Journal of Chromatographic Science 2012;50:666–672
doi:10.1093/chromsci/bms061 Advance Access publication May 17, 2012

Article

Characterization of Jamaican Agro-Industrial Wastes. Part II, Fatty Acid Profiling Using HPLC: Precolumn Derivatization with Phenacyl Bromide

Y.A. Bailey-Shaw[1]*, K.D. Golden[2], A.G.M. Pearson[2] and R.B.R. Porter[3]

[1]Product Research and Development Division (Natural Products Unit), Scientific Research Council, P.O. Box 350, Kingston 6, Jamaica W.I, [2]Department of Basic Medical Sciences (Biochemistry Section), and [3]Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica W.I

*Author to whom correspondence should be addressed. Email: yvonneb@src-jamaica.org

Received 30 August 2011; revised 28 October 2011

This paper describes the determination of fatty acid composition of coffee, citrus and rum distillery wastes using reversed-phase high-performance liquid chromatography (RP-HPLC). Lipid extracts of the waste samples are derivatized with phenacyl bromide and their phenacyl esters are separated on a C8 reversed-phase column by using continuous gradient elution with water and acetonitrile. The presence of saturated and unsaturated fatty acids in quantifiable amounts in the examined wastes, as well as the high percentage recoveries, are clear indications that these wastes have potential value as inexpensive sources of lipids. The HPLC procedures described here could be adopted for further analysis of materials of this nature.

Introduction

Jamaica's rum distilleries, coffee factories and citrus processors, although major players in economic growth and development, are also among the primary sources of water pollution and damage to economically important natural resources and ecosystems. These agro-industrial wastes are composed of a wide diversity of organic compounds, including carbohydrates, proteins, lipids, vitamins and minerals, thus accounting for the generation of large quantities of organic matter with high chemical and biochemical oxygen demand loads. These are used, to a limited extent, as animal feed and soil conditioners, or are otherwise discarded. Because they are primarily organic in nature, several options are available for biological recycling. However, evaluation of these options is possible only if the contents of the major biochemical groups are known.

Lipids, one of the major biochemicals present in agro-industrial wastes, are best characterized by their fatty acids. These fatty acids differ in chain length, the degree of unsaturation, configuration, the position of the double bonds, and the presence of other functionalities, thereby rendering their composition the most definitive characteristic of such lipids (1). Fatty acids are also important biological molecules, functioning primarily as sources of energy and structural components for cells (2).

Fatty acids are most frequently determined by capillary gas chromatography (GC) of the methyl esters, prepared by a variety of methods that have been thoroughly reviewed (1, 3). However, in recent times, the application of high-performance liquid chromatography (HPLC) for the separation of fatty acids has increased tremendously (4). The emergence of new derivatization techniques and advances in detection technology have been cited as the primary reasons for this increase (1, 5, 6).

HPLC offers several advantages; for example, a wide range of compounds can be isolated, including those that possess high molecular weights or are thermally labile, nonvolatile, polar, nonpolar or ionic. Additionally, samples can also be subjected to further analysis because they are not destroyed during the detection process (4), with the exception of samples analyzed by liquid chromatography–mass spectrometry. HPLC operates at ambient temperature, so there is relatively little risk to sensitive functional groups (7). In addition, many derivatives have been described for HPLC, whereas fatty acid profiling by GC is predominantly based on a single derivative, fatty acid methyl esters (FAMEs) (1, 4). Because fatty acids lack chromophores, prechromatographic derivatizations are required to increase detector sensitivity and selectivity to enhance separation of the compounds being eluted during HPLC analysis (4). Improvement in sensitivity may result in improvement of detectability (lower limits of detection).

A plethora of ultraviolet (UV)-absorbing and fluorescent derivatives have been described. Typical examples include: phenacyl, napthacyl, methoxyanilide, methoxyacetophenone, pentafluorobenzyl, phenylhydrazine, 9-aminophenanthrene, pyrenldiazomethane, methoxycoumarin, quinoxalinone and dansyl piperazides, among others, which have been comprehensively reviewed (1, 4). However, there is no consensus as to which is the best derivative from a chromatographic standpoint, with the choice depending on the detector available to the analyst. For the purpose of this study, phenacyl ester derivatives were prepared. These have been described as the most frequently used derivatives due to their easy detection using inexpensive fixed wavelength UV detectors. The high molar absorptivity (18,700 mol/cm at 254 nm) and the close proximity of the absorption maximum (257 nm in methanol for p-bromophenacyl esters) to 254 nm make these derivatives almost ideally suited for analysis with the previously mentioned detectors. In addition, these derivatives allow for greater sensitivity, because the detector responds only to the ester moiety, giving a quantitative molar response (1, 4, 7, 8).

