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Nonpolar Lipids of *Amaranthus palmeri* S. Wats. 1. Fatty Alcohols and Wax Esters (Saturated)

Oliver D. Dailey, Jr.,* Ray F. Severson, and Richard F. Arrendale

In order to isolate and identify the nonpolar lipids of *Amaranthus palmeri*, a common agronomically significant weed, the ground leaves and flowering parts of dried plants were extracted with hexanes. The extract was subjected to column chromatography, thin-layer chromatography, and finally capillary GC/MS studies. In this paper, the compositions of the saturated fatty alcohol and wax ester components will be discussed. These classes of compounds have not been characterized in any prior studies of *Amaranthus* species. The wax ester isolate consisted of a series of C_{36}–C_{56} homologues, with the C_{40}, C_{42}, C_{44}, C_{46}, and C_{48} homologues predominating. The major wax ester fatty acids were C_{16}, C_{18}, C_{20}, C_{22}, and C_{24}. Similar trends in carbon number distribution were found between free and bound fatty alcohols, with the C_{22}, C_{24}, C_{26}, C_{28}, C_{30}, and C_{32} homologues predominating.

Palmer amaranth (*Amaranthus palmeri* S. Wats) is a common agronomically significant weed whose soil-incorporated residues have been observed to inhibit the growth of certain crop plants, most notably carrot and onion. The weed residues are also autotoxic. (Menges, 1987).

Laboratory seed germination bioassays of crude organic solvent extracts of *A. palmeri* plant parts (including those discussed in this paper) indicated the presence of both promotive and inhibitory compounds (Bradow, 1985). Aqueous extracts of the leaves and thyrses (flowering parts) had no significant effect on any of the seeds tested (Bradow, 1985). In their studies of *A. palmeri*, Fischer and Quijano (1985) isolated phytol and chondrillasterol from a petroleum ether extract of aerial parts and fatty acids, chondrillasterol, 3-methoxy-4-hydroxynitrobenzene, vanillin, and 2,6-dimethoxybenzoquinone from a dichloromethane extract of ground roots.

There have been a number of investigations of the composition of the seed lipids of both vegetable amaranths [*Amaranthus caudatus* (*Amaranthus edulis*), *Amaranthus cruentus*, *Amaranthus dubius*, *Amaranthus tricolor* (*Amaranthus gangeticus*)] and weedy species (*Amaranthus hybridus*, *Amaranthus retroflexus*, *Amaranthus spinosus*) (Becker et al., 1981; Dixit and Varma, 1971; Fernando and Bean, 1985; Bressani et al., 1987; Lorenz and Hwang, 1985; Opute, 1979; Stoller and Weber, 1970). These studies dealt primarily with fatty acid composition.

Lakshminarayana et al. (1984) published a comprehensive report of the lipid and fatty acid composition of the leaves of young *A. gangeticus* (*A. tricolor*) plants. The nonpolar lipids consisted of pigments, hydrocarbons, sterols, ester waxes, fatty acid methyl esters, triglycerides, diglycerides, monoglycerides, and fatty acids. The fatty acid composition of the ester waxes in decreasing order was palmitic, linolenic, stearic, oleic, linoleic, and lauric.

The studies described in this paper were initiated as part of a research effort to isolate and identify allelopathic compounds of *A. palmeri*. In screening for potential al-

Southern Regional Research Center, USDA—ARS, P.O. Box 19687, New Orleans, Louisiana 70179 (O.D.D.), and Richard B. Russell Agricultural Research Center, USDA—ARS, P.O. Box 5677, Athens, Georgia 30613 (R.F.S., R.F.A.).

