Low-Molecular Weight Organic Composition of Acid Water from Coconut Oil Soapstock

Arunthathi Sivasothy and Peter J. Reilly*
Department of Chemical Engineering, Iowa State University, Ames, Iowa 50011

ABSTRACT: Soapstock from alkaline refining of coconut oil was acidified, and the resulting acid water after neutralization was subjected to gas chromatography, electron-ionization and chemical-ionization mass spectroscopy, and high-performance liquid chromatography. The chief low-molecular weight organic components were C_4–C_{18} fatty acids, hydroxylated acids, and sugar alcohols. The prevalence of acids and total absence of phosphate compounds make coconut acid water different in composition from the acid waters from other soapstocks.
JAOCS 73, 1305–1309 (1996).

KEY WORDS: Acid water, coconut oil, composition, fatty acids, gas chromatography, high-performance liquid chromatography, mass spectroscopy, soapstock.

We have been involved in the identification and quantitation of the low-molecular weight organic components of by-product streams from the processing of agricultural products, first the ethanol stillages from cornstarch (1), cane molasses, citrus waste, and sweet whey (2), and then the acid waters from canola, corn, cottonseed, peanut, soybean, and sunflower soapstocks (3). Soapstocks are produced by the alkaline extraction of vegetable oils, which for soybean oil is normally preceded by degumming with water. Acidification of soapstock, not immediately diverted to animal feed, produces organic and aqueous layers, the latter commonly known as acid water, and sometimes an intervening emulsion layer. The organic layer and some of the emulsion layer are used in animal feed, but the acid water is discarded. Because the latter has an extremely high biological oxygen demand, its disposal, either at the processing site or in a municipal facility, is expensive.

Our work on stillage and acid water had two objectives. The first was to identify components that could be recovered and reused. This appears to have been successful with the acid waters (3) but not with the stillages (1,2). The second objective was to learn more about the composition of the plant tissue from which the by-products were derived, because both stillages and acid waters contain components left behind after other materials present at higher concentrations were removed by distillation or formation of a second liquid phase.

Until the preceding study (3), no detailed knowledge of the low-molecular weight organic composition of acid waters existed. The six acid waters analyzed there were similar, with five phosphatide fragments and a number of sugars and sugar alcohols dominating. Acid water from coconut oil soapstock, derived from the seventh most commonly produced vegetable oil (4), has a totally different composition, and therefore, the results from its analysis are presented separately here.

EXPERIMENTAL PROCEDURES

Soapstock source and treatment. Soapstock from coconut oil, which was not subjected to degumming, was donated by Lou Ana Foods (Opelousas, LA). Three volumes of water per volume of soapstock had been added. Addition of 2.8N H_2SO_4 decreased its pH from an initial 10.5 to 2.2, adding 6% to the volume of the resulting acid water fraction. No emulsion formed after acidification. The mixture was centrifuged at 11,800 × *g* for 20 min, after which the acid water was decanted after cooling the mixture in an icewater bath. The acid water was centrifuged again at 11,800 × *g* for 20 min. This time, the remaining organic fraction was removed by syringe.

The separated acid water was neutralized with 5N NaOH to approximately pH 7.1, which added a further 0.4% to its volume, and was clarified with a 0.22-μm filter and stored at 4°C. For further analysis, 15 mL of neutralized solution on a glass plate was evaporated to dryness at 50°C for 2 h.

Other procedures. Proximate analyses, sample derivatization, gas chromatography (GC), electron-ionization mass spectroscopy (EIMS), chemical-ionization mass spectroscopy (CIMS), peak identification, and high-performance liquid chromatography (HPLC) were performed as described previously (3), except that total fatty acids were determined by AOCS Official Method G 4-40 (5).

RESULTS

Soapstock and acid water characterization. After soapstock acidification, the liquid separated into 73% acid water and 27% organic layer, the percentages corrected for dilution by the added H_2SO_4 solution. Acid water dry weight was a low

---

*To whom correspondence should be addressed at Department of Chemical Engineering, 2114 Sweeney Hall, Iowa State University, Ames, IA 50011-2230.

14.6 g/L. Proximate analyses of soapstock and acid water are shown in Table 1. Just over 2%, at most, of the fatty acids in the soapstock ended up in the acid water.

GC peak identification and quantitation. Gas chromatograms from a DB-5 capillary column of trimethylsilyl (TMS)-derivatized neutralized acid water obtained with 50°C→150°C and 150°C→250°C gradients are shown in Figure 1.