Therefore, the purpose of this study was to determine the fatty acid composition of the selected agro-industrial wastes to evaluate their potential for recycling.

Before this study, the use of HPLC has never been employed in the development of the fatty acid profiles of Jamaican agro-industrial wastes, making this work highly important.

© The Author [2012]. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

Experimental

Samples

Coffee processing wastes (coffee pulp, pulping water—water that takes the pulp from the pulper—and wash water with mucilage—water that takes the mucilage from the aquapulper) were obtained from a coffee factory (traditional aquapulper, wet processing) in St. Andrew, Jamaica.

Citrus wastes (juice extractor residues—orange and grapefruit pulp, citrus wash water—water used to wash fruits and floors—and citrus press liquor—waste from pressing of juice extractor residues during production of citrus meal) were obtained from a commercial citrus processing plant in St. Catherine, Jamaica.

Liquid distillery wastes [pot stills and fermentor bottoms (light and heavy) and continuous still effluent] were obtained from a sugar factory/distillery in Trelawny, Jamaica.

Reagents

All reagents used, unless otherwise stated, were analytical grade. Fatty acid standards, methanol and chloroform were obtained from Sigma Chemical Co. (St. Louis, MO). HPLC-grade acetonitrile was obtained from Acros (Morris Plains, NJ), and phenacyl bromide was obtained from British Drug Houses (BDH) (Poole, UK).

Water used for HPLC was deionized and then purified with a Milli-Q ultrapure water system. Glass distilled water was used for ordinary preparations.

HPLC apparatus

HPLC analyses were performed using a Beckman System Gold-Nouveau HPLC Unit (Beckman Instruments, Fullerton, CA) equipped with a 126 Programmable Solvent Module with a binary pump, a 168 photodiode array detector and a 508 autosampler fitted with a 20-µL loop, and utilizing Gold Nouveau Software.

Sample preparation

Before extraction, solid samples (coffee pulp and citrus juice extractor residues) were freeze-dried and stored in sealed plastic bags in a dessicator at 0–4°C.

Citrus press liquor, citrus wash water, coffee wash water and water containing mucilage were stored at −10°C without prior preparation. The distillery wastes, on the other hand, were separated into fractions of dissolved (supernatant) and suspended solids (pellets) by centrifugation procedures before analysis. Both fractions were stored at 4°C.

Extraction of total lipids

Total lipids were extracted from the waste samples according to the method of Bligh and Dyer (9), to which minor modifications were made. Approximately 1 g of sample was homogenized at high speed in a blender for 2 min with a mixture of water (8 mL), chloroform (10 mL) and methanol (20 mL). No water was added to liquid samples. A further 10 mL of chloroform was added and the mixture homogenized for 30 s, followed by further homogenization for 30 s with 10 mL of distilled water. To avoid inaccuracies of filtration and re-extraction of the residue, the whole mixture was centrifuged at 2,000–2,500 × g (IEC Centrifuge) and 0–4°C for 10 min. The volume of the lower chloroform layer (lipid extract) was then recorded and the layer was removed by aspiration and gravity filtered (Whatman No. 2 filter paper; Whatman International, Kent, UK).

Saponification of lipids

Virtually all procedures for the preparation of derivatives for fatty acid profiling by HPLC commence with the saponification of sample lipids (1). Saponification was therefore performed according to the method of Slaughter and Minabe (10), with minor modifications. Lipid extracts (5 mL) were evaporated to dryness under N_2 in screw-capped tubes. One milliliters of ethanolic KOH (100g/L) was then added to the residue and the tubes capped and heated in a water bath (95°C) for 1 h. Two milliliters (2 mL) of 2M HCl was added to the residues, which were then extracted twice with 2-mL portions of diethyl ether. The extracted portions were pooled and evaporated under N_2 and the residues derivatized for analysis.