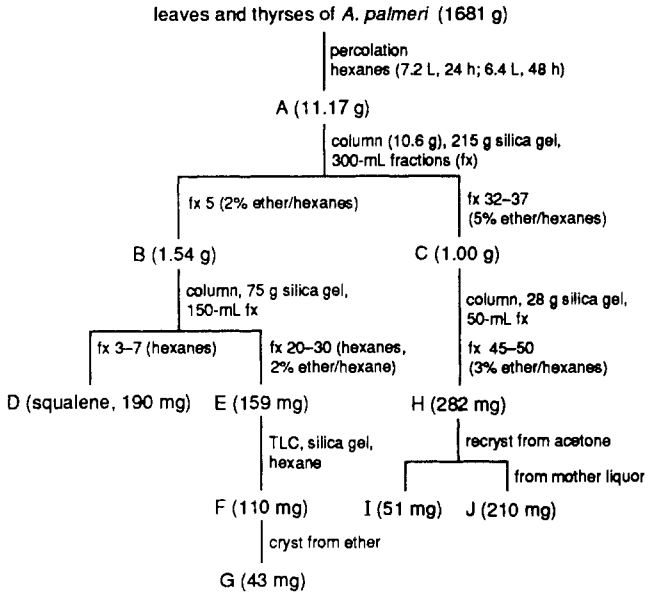

Figure 1. Scheme for the extraction and isolation of the free fatty alcohols and wax esters (saturated) from *A. palmeri* leaves and thyrses.

lelochemicals, crude extracts were subjected to a series of seed germination studies as described by Bradow (1985). The hexanes extract of the leaves and thyrses (flowering parts) significantly inhibited germination of carrot seeds (Bradow, 1985) and was chosen for further study. This is the first of a series of papers describing the composition of the nonpolar components of the leaves and thyrses of *A. palmeri*. In this paper, the composition of the saturated fatty alcohol and wax ester components, as determined by capillary gas chromatography/mass spectroscopy (GC/MS) analysis, will be presented.

EXPERIMENTAL SECTION

Plant Material. *A. palmeri* plants were provided by Dr. R. M. Menges, USDA—ARS, Weslaco, TX. They had been sown in Texas on September 30, 1982 and harvested on December 9, 1982 (41 cm tall with green seed heads). Approximately 3 kg of plant material was packed in dry ice and shipped to the Southern Regional Research Center. The whole plants were spread out and allowed to air-dry in a hood at ambient temperatures over a 3-day period. The dried plants were separated into three categories: roots; stems; leaves and thyrses.

Extraction of Plant Material. The ground leaves and thyrses (1681 g) were placed into four 2-L percolators and extracted with 1.8 L of hexanes for 24 h and with 1.6 L of hexanes for 48 h. The solvent was removed from the combined extracts to afford 11.2 g of A (Figure 1). Extract A inhibited germination of carrot seeds (Bradow, 1985). Individual constituents of this extract are not necessarily seed germination inhibitors.

Fractionation of the Hexane Extract. As shown in Figure 1, extract A (10.6 g) was chromatographed on a silica gel column by eluting first with hexanes and then with increasing percentages of ether in hexanes (300-mL fractions). Fraction 5 was eluted with 2% ether/hexanes and afforded 1.54 g of white solid plus orange oil (B). Subsequent elution with 5% ether/hexanes yielded 1.00 g of white solid plus yellow oil (C). Sample B was rechromatographed on a silica gel column; the column was eluted with 4200 mL of hexanes followed by 1500 mL of 2% ether/hexanes. Fractions 3–7 furnished 190 mg of D, which was determined to be 97+% pure squalene by capillary GC. Its GC retention time and MS data were identical with those of authentic squalene. Fractions 20–30 provided 159 mg of wax ester isolate E. Preparative TLC and crystallization from ether afforded 43 mg of G, mp 70.5–72 °C. Sample C was subjected to column chromatography, and 50-mL fractions (fx) were eluted as follows: fx 1–12, 100% hexanes; fx 13–27, 1% ether/hexanes; fx 28–40, 2% ether/hexanes; fx 41–109, 3% ether/hexanes. Fractions 45–50 yielded

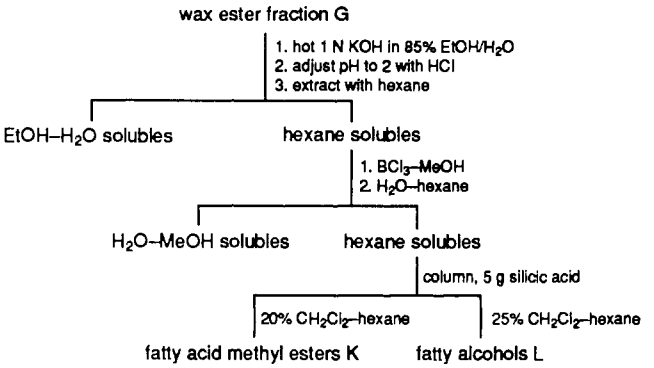

Figure 2. Scheme for the hydrolysis of wax ester isolate G and the isolation of fatty alcohol and fatty acid (as methyl esters) fractions.

