Long-Term Stability Studies on Stored Glycerol Monostearate (GMS)-Effects of Relative Humidity

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The long-term hydrolysis and isomerizations that occurred in commercial glycerol monostearate (GMS) have been studied under controlled low relative humidity (ca. 30%) and high relative humidity (ca. 90-100%). Acid number values increased 2-3 units over an 84-day period in high relative humidity, and only 0.5 unit in low humidity.

Changes in the total α-monoglyceride, β-monoglyceride, α,α′- and α,β-diglyceride concentrations with time offered a method to observe the processes involved. Changes in monoglyceride and diglyceride concentrations of GMS were monitored using the BSTFA [(N,O)-bis(trimethylsilyl)trifluoroacetamide] derivative and fused silica capillary gas chromatography. Isomerization was shown to occur before any substantial hydrolysis. Decreases in concentration of α,β or α,α′-diglyceride (after isomerization was essentially complete) corresponded to the acid number rises observed.

Preparations of monoglycerides have been well documented in the literature (1-3), and many instances of enzyme-catalyzed hydrolysis of glycerides, mainly triglycerides (4-9), are known. However, few references were cited involving the long-term hydrolysis (nonenzymatic) of glycerides (10). One such article describes the long-term rancidity of stored wheat flour. It was concluded that the rancidity development was due to the oxidation of the double bonds of fatty acids and the hydrolysis of glycerides. An acid number increase was observed during rancidity development. However, it was stated that there was no quantitative relationship between the increase in acid number and the degree of rancidity. Similarly, various literature references (11-13) have shown that isomerization of glycerides can occur in the solid state (i.e., α,β-diglycerides to α,α′-diglycerides and β-monoglycerides to α-monoglycerides).

This study is focused on the cause for the gradual rise in the acid number of commercial glycerol monostearate (GMS) with time (3 mo). Commercial GMS is a mixture of α- and β-monoglycerides, α,α′ and α,β-diglycerides and triglycerides. Commercial GMS samples that have exhibited acid number increases were submitted for biological (microbial, bacterial) analysis (Hess Environmental Services, Inc., and Woodson-Tenent Laboratories, Inc., Memphis, Tennessee). No indication of partial hydrolysis through biological means was found. To determine the effect of relative humidity on GMS (initial AN 1.2), stability tests were done at two temperature ranges. Shelf-life stability was done at 20-25 C in 90-100% relative humidity. Stability tests were also done at 40-45 C at low and high relative humidity (30% and 90-100%, respectively).

EXPERIMENTAL

Materials. GMS was supplied by Humko Chemical Division of Witco Corp. The pure (99+%) compounds listed in Table 1 were used to determine response factors required for the calculation of concentrations. These compounds and the internal standard tridecanoin were obtained from NuChek Prep., Inc. (Elysian, Minnesota). The derivatizing agent BSTFA [(N,O)-bis(trimethylsilyl)trifluoroacetamide] was purchased from Supelco, Inc. (Bellefonte, Pennsylvania).

Equipment. Fused silica capillary gas chromatography was conducted with a Hewlett Packard 5710 equipped with flame ionization detector and split capillary injector. The chromatograph was interfaced with a Hewlett Packard Model 3392A integrator-recorder.

Chromatographic conditions. Separations were done on a 0.33 mm × 3 M fused silica CP® Sil5, WCOT capillary column. The initial oven temperature upon injection was 180 C with an initial hold time of 4 min. The temperature was increased at a rate of 32 C/min until a final oven temperature of 320 C was obtained. This final temperature was maintained for 8 min. The purge flow rate was 1 ml/min with an inlet pressure of 4 psi He. The split vent flow rate, split ratio and chart speed were 26 ml/min, 25:1 and 2.0 cm/min, respectively.

Analyses of GMS—comparison of capillary GC method to periodate wet method. Formulated samples of GMS with various α-monoglyceride concentrations (Table 2) were analyzed by both the capillary GC method (14) and the periodate wet method (AOCS Official Method

TABLE 1

Primary Glyceride Standards

Component[a,c]	Identification
Myristic acid	C14FA
Palmitic acid	C16FA
Margaric acid	C17FA
Stearic acid	C18FA
Arachidic acid	C20FA
Monomyristin	C14 α-mono
Monopalmitin	C16 α-mono
Monomargarin	C17 α-mono
Monostearin	C18 α-mono
Dimyristin[b]	C14 α,α′-di
Dipalmitin	C16 α,α′-di
Dimargarin	C17 α,α′-di
Distearin	C18 α,α′-di
Dipalmitin	C16 α,β-di
Distearin	C18 α,β-di
Trimyristin	C14 tri
Tripalmitin	C16 tri
Trimargarin	C17 tri
Tristearin	C18 tri
Triarachidin	C20 tri

[a] The C20 monoglyceride and the C17 α,β-diglyceride were not available.