Derivatization of lipids

Phenacyl ester derivatives of the fatty acids were prepared according to the method of Borch (11), to which minor modifications were made. Approximately 5 mg of each fatty acid, 500 µL of phenacyl bromide (12 mg/mL in acetone) and 500 µL of triethylamine (10 mg/mL in acetone) were combined in a small screw-cap vial and heated for 30 min at 100°C. The formed derivative was then filtered through a 0.45-µm Millipore filter membrane and an aliquot was injected onto the column. The standard mixture used contained saturated (C6–C22) and unsaturated (C16.1, C18.1, C18.2, C18.3 and C24.1) fatty acids. Nervonic acid (C24:1) was used as the internal standard.

The residues of previously prepared saponified sample extracts were derivatized in a similar manner. However, 100 µL of C24.1 at a concentration of 5 mg/mL (prepared in acetone) was added to each sample residue before derivatization.

Recovery analysis

A 10-mg/mL mixture (1 mL) containing the saturated and unsaturated fatty acids described previously was used to replace 1 mL of chloroform used in the extraction of lipids. Lipids were extracted, purified and estimated as described previously and the recoveries calculated based on the difference between the total lipid content in the spiked samples and the amount in the non-spiked samples.

HPLC analysis

The phenacyl ester derivatives were separated at room temperature (26°C) using a Phase Sep S5 C8, 5 µm, 250 × 4.6 mm column (Phase Separations Inc.; Norwalk, NJ). The solvent system consisted of water and acetonitrile. A continuous gradient elution of 70–100% acetonitrile was employed at a flow

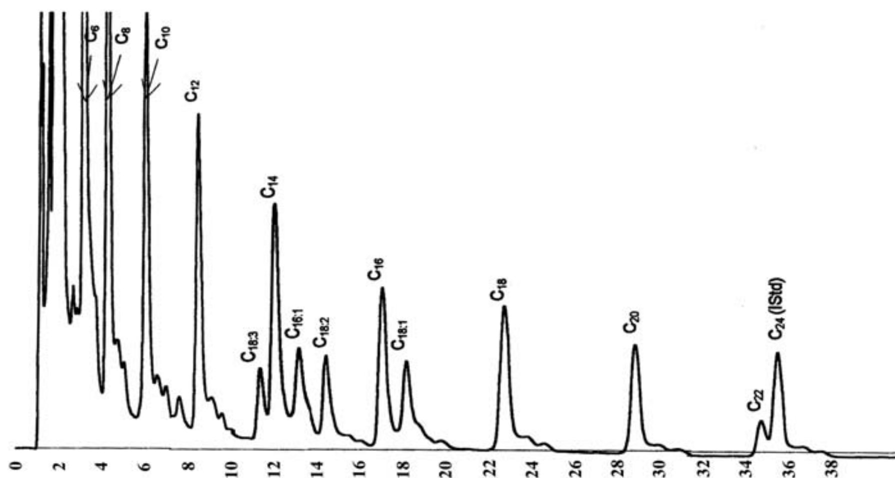

Figure 1. Chromatogram of phenacyl esters of standard fatty acids. C6, n caproic acid; C8, caprylic acid; C10, capric acid; C12, lauric acid; C18.3, linolenic acid; C14, myristic acid; C16.1, palmitoleic acid; C18.2, linoleic acid; C16, palmitic acid; C18.1, oleic acid; C18, stearic acid; C20, arachidic acid; C22, behenic acid; C24.1, nervonic acid (ISTD: internal standard).