Identified components consisted of all the straight-chain saturated fatty acids with even numbers of carbon atoms from C_4 to C_{18}, two unsaturated fatty acids (palmitoleic and oleic),

TABLE 1
Proximate Analysis of Coconut Soapstock and Neutralized Acid Water

Soapstock (%)	
Moisture	95
Protein	0.52
Total fatty acids	3.3
Phosphatides from total phosphate	0.126
Neutralized acid water (%)	
Moisture	98.6
Protein	0.64
Fat	<0.10
Ash	1.40
Carbohydrate	0.0

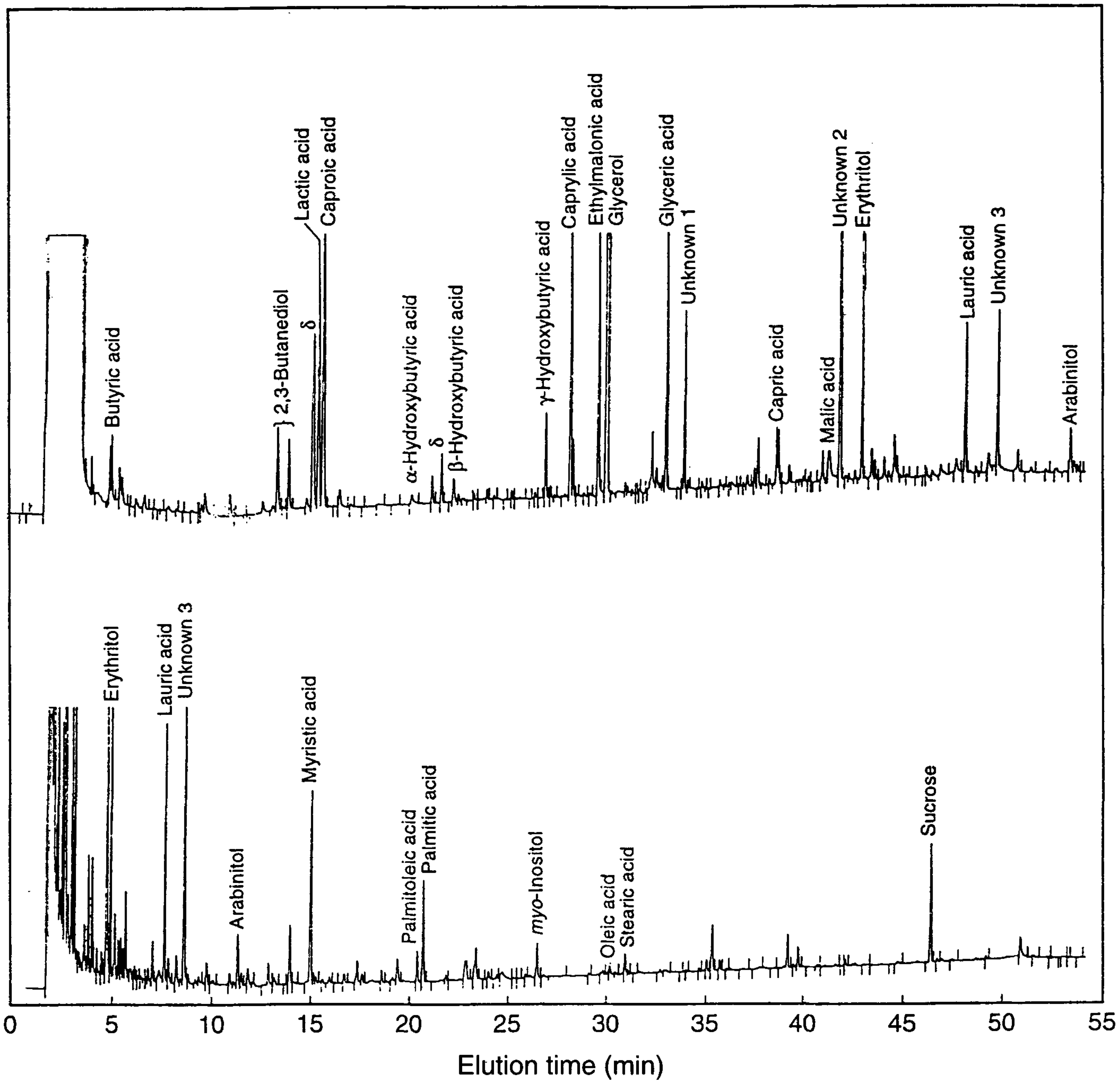

FIG. 1. Gas chromatograms of trimethylsilyl-derivatized neutralized coconut acid water. Upper: 50°C→150°C gradient. Lower: 150°C→250°C gradient.

a number of hydroxylated monobasic acids, two dibasic acids (ethylmalonic and malic), one aldehydo acid (glyoxylic), several sugar alcohols, and one sugar (sucrose).