[b] The C14 α,β-diglyceride was not used due to similarities in retention times with the internal standard (tridecanoin).

[c] Triglyceride percentages did not vary appreciably from sample to sample (3-6%) and are not listed in Figures 3-5.

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TABLE 2

α-Monoglyceride Determination on Formulated GMS Samples by GC Method and Periodate Wet Method

GC Method (%, n = 3)	Std Dev	Periodate Method (%, n = 5)	Std Dev
89.2	0.6	90.6	1.0
81.3	0.6	81.5	0.7
67.5	0.8	66.7	0.9
53.7	0.8	54.8	0.6
46.5	0.9	45.1	0.5

Cd11-57). Results indicate that for the purposes of this study, the capillary GC method is reliable for observing the changes that occur in GMS with time.

Stability test (40-45 C) of GMS—effect of internal moisture on acid number. GMS initially containing 0.10% moisture (See Table 3 for typical analysis.) was formulated with water to give moisture levels of 0.18%, 0.5% and 2.2%. The calculated amount of water was added to molten GMS in a jar. The jar was capped and shaken vigorously until the mixture had solidified. The samples were analyzed for percent moisture and then placed in an oven at 40-45 C. The samples were analyzed for acid number.

Stability test (40-45 C) of GMS at low relative humidity (ca. 30%). Samples of GMS were placed in containers having aluminum foil tops with holes. The temperature of the oven was set at 40-45 C. Relative humidity was measured with a psychrometric probe (Cole-Parmer Instrument Company). Samples were analyzed for acid number, percent α-monoglyceride [by both the periodate wet method (AOCS Official Method Cd11-57) and capillary GC analysis], percent β-monoglyceride, percent α,β-diglyceride, percent α,α′-diglyceride and percent triglyceride (all by capillary GC).

Stability test (20-25 C and 40-45 C) of GMS at high relative humidity (ca. 90-100%). The GMS samples in open containers were placed in 2-l beakers containing water. The tops of the beakers were sealed with aluminum foil. A GMS sample was placed in the oven at 45-50 C, and another sample was left out at room temperature (20-25 C). Samples were analyzed for acid number, percent α-monoglycerides (by both the periodate wet method and capillary GC), percent β-monoglycerides, percent α,β-diglycerides, percent α,α′-diglycerides and percent triglycerides (all by capillary GC).

Sample preparation for fused silica capillary GC analysis (14). All pure compounds used in determining response factors and all GMS samples were converted into the BSTFA [(N,O)-bis(trimethylsilyl)trifluoroacetamide] derivative. Solutions for GC injections were prepared by dissolving 0.1 g of sample in 10 ml of methylene chloride. Internal standard (0.5 ml of 1.2262 g tridecanoin/100 ml) was added, and the mixture was evaporated to dryness under a stream of nitrogen gas. BSTFA (1 ml) was added, and the resulting solution was heated at 100 C for 5 min. The derivative (0.2 μl) was then injected on the capillary GC.

Response factors were calculated using the following formula:

TABLE 3

Typical Initial Analyses of GMS

Analyses	
IV	0.3
Color (Gardner)	1
Acid number	1.2
Saponification No.	172.7
% α-Monoglyceride	43.4
% Free glycerine	3.0
% Moisture	0.1
Capillary MP (C)	58.0
Composition	
C14	2.0
C15	0.4
C16	50.2
C17	2.1
C18	45.2

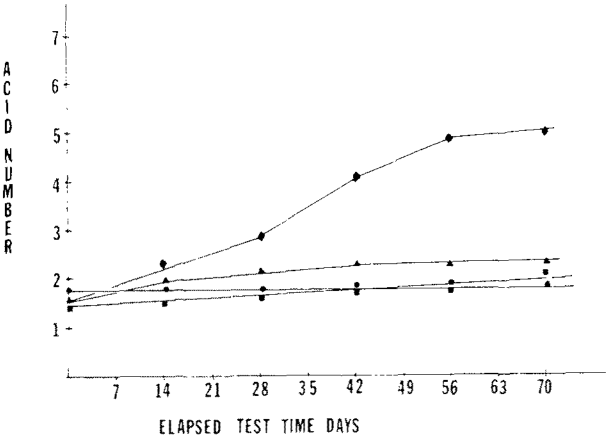


FIG. 1. Acidity development in GMS at various levels of moisture (40-45 C). ●, 0.05-0.1% moisture; ■, 0.18%; ▲, 0.5%; ◆, 2.2%.