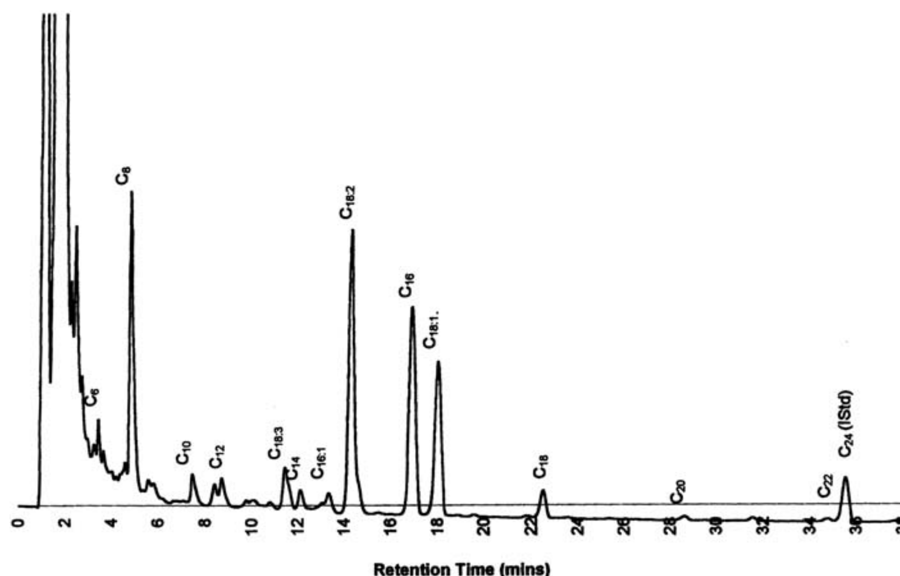

Figure 2. Chromatogram of phenacyl esters of grapefruit pulp. Peak labels are the same as in Figure 1.

rate of 2 mL/min and the detector wavelength was set at 254 nm (12). A sample volume of 20 μL was injected. All determinations were conducted in duplicate.

Results and Discussion

Phenacyl esters were easily prepared and separation was shown to be satisfying in terms of peak resolution. The elution profile of standard unsaturated and saturated fatty acids is presented in Figure 1. Retention time was observed to increase with chain length (C6 elutes before C8 or C10). However, increasing unsaturation resulted in a decrease in retention time (C18:3 elutes before C18:2). This was not surprising, because in reversed-phase (RP)-HPLC, fatty acids are separated by both chain length and the degree of unsaturation (7, 11).

The trends observed in peak elution of the standard mixture were identical to those in the samples. Representative chromatograms of some wastes are presented in Figures 2–3.

Total analysis time for each sample was approximately 36 min, a significant improvement on previously reported separation times of 56 min, 80 min and 4 h (11, 13, 14). This was because gradient elution was optimized by the use of a continuous gradient of 70–100% acetonitrile on a C8 reversed-phase column (12). Other investigators (11, 13, 14) employed the use of C18 reversed-phase columns [Borch (11) used a column 900 mm in length], and varying gradient elution systems of acetonitrile and water (80–90%) and methanol, acetonitrile and water. Methanol and acetonitrile and their aqueous mixtures are commonly used eluents in separations on nonpolar stationary phases. Engelhardt and Elgass (12), in

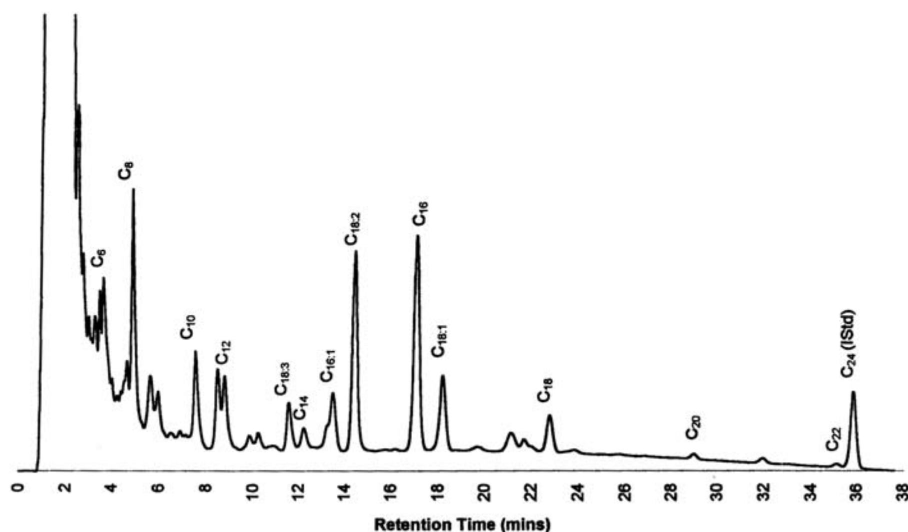

Figure 3. Chromatogram of phenacyl esters of light pot still effluent suspended solid (pellet). Peak labels are the same as in Figure 1.