282 mg of white solid H. Recrystallization from acetone gave 51 mg of I, mp 81–82 °C, and 210 mg of J was recovered from the mother liquor. Components isolated were characterized by GC retention and/or GC/MS data.

Hydrolysis of the Wax Ester Isolate. Wax ester isolate G was hydrolyzed and separated into alcohols and fatty acids (as methyl esters) as described by Arrendale et al. (1988) (Figure 2). About 2 mg of G was placed in an 8-mL capped test tube containing 3 mL of 1 N KOH in 85% EtOH/H_2O and the mixture heated at 76 °C for 4 h. After cooling in ice, the pH was adjusted to about 2 with concentrated HCl and the mixture was extracted with hexane (2 × 2 mL). The hexane extracts were washed with water until neutral and dried over anhydrous sodium sulfate, and the solvent was removed. The residue was treated with 3 mL of BCl_3–MeOH for 4 h at 76 °C in a capped test tube. After cooling, water (1 mL) was added and the mixture was extracted with hexane as above. The residue from the hexane extract was chromatographed on a silicic acid column, eluted with 1:4 methylene chloride/hexane to yield a fatty acid methyl ester fraction (K) followed by 3:1 methylene chloride/hexane to yield a fatty alcohol fraction (L).

Capillary Gas Chromatography (GC-2) and GC/MS. Fractions D, G, and K were dissolved in isooctane. Analysis of the wax ester isolate G was conducted on a Hewlett-Packard 5890 equipped with a 20 m × 0.3 mm (i.d.) thin film (about 0.1 μm) SE-54 fused silica capillary column, splitless injection port (250 °C), and flame ionization detector (350 °C) using hydrogen carrier gas. Fatty alcohol isolates I, J, and L were converted to their trimethylsilyl ethers by treatment with *N,O*-bis(trimethylsilyl)-acetamide (BSA) at 76 °C for 30 min. Fatty acid methyl esters and alcohol trimethylsilyl ethers were analyzed on a HP 5720 GC equipped with autosampler, split injector, flame ionization detector, and a 30 m × 0.3 mm (i.d.) thin film SE-54 fused silica capillary column using hydrogen carrier gas. Peak areas were corrected for the difference in chromatographic response by conventional internal standard calibration methods and authentic standards. GC/MS data were obtained on a Hewlett-Packard 5985B, modified for capillary GC/MS application (Arrendale et al., 1984) under the same conditions as above except helium carrier gas was used.

RESULTS AND DISCUSSION

Approximately 0.67% of the air-dried weight of *A. palmeri* leaves and thyrses was extracted with hexanes. Chromatography and/or recrystallization (Figure 1) produced several fractions, four of which (D, G, I, J) were fully characterized. Fraction D was essentially pure squalene (97+% by GC). Squalene has been found in relatively large amounts in the seeds of several *Amaranthus* species (Becker et al., 1981).

The composition of wax ester isolate G was determined from GC retention and GC/MS data and by hydrolysis and analysis of the resulting acid and alcohol moieties. The capillary gas chromatogram of the wax ester fraction G is shown in Figure 3. The C_{36}–C_{42} esters had retention data identical with those of synthesized standards (Arrendale

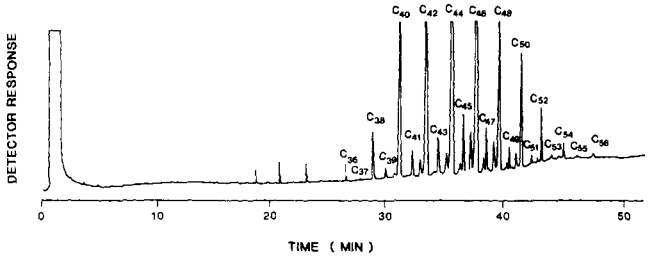

Figure 3. Capillary gas chromatogram of wax ester fraction G.