Concentrations of identified components are found in Table 2. Those of highest concentration were lauric acid, glycerol, malic acid, myristic acid, glyoxylic acid, caprylic acid, lactic acid, and glyceric acid.

An extensive but unavailing effort was made to identify the three major unknowns. EIMS suggested that TMS-derivatized Unknown 1 was a TMS-derivatized hydroxylated saturated fatty acid. CIMS with NH_3 ionizing gas indicated a molecular weight of 259 or 260 Da, with the uncertainty caused by multiple M + 18 peaks. Subtracting the two TMS groups gave a molecular weight of 115 or 116 Da. This suggests a C_5 hydroxylated fatty acid, whose molecular weight is 118 Da.

EIMS suggested that TMS-derivatized Unknown 2 was a TMS-derivatized straight-chain or branched saturated dibasic acid. CIMS indicated a molecular weight of 287 or 288 Da. Taking the two TMS groups into account, the resulting molecular weight would be 143 or 144 Da, suggesting a C_6 dibasic acid, which however has a molecular weight of 146 Da.

TABLE 2
Retention Times and Concentrations of the Low Molecular Weight Components of Neutralized Coconut Acid Water

Component	Relative retention time		Concentration (g/L)
	50→150°C[a]	150→250°C[b]	
Saturated fatty acids			
Butyric[c]	0.17		0.021
Caproic[c]	0.52		0.028
Caprylic[c]	0.94		0.053
Capric[c]	1.29		0.016
Lauric[c]	1.60	0.30	0.233
Myristic		0.57	0.106
Palmitic		0.88	0.015
Stearic acid		1.17	0.025
Unsaturated fatty acids			
Palmitoleic		0.86	0.015
Oleic		1.15	0.003
Hydroxylated acids			
Lactic	0.52		0.050
Glycolic	0.55		0.010
α-Hydroxybutyric	0.67		0.013
β-Hydroxybutyric	0.74		0.032
γ-Hydroxybutyric[c]	0.90		0.023
Glyceric[c]	1.10		0.050
Aldehydo acid			
Glyoxylic	0.94		0.068
Dibasic acids			
Ethylmalonic	0.98		0.021
Malic	1.37		0.124
Alcohols			
2,3-Butanediol[c]	0.45		0.041
Glycerol[c]	1.00		0.133
Erythritol[c]	1.43	0.19	0.045
Arabitol	1.78	0.43	0.007
myo-Inositol		1.00	0.004
Sugar			
Sucrose		1.74	0.019

[a] Relative to gas chromatography (GC) retention time of trimethylsilyl (TMS)-glycerol (30.08 min).
[b] Relative to GC retention time of hexa-TMS-*myo*-inositol (26.84 min).
[c] Identity confirmed by chemical-ionization mass spectroscopy as well as by electron-ionization mass spectroscopy and GC retention time.

By EIMS, TMS-derivatized Unknown 3 was a TMS-derivatized hydroxylated dibasic acid. CIMS suggested a molecular weight of 315 or 316 Da. After subtracting three TMS groups, Unknown 3 would have a molecular weight of 99 or 100 Da, too low for the structure suggested by EIMS.

Given that all the suggestions from the EIMS on-line library for the unknowns were of relatively low fit to the original mass spectra, the GC retention times of the three TMS-derivatized unknowns were compared not only with those of the many TMS-derivatized compounds obtained earlier (1–3), but also with sixty newly TMS-derivatized organic acids, most from Aldrich (Milwaukee, WI) with a few from Sigma (St. Louis, MO). Some of these were selected by using Molecular Fragment Calculator (James E. Deline, Livermore, CA), an algorithm that suggests logical molecular formulae based on molecular weights and elements present. However, no matches occurred.