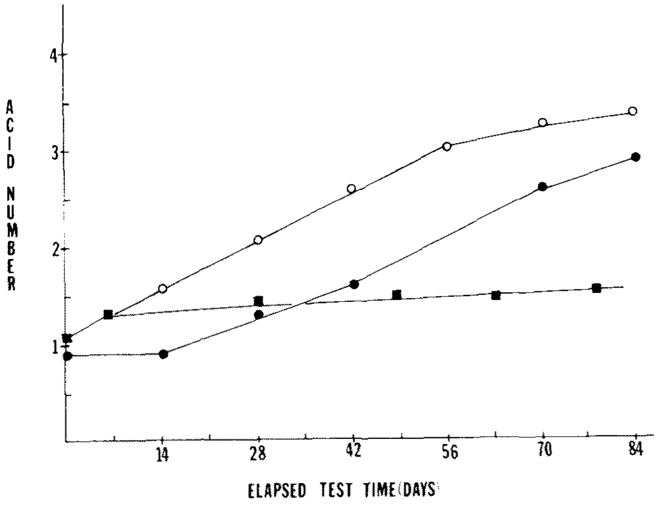


FIG. 2. Acidity development in GMS (0.1% initial moisture). ○, 20-25 C; ●, 40-45 C, both at 90-100% relative humidity; ■, 40-45 C and 30% relative humidity.

RF = (WIS × AS) / (WS × AIS)
RF = Response factor
WIS = Weight of internal standard (grams)
AS = Area of GC peak assigned to sample
WS = Weight of sample (grams)
AIS = Area of GC peak assigned to internal standard

Determination of monoglyceride, diglyceride and triglyceride content. The percent component was calculated using the following formula:

%C = (AC × WIS × 100)/(RF × AIS × WS)
%C = Percent of component (α-mono or β-monoglyceride; α,β or α,α'-diglycerides or triglycerides)
AC = Area of GC peak assigned to component
WIS = Weight of internal standard (grams)
RF = Response factor
AIS = Area of GC peak assigned to internal standard
WS = Weight of sample (grams)

Mono-, di-, and triglyceride contents are reported on a percent normalized basis using the previously determined response factors. The total α-monoglyceride content of a sample was obtained by summing the concentration of α-monoglycerides with varying chain lengths (i.e., C14 + C15 +C16 + C17 + C18 α-monoglyceride). The other total components were calculated similarly.

Actual percentages of α-monolycerides, β-monoglycerides, α,β-diglycerides and α,α'-diglycerides are shown in Figures 3–5 at the times indicated (in days).

RESULTS AND DISCUSSION

The effect of initial moisture content on the acid number of GMS. Greater acid number increase occurred in GMS at 0.5%+ initial moisture than at moisture levels of 0.05–0.10% (Fig. 1). At 0.05–0.10% moisture no acid number increase was observed over 70 days, and a small gradual increase was observed in GMS containing 0.18%–0.5% moisture (0.4 rise). However, as moisture content increased even further (2.2%), acid number increases were greater (3.3 rise).

The effect of relative humidity on the acid number of GMS. Greater acid number increase occurred in GMS samples at high relative humidity (ca. 90–100%, Fig. 2). At 20–25 C, GMS showed an acid number increase of 2.3 and 2.0 at 40–45 C after 84 days. Acid number drift was not as pronounced at 30% relative humidity and 40–45 C. Acid number increased by only 0.4 unit after 89 days.

Since the initial concentrations of moisture in all the samples were the same (0.1%), the higher acid number rises observed (Fig. 2) can be attributed to the high relative humidity. The acid number increases observed in GMS at 90–100% relative humidity can be attributed to the acid catalyzed hydrolysis of α,β and α,α'-diglyceride to α-monoglycerides and fatty acids. Ideally for each unit acid number rise (α,α' or α,β-diglyceride to α- or β-monoglyceride) there is a 1.1% drop in diglyceride concentration.