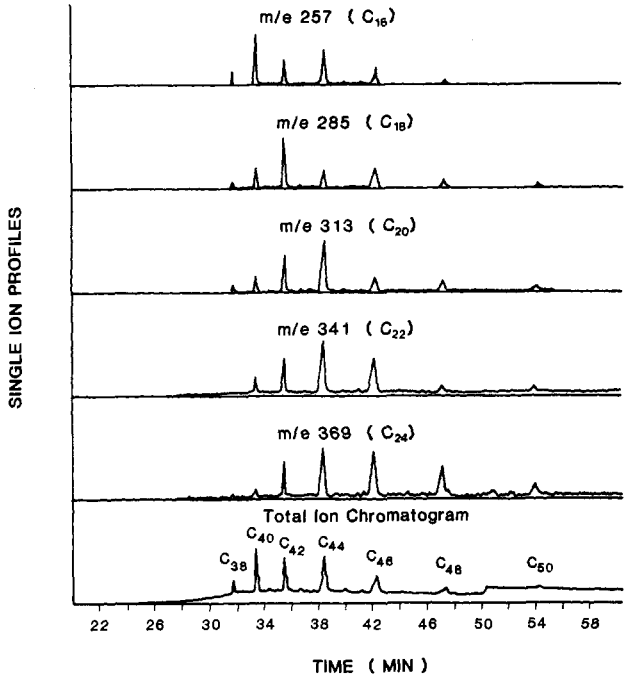

Figure 4. Comparison of capillary GC/MS single ion profiles of major wax ester fatty acids (acid + 1) and total ion chromatogram.

et al., 1988), and all carbon numbers shown were confirmed by GC/MS. The carbon number of each wax ester peak was calculated from molecular ion data. Fragment ions related to the alcohol (alcohol − 18 ion) and acid (acid + 1 ion) moieties can be used to determine the major isomers present in each carbon number (Arrendale et al., 1988); Silverstein and Bassler, 1976). The ion representing the acid moiety (acid + 1 ion) is generally the base peak in the spectra. The single ion profiles of the acid + 1 ions of the major wax ester homologues are shown in Figure 4. Note the difference in the retention times of the same carbon number esters as shown in Figures 3 and 4. The change in retention times is due to differences in linear flow rates of the carrier gases in the analytical chromatogram (H_2, Figure 3) and the total ion GC/MS chromatogram (He, Figure 4).

As shown in Figure 2, ester fraction G was hydrolyzed, free acids were converted to methyl esters, and silicic acid chromatography yielded fatty acid methyl ester fraction K and fatty alcohol fraction L. Gas chromatography (Figure 5) and GC/MS of K showed that the wax ester fatty acids ranged from C_{14} to C_{30} with major homologues in the C_{16}–C_{24} range. The wax ester fatty alcohols ranged from C_{18} to C_{34} (Figure 6). In agreement with wax ester GC and GC/MS data (Figures 3 and 4), the even carbon number esters were predominantly produced by combinations of even carbon number acids and alcohols.

The gas chromatograms of the recrystallized free fatty alcohol fraction, I, and material recovered from the mother liquor, J, are compared in Figure 7. The alcohols of I

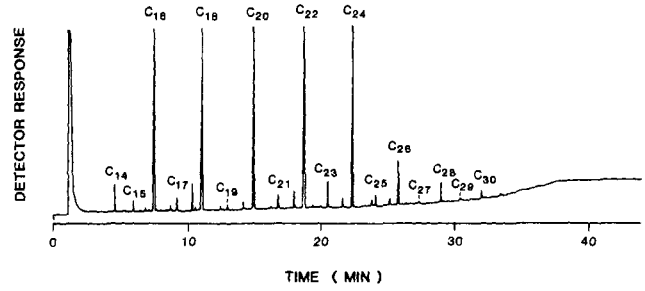

Figure 5. Capillary gas chromatogram of wax ester fatty acid methyl ester fraction K.

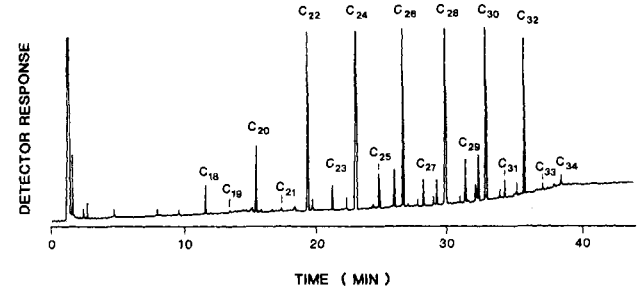

Figure 6. Capillary gas chromatogram of silylated wax ester fatty alcohol fraction L.

