAG preparations were not very effective in stabilizing these emulsions relative to the influence that a more hydrophilic emulsifier, tween 80, had on stabilizing these emulsions.

Starch-complexing activity was evaluated using a 0.19% amylose solution to which 0.012, 0.024 and 0.048% different MAG preparations (84-90% purity in MAG) were added. Amylose-complexing ability of the MAG preparations derived from the LMF and MMF of butteroil was greater than or equal to that of the commercial MAG product at all levels evaluated, whereas the MAG preparation derived from the HMF of butteroil was least effective (Figure 4). These results indicate the ability of the butteroil-derived MAG preparations to be effective anti-staling or crumb-softening agents in baked goods.

Objective 4: Preliminary studies indicated that the butteroil-derived MAG was equally as effective as a commercial emulsifier blend when used in ice cream (these results evolved into a project to focus specifically on this application - see Final Project Report by Hartel and Parkin).

Figure 1. Palatase-mediated partitioning of fatty acyl groups

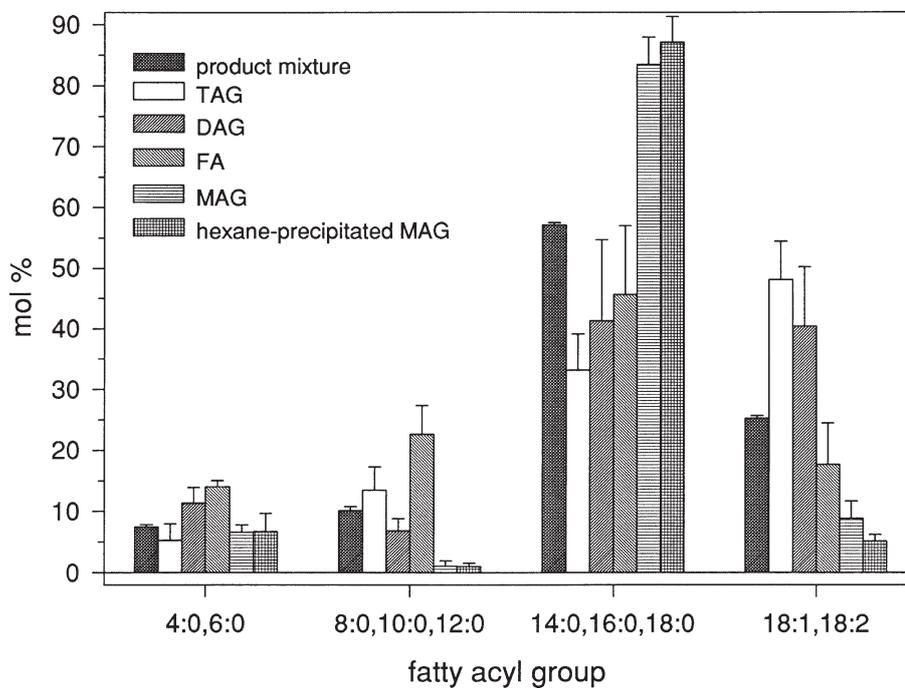
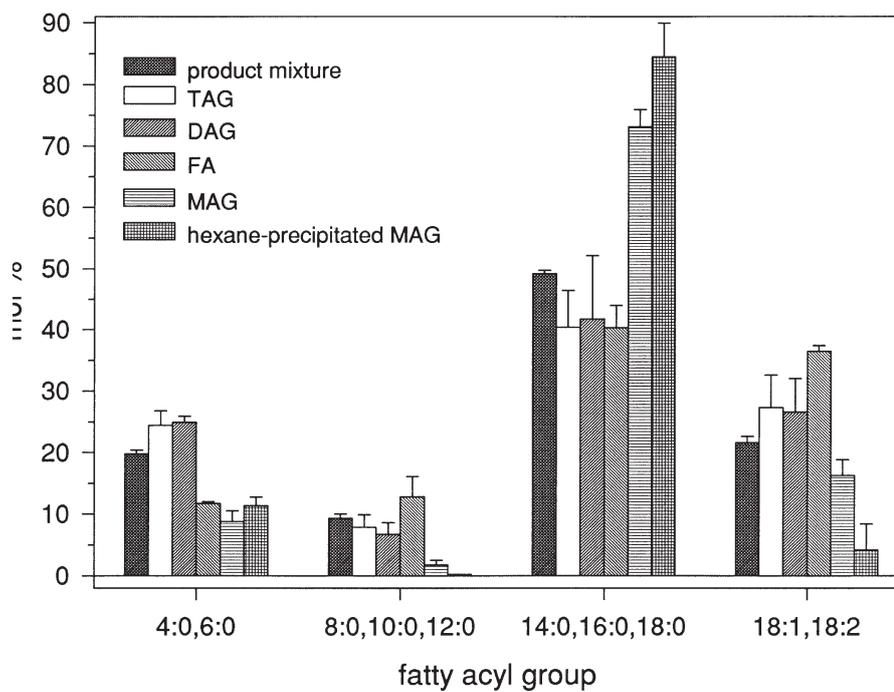


Figure 2. PS30-mediated partitioning of fatty acyl groups



The effect of the MAG-rich fractions incorporated into a simple cake product (48% each water and flour, 2.8% oil and 0.48% MAG preparation) indicated that staling (textural hardness as measured with a TA.XT2 analyzer) took place to similar extents over a 7 day period at 4°C for samples formulated with MAG-rich fractions from butteroil, butteroil fractions or a commercial preparation. Preliminary studies with reduced fat (40, 60%) table spreads also indicated a similar degree of emulsion stabilization by MAG-rich prepared from butteroil compared to commercial MAG products. Samples of MAG-rich preparations derived from butteroil were also supplied to Dr. W. James Harper at The Ohio State University for applications testing in baked goods.

### Significance to the dairy industry

These studies indicate that food-grade MAG-rich preparations can be enzymically derived from butteroil and/or butteroil fractions, and that these preparations have physicochemical and functional properties similar to commercial MAG products available from other sources. Thus, the opportunity exists to increase the utilization of butteroil-derived resources in markets that are conventionally dominated by nondairy resources. Focus in future product development efforts should be placed on 1) identifying appropriate products/markets in which to utilize butteroil-derived MAG, and 2) exploiting any unique (or dual, including any flavoring impact of butteroil) functionality that butteroil-derived MAG can offer.

Figure 3. Melting behavior

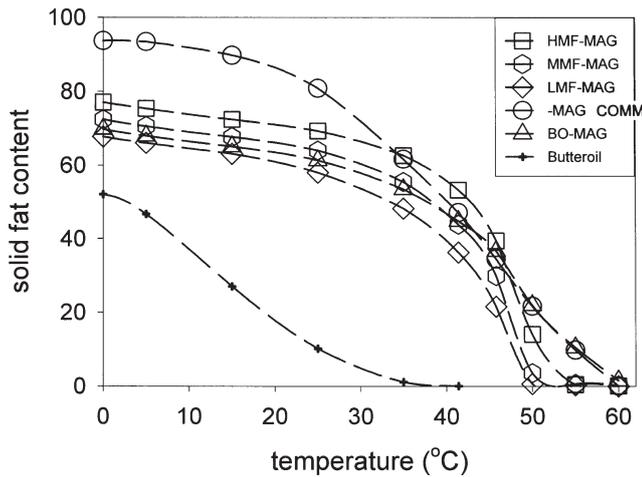


Figure 4. Amylose-complexing properties