Table I
Fatty Acid Content and Recoveries of Coffee Processing Wastes as Determined by RP-HPLC of Phenacyl Esters*

Fatty acid	Coffee pulp (mg/100 g dry weight)	Recovery (%)	Coffee wash water (mg/100 g)	Recovery (%)	Wastewater with mucilage (mg/100 g)	Recovery (%)
C6	2.38 ± 0.02	99.6	0.199 ± 0.001	100.0	0.238 ± 0.001	100.0
C8	3.49 ± 0.02	99.1	0.70 ± 0.02	100.0	1.060 ± 0.003	100.0
C10	1.403 ± 0.002	98.8	0.092 ± 0.004	99.9	0.06 ± 0.005	99.9
C12	1.15 ± 0.03	98.6	0.32 ± 0.03	99.9	0.10 ± 0.02	99.9
C18.3	3.09 ± 0.07	100.0	0.38 ± 0.02	100.0	0.18800 ± 0.00005	100.0
C14	0.99 ± 0.07	98.8	0.02 ± 0.003	99.9	0.010 ± 0.002	99.9
C16.1	3.12 ± 0.03	99.8	0.19 ± 0.008	100.0	0.100 ± 0.002	100.0
C18.2	5.3 ± 0.1	99.7	0.01 ± 0.001	100.0	0.01820 ± 0.0004	100.0
C16	3.7 ± 0.3	98.5	0.03 ± 0.007	100.0	0.030 ± 0.005	99.9
C18.1	1.1 ± 0.1	98.5	0.25 ± 0.02	100.0	0.085 ± 0.002	99.9
C18	1.16 ± 0.02	98.7	0.006 ± 0.001	100.0	0.00480 ± 0.00006	99.9
C20	0.46 ± 0.04	98.6	ND	99.9	0.00371 ± 0.00005	99.9
C22	0.108 ± 0.007	97.9	ND	99.8	ND	99.8
Total	27.50		2.20		1.90	
% UFA	45.70		37.70		20.50	
% SFA	54.30		62.30		79.50	

*Values in the table represent the mean of two determinations ± standard error of the mean. ND: not detected, UFA: unsaturated fatty acid, SFA: saturated fatty acid, C6, *n*-caproic acid; C8, caprylic acid; C10, capric acid; C12, lauric acid; C18.3, linolenic acid; C14, myristic acid; C16.1, palmitoleic acid; C18.2, linoleic acid; C16, palmitic acid; C18.1, oleic acid; C18, stearic acid; C20, arachidic acid; C22, behenic acid.

comparing the separation of phenacyl esters of even-numbered saturated fatty acids with water–methanol and water–acetonitrile gradients under otherwise identical conditions, showed that with water–methanol gradients, the resolutions of the acids eluted after the C8 compound diminished more markedly than with water–acetonitrile. Consequently, in the C12–C20 region, where most of the common unsaturated fatty acids appear, the former gradient achieved only approximately half the resolution of the latter. These investigators also proved that satisfactory separation could be achieved on a C8 instead of a C18 reversed- phase column, which in the case of the latter, had the highest relative retentions, but could not separate oleic and palmitic acids.

Fatty acid contents were calculated using the nervonic acid internal standard method (15), and the percentage recoveries were calculated based on the difference between the total amount determined in the spiked and that in the non-spiked samples. These values are reported in Tables I–IV.

Coffee wastes

Coffee wastes were found to be predominantly composed of saturated fatty acids, with linoleic (5.27 mg/100 g dry weight) and palmitic (3.71 mg/100g) acids identified as the two major fatty acids occurring in coffee pulp. While information reported in literature does not directly address coffee wastes, indications are that most of the lipids, the coffee oil, are located in the endosperm of the coffee bean, with only a small amount (0.2–0.3%), the coffee wax, in the outer layers (16). Coffee wax contains a relatively high percentage of saturated

Table II
Fatty Acid Content and Recoveries of Citrus Processing Wastes as Determined by RP-HPLC of Phenacyl Esters*