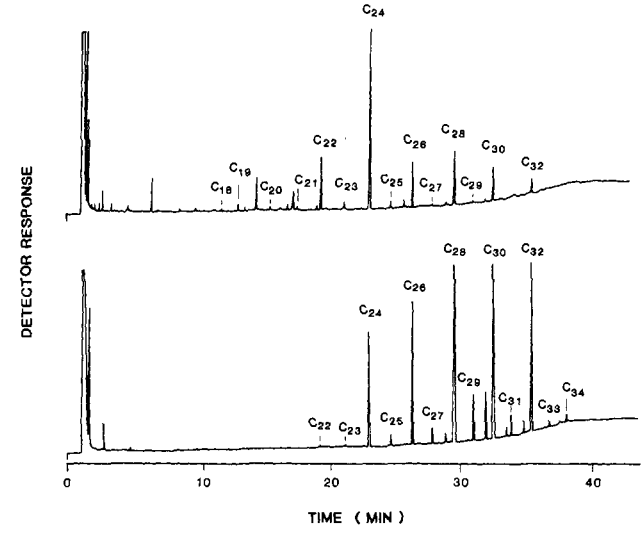

Figure 7. Comparison of capillary gas chromatograms of silylated free fatty alcohol fractions I (bottom) and J (top).

ranged from C_{22} to C_{34} whereas those of J ranged from C_{18} to C_{32}, indicating that the recrystallization resulted in concentration of the higher molecular weight homologues. The compounds were characterized by GC retention and GC/MS data of their trimethylsilyl ethers. An important diagnostic fragment ion in the mass spectrum of the silyl ethers of the fatty alcohols is the M − 15 ion. In Figure 8, the single-ion profiles of the ion for the M − 15 ions of the C_{24}–C_{32} homologues are compared with the total ion chromatogram of fraction I. The C_{30} alcohol single-ion profile (m/e 495) indicated the presence of two isomeric alcohols. The GC retention and mass spectral data for the major compound were identical with the silyl ether of the standard 1-triacontanol with a base peak at m/e 75 due to the dimethylhydroxysilyl cation. The base peak of the minor isomer occurred at m/e 43, indicative of an isopropyl end group (Arrendale et al., 1988; Severson et al., 1981). Thus, the minor C_{30} alcohol is probably a methyl iso-branched isomer.

Data on the relative distribution of homologues of the free fatty alcohol fractions (J, I), the bound fatty alcohols

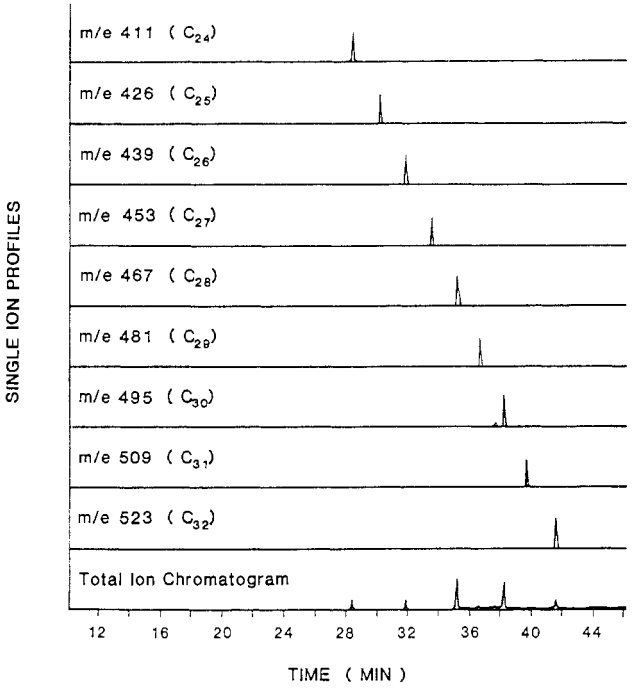

Figure 8. Comparison of the capillary GC/MS single-ion profiles of fatty alcohol trimethylsilyl ether M − 15 ions and total ion chromatogram of silylated fatty alcohol fraction I.