Isomerization and hydrolysis of GMS. Low humidity case: Isomerizations of β-monoglyceride to α-monoglyceride and α,β-diglyceride to α,α'-diglyceride occurred. The level of α-monoglyceride rose by 16.9%, while the concentration of β-monoglyceride dropped 17.0% after 31 days (Fig. 3). Isomerization of α,β-diglyceride to α,α'-diglyceride decreased its concentration by 6.8% after 31 days, while α,α'-diglyceride content increased 7.0%. The concentration of monoglycerides and diglycerides remained relatively constant for the 31- 84-day period, indicating the state of equilibrium.

High humidity case: The observed rise in α,α'-diglyceride concentration (28 days, Fig. 4) can be attributed to either the interesterification of β-monoglyceride to α,β-diglyceride followed by isomerization (Scheme I, Paths D, B), or the interesterification of β-monoglyceride with α-monoglyceride (Scheme I, Path E). In both cases free glycerine would be a by-product. The acid number rise observed can be explained by the hydrolysis of α,α'-diglyceride to α-monoglyceride and fatty acids. At 90–100% relative humidity, hydrolysis was enhanced while isomerization decreased slightly. In the higher humidity environment, the increased amount of water caused the hydrolysis reactions to compete favorably with the isomerization reactions.

In oven stability tests with GMS at 40–45 C (Fig. 4), isomerization occurred predominantly in the first 28 days of oven testing (acid number rose by only 0.4 unit). α-

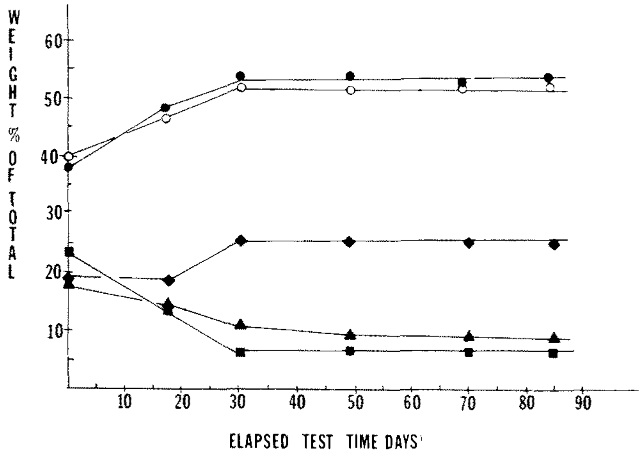


FIG. 3. The isomerization of GMS at 30% relative humidity and 40–45 C. ●, α-monoglyceride; ■, β-monoglyceride; ▲, α,β-diglyceride; ◆, α,α'-diglyceride (analyzed by GC method); ○, α-monoglyceride (analyzed by periodate wet method).

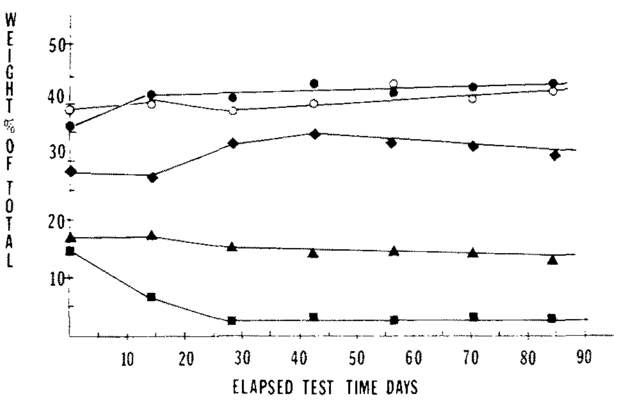


FIG. 4. The isomerization and subsequent hydrolysis of GMS at 90–100% relative humidity and 40–45 C. ●, α-monoglyceride; ■, β-monoglyceride; ▲, α,β-diglyceride; ◆, α,α'-diglyceride (analyzed by GC method); ○, α-monoglyceride (analyzed by periodate wet method).