HPLC. HPLC was mainly concerned with finding components that are too volatile to be detected by GC, many of which elute late from a strong-acid ion exchange HPX-87H column (1,2). From the HPLC retention times of all components identified by GC in this work and the previous one (3), plus those measured earlier (1,2), a number of peaks were tentatively identified. They included acetic acid and either propionic acid or acetaldehyde, whose TMS-derivatized forms were not separated from the void volume peak by GC, and glyceric acid, either lactic acid or ethylmalonic acid or both, and glycerol, which were identified by GC/MS. Fatty acids larger than C_6 did not exit the HPLC column, helping to cause a majority of the components detected by GC not to appear by HPLC.

One large peak detected by both ultraviolet and differential refractive index detectors, eluting at approximately 47 min (Unknown A), excited interest. This peak was collected and applied to GC, but did not yield a peak there. Electrospray MS was attempted, but the resulting spectrum was unusable.

DISCUSSION

No phosphate compounds were found in coconut acid water, unlike the acid waters from oils of field crops (3). Considering that the crude coconut oil was not subjected to degumming to remove lecithins, and that the phosphatide content in coconut soapstock is low (Table 1), also opposite to the soapstocks from other vegetable oils (3), its phosphatide level was clearly low.

Nearly all previous work on the composition of coconut oil has concentrated on its fatty acid profile. The profile found in acid water is slightly enriched in the shorter fatty acids, which are more water-soluble, compared to the average composition of fatty acids found in 21 oils from five countries (6) (Table 3).

TABLE 3
Fatty Acid Composition of Coconut Oil and Acid Water (%)

	Oil[a]	Acid water
Butyric	—	4
Caproic	1	5
Caprylic	8	10
Capric	7	3
Lauric	47	45
Myristic	18	21
Palmitic	9	3
Palmitoleic	—	3
Stearic	3	5
Oleic	6	<1
Linoleic	2	—
Arachidic	<1	—

[a] Reference 6.

Perhaps the most interesting discovery from this work is the relatively high amounts of highly water-soluble alcohols and hydroxylated, aldehydo, and dibasic acids, and the relative absence of sugars in coconut acid water (Figure 2). Only one sugar, sucrose, appeared, compared to seven (fructose, galactose, glucose, melibiose, palatinose, sucrose, and raffinose) in one or more of the six acid waters previously analyzed (3). Of the alcohols and acids found in coconut acid water, all appear to arise from coconut tissue, except for lactic acid, which is almost surely the product of fermentation after the oil had been isolated. Apparently, no accumulation of these alcohols or acids had been previously noted in coconut tissue.

Analysis of the acid water from coconut oil is a sensitive probe of the metabolism of the plant itself because water-soluble materials present in low concentrations in the crude oil, where they are not detected, are removed first into soapstock and then into an aqueous phase, where they are easily detected. A parallel exercise has been conducted on palm acid oil (7), the primarily fatty acid layer that results from the acidification of palm oil soapstock. In that case, steam distillation of the acid oil into pentane, followed by GC of the distillate after the pentane had been evaporated, yielded a mixture of aldehydes, ketones, and hydrocarbons, along with a few of the fatty acids prominent in soapstock. Such a study has apparently not occurred with coconut acid oil, but would likely give much the same product pattern, given the similarities between the two plant species.

ACKNOWLEDGMENTS

The authors thank the U. S. Department of Agriculture for its generous financial support through the Biotechnology Byproducts Consortium, a partnership of Iowa State University, the University of Iowa, and the City of Cedar Rapids, Iowa. The authors are grateful to Duncan Guy of Lou Ana Foods for donating coconut soapstock, to Walter Trahanovsky of Iowa State University for his advice on