Table I. Relative Distribution of Free Fatty Alcohols and Bound Fatty Alcohols and Acids from Wax Ester Fraction

	relative distribution (×100)[a]				
	free fatty alcohols			bound G[b]	
isomer (normal C_X)	mother liquor[c] J	recryst[c] I	total[d] J + I	alcohol[c] L	acid[e] K
14	-[f]	-	-	-	2.2
15	-	-	-	-	1.1
16	-	-	-	-	100.0
17	-	-	-	-	3.3
18	7.0	-	4.2	5.5	43.9
19	7.3	-	4.4	0.1	9.0
20	11.4	-	6.9	12.4	44.6
21	7.3	-	4.4	0.9	2.0
22	94.6	0.3	57.3	41.9	44.1
23	13.8	0.1	8.2	4.4	3.5
24	355.0	22.3	224.0	105.0	28.8
25	14.0	2.1	17.0	6.1	1.6
26	15.1	28.3	20.9	52.0	6.1
27	6.7	3.2	7.4	3.9	0.3
28	100.0	100.0	100.0	100.0	3.4
29	6.6	8.9	15.0	7.0	0.1
30	66.4	87.9	77.0	61.6	1.0
31	1.0	4.1	2.2	2.9	-
32	29.8	41.6	36.9	26.0	-
33	-	0.5	0.2	2.1	-
34	-	1.7	0.7	1.8	-

[a] Relative to octacosanol or hexadecanoic acid. [b] After hydrolysis of wax ester isolate. [c] Analyzed as TMS derivatives. [d] Calculated from J and I fraction weights and GC data assuming 81% of fraction J was GC-volatile alcohols. [e] Analyzed as methyl esters. [f] Absent or less than 0.1.

(L), and fatty acids (K) are given in Table I. The distribution of total free alcohols was estimated from the fraction weights of I and J and GC data. GC analysis of fraction I indicated that 81% of the GC volatiles were fatty alcohols. Similar trends in carbon number distribution were found between free and bound alcohols. The C_{22}, C_{24}, C_{26}, C_{28}, C_{30}, and C_{32} were the most abundant alcohol chain lengths, and even carbon numbers predominated. The major acids present after hydrolysis were in good agreement with the GC/MS data on the wax ester fraction (Figure 4). The major wax ester fatty acids were C_{16}, C_{18}, C_{20}, C_{22}, and C_{24}. Possible combinations of the alcohols and acids described in Table I readily explain the carbon number distribution in the wax ester fraction. Combinations of the C_{16} acid–C_{20} alcohol produced the C_{36} wax ester (Figure 3) and the C_{24} acid–C_{32} alcohol produced the C_{56} ester. The major wax ester homologues, C_{40}–C_{48}, are produced by combinations of the even carbon number C_{16}–C_{24} acids and C_{22}–C_{28} alcohols. The predominance of the even carbon numbered acids, alcohols, and wax esters and the lack of the normal internal plant unsaturated C_{18} acids indicated the alcohols and wax esters are cuticular components (Hadley, 1980; Arrendale et al., 1988).

In this paper, we have described the compositions of the saturated fatty alcohol and wax ester components of the leaves and thyrses of *A. palmeri*. These classes of compounds have not been characterized in any previous studies of *Amaranthus* species. In a subsequent paper, we will discuss the composition of esters containing unsaturated acyl moieties, including glycerides, steryl esters, and terpenol esters.

ACKNOWLEDGMENT

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Registry No. C_{18} alcohol, 112-92-5; C_{19} alcohol, 1454-84-8; C_{20} alcohol, 629-96-9; C_{21} alcohol, 15594-90-8; C_{22} alcohol, 661-19-8; C_{23} alcohol, 3133-01-5; C_{24} alcohol, 506-51-4; C_{25} alcohol, 26040-98-2; C_{26} alcohol, 506-52-5; C_{27} alcohol, 2004-39-9; C_{28} alcohol, 557-61-9; C_{29} alcohol, 6624-76-6; C_{30} alcohol, 593-50-0; C_{31} alcohol, 544-86-5; C_{32} alcohol, 6624-79-9; C_{33} alcohol, 71353-61-2; C_{34} alcohol, 28484-70-0.