Fatty acid	Grapefruit waste (mg/100 g)	Recovery (%)	Orange waste (mg/100 g)	Recovery (%)	Press liquor (mg/100 g)	Recovery (%)	Wash water (mg/100 g)	Recovery (%)
C6	2.95 ± 0.36	99.8	4.87 ± 0.02	99.8	0.0800 ± 0.002	100.0	0.151 ± 0.008	100.0
C8	4.32 ± 0.07	99.7	1.96 ± 0.33	98.8	0.0400 ± 0.0003	99.9	0.107 ± 0.002	99.9
C10	1.83 ± 0.36	99.0	2.46 ± 0.27	98.6	0.05 ± 0.01	99.9	0.07897 ± 0.00006	99.9
C12	2.07 ± 0.37	98.9	1.80 ± 0.01	98.3	0.0980 ± 0.0007	99.9	0.064 ± 0.007	99.9
C18.3	7.91 ± 0.07	100.0	4.1 ± 0.2	100.3	ND	100.0	ND	100.0
C14	1.030 ± 0.007	99.1	0.75 ± 0.12	98.7	0.0193 ± 0.0002	99.9	0.0090 ± 0.0001	99.9
C16.1	5.68 ± 0.04	100.0	4.63 ± 0.08	99.3	0.153 ± 0.002	100.0	0.0948 ± 0.0002	100.0
C18.2	28.05 ± 0.02	100.9	7.11 ± 0.09	100.0	0.048 ± 0.002	100.0	0.029 ± 0.005	100.0
C16	9.63 ± 0.02	99.4	1.95 ± 0.05	98.5	0.01927 ± 0.00008	99.9	0.0090 ± 0.0007	99.9
C18.1	12.96 ± 0.07	99.4	3.00 ± 0.01	98.3	0.0400 ± 0.0009	99.9	0.025 ± 0.003	99.9
C18	1.40 ± 0.22	99.1	0.24 ± 0.009	98.4	0.00963 ± 0.00008	99.9	0.0027 ± 0.0001	99.9
C20	0.2600 ± 0.0006	99.0	0.119 ± 0.005	98.3	ND	99.9	ND	99.9
C22	0.16 ± 0.01	98.0	0.0678 ± 0.0005	97.4	ND	99.8	ND	99.8
Total	78.25		33.20		0.60		0.60	
% UFA	69.80		57.10		40.00		23.30	
% SFA	30.20		42.90		60.00		76.70	

*Values in the table represent the mean of two determinations ± standard error of the mean.

Table III
Fatty Acid Content and Recoveries of Distillery Wastes (Dissolved Solids) as Determined by RP-HPLC of Phenacyl Esters*

Fatty acid	CS (mg/100 g)	Recovery (%)	LPS (mg/100 g)	Recovery (%)	HPS (mg/100 g)	Recovery (%)	LFB (mg/100 g)	Recovery (%)	HFB (mg/100 g)	Recovery (%)
C6	0.27 ± 0.02	100.0	0.350 ± 0.002	100.0	0.186 ± 0.003	100.0	0.240 ± 0.007	100.0	0.2228 ± 0.0002	100.0
C8	0.120 ± 0.003	100.0	0.194 ± 0.007	99.9	0.25 ± 0.01	99.9	0.14 ± 0.04	99.9	0.1600 ± 0.0004	100.0
C10	0.12 ± 0.02	99.9	0.200 ± 0.002	99.9	0.1626 ± 0.0005	99.9	0.1100 ± 0.0001	99.9	0.18000 ± 0.00003	99.9
C12	0.110000 ± 0.000008	99.9	0.221 ± 0.004	99.9	0.17 ± 0.01	99.8	0.0900 ± 0.0001	99.8	0.25 ± 0.03	99.9
C18.3	ND	99.8	ND	99.8	ND	99.8	0.0900 ± 0.0003	99.8	0.236 ± 0.004	99.9
C14	0.02 ± 0.001	100.0	0.050 ± 0.002	100.0	0.040 ± 0.007	99.9	0.020 ± 0.002	100.0	0.066 ± 0.004	100.0
C16.1	0.20 ± 0.02	99.9	0.407 ± 0.003	99.8	0.03000 ± 0.00007	99.8	0.20 ± 0.01	99.9	0.51 ± 0.08	99.9
C18.2	0.17 ± 0.03	100.0	0.300 ± 0.002	100.0	0.0427 ± 0.0005	100.0	0.105 ± 0.002	100.0	0.40 ± 0.02	100.0
C16	0.077 ± 0.001	100.0	0.135 ± 0.004	100.0	0.12 ± 0.01	99.9	0.045 ± 0.004	100.0	0.232 ± 0.002	100.0
C18.1	0.106 ± 0.004	99.9	0.25 ± 0.01	99.9	0.26 ± 0.03	99.9	0.043 ± 0.007	99.9	0.47 ± 0.01	99.9
C18	0.0072 ± 0.0003	99.9	0.150 ± 0.001	99.9	0.207 ± 0.001	99.9	0.00800 ± 0.00006	99.9	0.118 ± 0.002	99.9
C20	0.0035 ± 0.0003	99.9	ND	99.8	0.190 ± 0.005	99.9	ND	99.9	0.237 ± 0.002	99.9
C22	0.00100 ± 0.00003	99.9	0.0030 ± 0.0002	99.8	ND	99.9	ND	99.9	0.0081 ± 0.0004	99.9
Total	1.20		2.20		1.60		1.10		3.10	
% UFA	39.20		43.20		21.30		39.10		51.90	
% SFA	60.80		56.80		78.20		60.90		48.10	