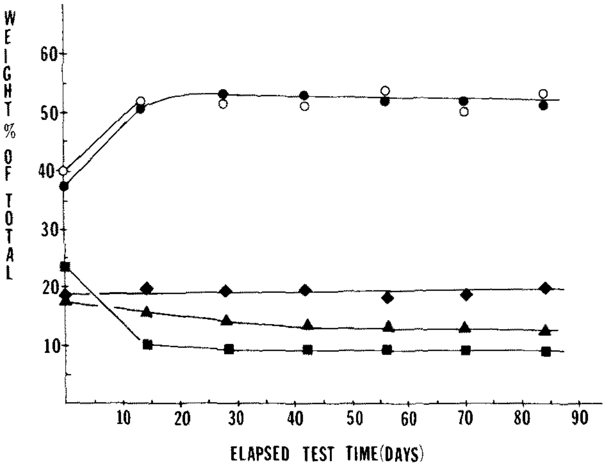
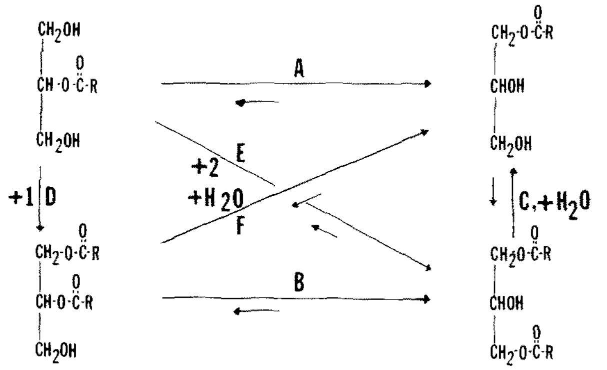


FIG. 5. The isomerization and subsequent hydrolysis of GMS at 90–100% relative humidity and 20–25 C. ●, α-monoglyceride; ■, β-monoglyceride; ▲, α,β-diglyceride; ◆, α,α′-diglyceride (analyzed by GC method); ○, α-monoglyceride (analyzed by periodate wet method).

Monoglyceride increased 6.5%, while β-monoglyceride decreased 12.0%. α,α′-Diglyceride increased 5.0%, accounting for the remaining decrease in β-monoglyceride (interesterification). α,β-Diglyceride stayed fairly constant. From 28 to 84 days acid number rose by 1.6 units. This rise in acid number corresponded to a calculated 1.8% drop in α,α′-diglyceride (hydrolysis). α,α′-Diglyceride actually decreased 2.1%. Overall, the total percent fatty acid (determined by capillary GC) rose by 0.85% (from 0.55% to 1.40%) giving an actual acid number rise of 2.0. The calculated acid number rise is 1.7.

At high humidity and ambient temperature (20–25 C), hydrolysis of α,β-diglyceride to α-monoglycerides appears to have occurred (Fig. 5). The acid number rise in GMS (2.3, Fig. 2) corresponded to a 2.6% drop in α,β-diglyceride after 42 days. β-Monoglyceride level dropped 13.6% in 42 days while the α-monoglyceride level increased 16.4%. After the isomerization of β-monoglyceride to α-monoglyceride (13.6%) was subtracted, the remaining 2.8% corresponded to the decrease in α,β-diglyceride, suggesting that α,β-diglyceride hydrolyzed to α-monoglyceride. Total percent fatty acid (by capillary GC) rose by 1.1% (from 0.4% to 1.5% after 84 days), giving an actual acid number rise of 2.3. The calculated acid number rise is 2.2, which agrees well with the observed acid number rise.

In summary, this paper describes the transformations occurring in commercial GMS, a mixture of monoglycerides, diglycerides and triglycerides. β-Monoglycerides, α,β-diglycerides and α,α′-diglycerides demonstrated a tendency to convert to α-monoglycerides during storage over 3 mo. At low relative humidity (30–32%) and 40–45 C, β-monoglyceride isomerized to α-monoglyceride (Path A, Scheme 1) and α,β-diglyceride to α,α′-diglyceride (Path B, Scheme 1). These isomerizations occurred most likely through an intramolecular transesterification involving a 5-membered ring orthoester intermediate (12).



SCHEME 1. Transformations occurring in commercial GMS which cause acid number rise over a 3-mo period. R, fatty acid chain (C14–18); 1, β-monoglyceride; 2, α-monoglyceride.

At high relative humidity (90–100%) and 40–45 C not only was isomerization of β-monoglyceride to α-monoglyceride observed (Path A, Scheme 1), but an increase in concentration of α,α′-diglyceride with a corresponding decrease in the concentration of β-monoglyceride also was observed. This suggested an interesterification of β-monoglyceride to α,β-diglyceride (Path D, Scheme 1) followed by isomerization to α,α′-diglyceride (Path B, Scheme 1) or interesterification with α-monoglyceride to directly give α,α′-diglyceride (Path E, Scheme 1). Finally, the hydrolysis of α,α′-diglyceride to α-monoglyceride (Path C, Scheme 1) would result in the release of a free fatty acid, accounting for the rise in acid number. Similarly, at high relative humidity and 20–25 C, α,β-diglyceride appeared to hydrolyze to α-monoglyceride, conceivably via Paths B and C (Scheme 1) or Path F (Scheme 1).

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