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Stability of Aqueous Foams: Analysis Using Magnetic Resonance Imaging

J. Bruce German* and Michael J. McCarthy

Aqueous foams differ widely in their structure and kinetic stability. Understanding and particularly assigning specific functional roles to individual components in the foams has been severely limited by the inability to analyze such unstable colloidal systems. We report the use of magnetic resonance imaging techniques in the description of foam structure and collapse. Foams from cream, egg white and beer were imaged, and the signal intensities contributed by aqueous protons were recorded sequentially over the lifetime of the foams. We were able to estimate densities, drainage rates, and collapse throughout the foam structure, noninvasively from actual food foams. This technique will be of use in the analysis of a variety of colloidal systems.

Foams are thermodynamically unstable colloidal systems in which gas is transiently maintained as a distinct dispersed phase in a liquid matrix (Adamson, 1982). These forms of matter are characterized by their low density, high viscosity and ultimately finite stability (Bikerman, 1973). There are a great many practical uses of aqueous foams in foods, pharmaceuticals, and engineering, and the number of novel applications is increasing rapidly (Aubert et al., 1986). Each foam system is unique in terms of its chemical composition and formation, and these differences lead to quite different macroscopic properties. For example, egg white protein stabilizes very high density foams in cakes, meringues, and candies (Cumper, 1953). Alternatively, novel surfactant solutions have proven to form effective very low density fire-fighting foams (Bikerman, 1973).

The formation, density, and ultimate stability of aqueous foams are critical parameters that must be characterized both in studying the basic mechanisms underlying foam chemistry and in practice estimating changes due to composition, i.e., food reformulation (Halling, 1981). Due to their inherent instability, sampling and measuring foams have proven very difficult (Halling, 1981; German et al., 1985). Model systems have been developed to examine foam collapse utilizing very precise measurements of pressure and conductance (Nishioka, 1986). These methods, though providing information for theoretical background, are not applicable to complex mixtures of surfactants and thus are inappropriate for studying most real foams. Noninvasive methods for describing foam development and collapse are critical to increasing our understanding of these events.

Magnetic resonance imaging (MRI) is a novel technology in which the strength of a signal associated with a resonant magnetic nucleus, i.e., proton ^1H, in a magnetic field can be assigned to a particular volume element in that magnetic field (Lauterbur, 1973). This allows one to generate images based not only on the presence of a nucleus in space but on its chemical structure and environment (Morris, 1986). The strength of MRI is that the image can provide density measurements as well as information on the local chemical environment (Morris, 1986). In addition to the spectacular breadth of chemical information available by this technique, it is also nondestructive and noninvasive. Sequential scans of materials can thus be used to follow changes in the chemical properties of matter dynamically. Foams are an intriguing model in which to apply this technology. In foam research one is interested in estimating densities and drainage rates without perturbing the structure of the foam to do it. MRI is uniquely suited to realize these goals. Our objective in this study was to employ MRI to monitor drainage from foams typical of food systems.

MATERIALS AND METHODS

Foams. Foams were generated with three well-known food systems: egg white, heavy cream, and beer. Egg whites were separated and 40-mL portions whipped for various times in a double-beater mixer (Sunbeam maximum speed). Typical final densities of the foams were approximately 0.15 g/mL. Heavy cream (40% fat) was chilled and again 40 mL whipped as above to densities of 0.3–0.4. Cylinders were punched from these foams using a Plexiglass chamber and immediately placed in the imaging coil of the NMR. For beer foams slightly different protocols were used. Commercial samples (30 mL) were carefully poured into a 100-mL glass beaker, and this was placed in an ultrasonic bath (Bransonic, Shelton, CN) and sonicated for 2 s. This effectively nucleated the dissolved CO_2 and generated approximately 50 mL of foam on top of 10–20 mL of liquid. Initial densities were therefore on the order of 0.3, although the very rapid drainage of these foams highlighted difficulties of the more traditional measurements of density. The glass beaker was then placed in the imaging coils.

Spectroscopy. A 2-T (General Electric, CSI) imaging spectrometer, with a 15-cm-i.d. imaging coil was used to measure ^1H signals at 85.5 MHz. A Fourier imaging spin-echo pulse sequence was used to generate the two-dimensional image. The same sequence with the pulse encode gradient turned off was used to

Department of Food Science and Technology, University of California, Davis, Davis, California 95617.

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