*Values in the table represent the mean of two determinations ± standard error of the mean. Continuous still effluent: CS; light pot still: LPS; heavy pot still: HPS; light fermentor bottom: LFB; heavy fermentor bottom: HFB.

fatty acids (17). The predominant distribution of saturated fatty acids in the wastes suggests that some amount of dewaxing could have occurred during pulping of the beans, thereby contributing to observed results. However, this is not conclusive, because the water solubility of the main constituents of the wax would have to be investigated. Coffee (*C. arabica*) is also reported to contain a high percentage of linoleic (46.9–55.8%) and palmitic (29.7–41.2%) acids, but lower values for stearic (3.8–7.1%), oleic (4.2–7.1%), arachidic (0.9–1.9%) and linolenic acids (0.6–1.4%), findings that are in keeping with results observed regarding coffee pulp (17).

Citrus wastes

Citrus solid wastes were found to contain primarily unsaturated fatty acids (grapefruit 69.8%, orange 57.1%), whereas the opposite was true for the press liquor and wash water. This is not surprising, because the lipid contents of the solid wastes are suspected to be linked to the presence of citrus seeds, some of which remain with the juice extractor residues after juice extraction. The fatty acids of citrus seed have been found to consist of saturated acids: palmitic (20.7%), stearic (4.7%) and arachidic (0.9%); and unsaturated acids: linolenic (0.6%), linoleic (36.5%) and oleic (36.6%) (18). These data conform to the observed results, indicating a similar trend with respect to the occurrence of the high levels of unsaturated versus saturated fatty acids.

Distillery wastes

The dissolved solids of distillery effluents were predominantly composed of saturated fatty acids. On the other hand, the

Table IV
Fatty Acid Content and Recoveries of Distillery Wastes (Suspended Solids) as Determined by RP-HPLC of Phenacyl Esters*

Fatty acid	CS (mg/100 g)	Recovery (%)	LPS (mg/100 g)	Recovery (%)	HPS (mg/100 g)	Recovery (%)	LFB (mg/100 g)	Recovery (%)	HFB (mg/100 g)	Recovery (%)
C6	3.5 ± 0.1	100.0	2.07 ± 0.01	100.0	1.76 ± 0.04	100.0	0.624 ± 0.009	100.0	0.338 ± 0.005	100.0
C8	1.99 ± 0.03	98.9	1.215 ± 0.002	99.3	1.2 ± 0.1	98.6	0.33 ± 0.02	99.9	0.16 ± 0.02	99.9
C10	2.28 ± 0.01	97.5	2.120 ± 0.002	99.0	1.42 ± 0.05	98.6	0.28 ± 0.01	99.8	0.15 ± 0.02	99.8
C12	3.04 ± 0.02	97.2	2.170 ± 0.003	98.8	1.310 ± 0.002	98.3	0.461 ± 0.002	99.7	0.67 ± 0.01	99.7
C18.3	5.26 ± 0.02	96.5	3.600 ± 0.003	98.3	4.4 ± 0.2	97.7	ND	99.7	0.240 ± 0.006	99.7
C14	1.37 ± 0.01	100.5	0.600 ± 0.002	100.2	0.517 ± 0.006	100.1	0.077 ± 0.002	100.0	0.042 ± 0.002	100.0
C16.1	10.343 ± 0.003	97.2	5.120 ± 0.009	98.6	3.353 ± 0.005	98.7	0.79 ± 0.02	99.8	0.48 ± 0.02	99.7
C18.2	10.34 ± 0.02	100.2	7.100 ± 0.004	100.1	12.76 ± 0.03	99.9	0.65 ± 0.02	100.0	0.610 ± 0.002	100.0
C16	4.18 ± 0.02	100.3	3.97 ± 0.01	100.0	5.6 ± 0.3	99.8	0.84 ± 0.01	100.0	0.560 ± 0.004	99.9
C18.1	7.82 ± 0.02	97.2	5.35 ± 0.02	98.6	6.32 ± 0.02	98.3	0.920 ± 0.003	99.8	0.473 ± 0.002	99.7
C18	6.341 ± 0.006	97.4	3.77 ± 0.02	99.1	1.67 ± 0.01	98.7	0.14 ± 0.03	99.8	0.12 ± 0.01	99.8
C20	3.792 ± 0.001	97.4	2.000 ± 0.005	99.0	1.060 ± 0.003	98.4	0.0250 ± 0.0003	99.7	0.016 ± 0.002	99.7
C22	0.271 ± 0.002	97.2	0.24 ± 0.02	99.1	0.2700 ± 0.0006	98.5	ND	99.7	0.010 ± 0.0002	99.7
Total	60.60		39.30		41.60		5.10		3.20	
% UFA	56.00		53.80		64.50		46.30		55.90	
% SFA	44.00		46.20		35.50		53.70		54.10	