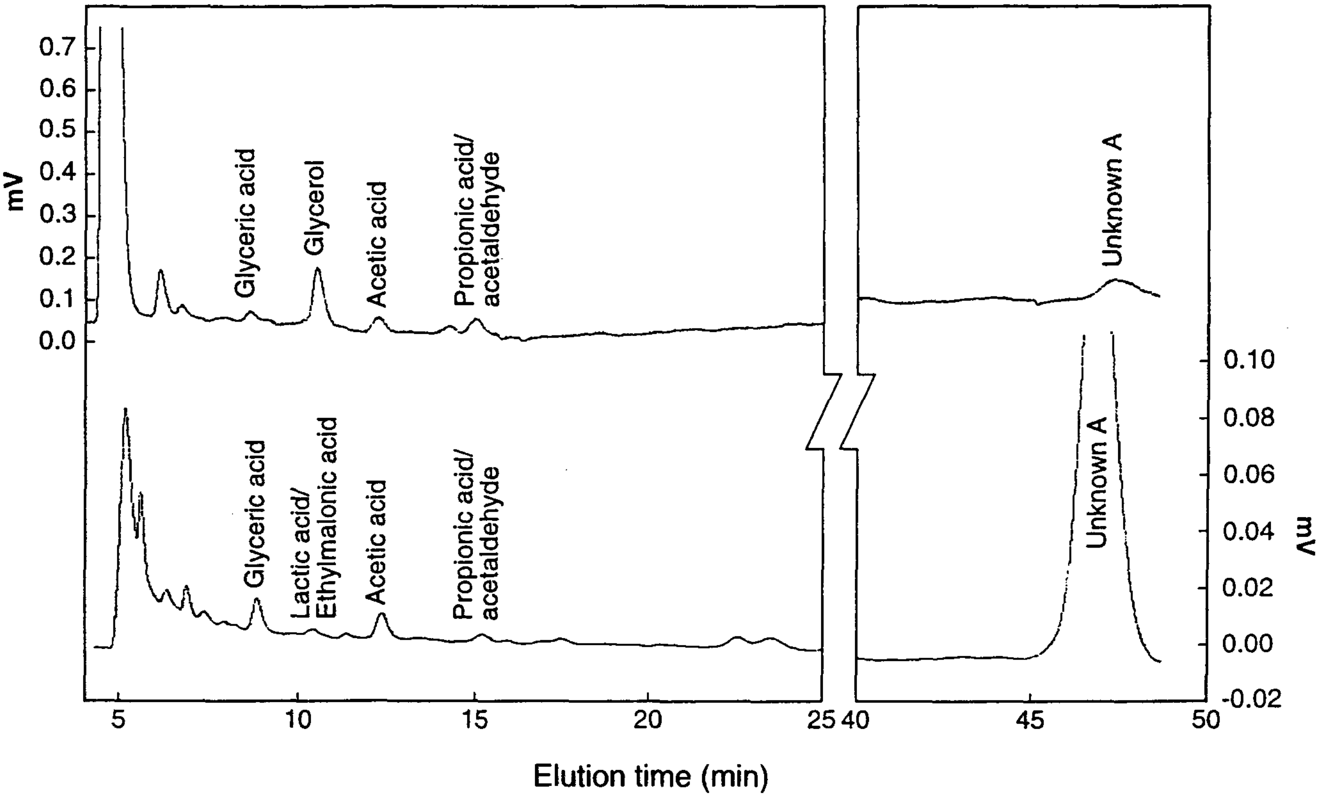

FIG. 2. High-performance liquid chromatographic chromatograms of neutralized coconut acid water. Upper: differential refractometric detection. Lower: ultraviolet absorption detection.

sample identification, and to undergraduate students Jana Mentzer, Michael Stratton, and Raymond Turner for help with experimental work. Thanks are also due to Kamel Harrata of the ISU Department of Chemistry Instrument Services Group, who helped with the MS work.

REFERENCES

1. Dowd, M.K., P.J. Reilly, and W.S. Trahanovsky, Low Molecular Weight Organic Composition of Ethanol Stillage from Corn, *Cereal Chem. 70:*204–209 (1993).
2. Dowd, M.K., S.L. Johansen, L. Cantarella, and P.J. Reilly, Low Molecular Weight Organic Composition of Ethanol Stillage from Sugar Cane Molasses, Citrus Waste, and Sweet Whey, *J. Agric. Food Chem. 42:*283–288 (1994).
3. Johansen, S.L., A. Sivasothy, M.K. Dowd, P.J. Reilly, and E.G. Hammond, Low Molecular Weight Organic Compositions of Acid Waters from Vegetable Oil Soapstocks, *J. Am. Oil Chem. Soc. 73:*1275–1286 (1996).
4. Howard, B., *Oils and Oilseeds to 1996: The New Patterns of Supply and Demand.* Special Report No M703, The Economist Intelligence Unit, London, 1993, p. 29.
5. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* edited by D. Firestone, 4th edn., Vol. 1, Champaign, 1989.
6. Rossell, J.B., B. King, and M.J. Downes, Composition of Oil, *J. Am. Oil Chem. Soc. 62:*221–230 (1985).
7. Kuntom, A., W.-L. Siew, and Y.-A. Tan, Characterization of Palm Acid Oil, *Ibid. 71:*525–528 (1994).

[Received February 19, 1996; accepted June 27, 1996]