*Values in the table represent the mean of two determinations ± standard error of the mean.

suspended solids of distillery effluents, with the exception of light fermentor bottom (46.3% unsaturated fatty acids), were found to contain mostly unsaturated fatty acids. These observations were not surprising, because distillery effluents contain dead yeast cells of Saccharomyces. Fatty acids may constitute 70–90% of total yeast lipids consisting primarily of the C12 to C18 straight chain derivatives with an even number of carbon atoms (19). Additionally, palmitoleic (C16.1) and oleic (C18.1) acids have been identified as the predominant unsaturated fatty acids of Saccharomyces (20), further confirming the results that indicate high levels of these fatty acids in the suspended solids of distillery effluents in particular. Additionally, fatty acid profiles of distillery effluent developed using GC reported in a previous study (21) identified the sole occurrence of saturated fatty acids (C9, C12, C14, C16, C17, C18 and C19) in suspended solids. Additionally, thin layer chromatography of lipid extracts tested in the same study showed no evidence of lipids in fractions of dissolved solids. This clearly highlights the greater sensitivity of the methods of extraction and analysis used in this study, which were not only able to identify and quantify saturated (C6–C22) and unsaturated (C16 and C18) fatty acids in suspended solids, but also in fractions of dissolved solids.

Importantly, the major fatty acid occurring in all three solid wastes was linoleic acid (5.27 mg/100 g in coffee pulp, 28.05 mg/100g in grapefruit waste and 7.11 mg/100 g in orange waste). Linoleic and linolenic acids are the most common polyunsaturated fatty acids (PUFAs) occurring in plant lipids (3). In addition, they are recognized as essential, because they cannot be synthesized by animals and therefore must come from plants by way of the diet. This augers well for the inclusion of these wastes into animal diets.

Recoveries of fatty acids between 96.5–100.5% were obtained overall, suggesting that the methods of extraction of total lipids and the estimation of fatty acids were highly efficient, thus the results can be used with confidence. This is particularly important, because a search of the literature did not reveal any other studies in which percentage recoveries were investigated.

Conclusion

Jamaican coffee, citrus and rum distillery wastes have potential value as inexpensive, recoverable sources of lipids. In addition, HPLC determination of lipid extracts from the wastes as their phenacyl esters can undoubtedly be used in the development of the fatty acid profiles of wastes of this nature.

Acknowledgments

This study was financed by a grant from the Environmental Foundation of Jamaica (EFJ). The authors would also like to thank Dr. Lawrence Willliams, who assisted with editing the manuscript and Mr. Timon Waugh, Dr. Percival Miller and Ms. Paulette Tavares who facilitated sample collection and provided technical information relevant to the coffee and citrus industries respectively.

References

1. Gutnikov, G.; Fatty acid profiles of lipid samples; *Journal of Chromatography B*, (1995); 671: 71–89.
2. Yaqoob, P.; Fatty acids as gatekeepers of immune cell regulation; *Trends in Immunology*, (2003); 24(12): 639–645.
3. Christie, W.W.; Preparation of ester derivatives of fatty acids for chromatographic analysis. In *Advances in lipid methodology, Volume 2*. Christie, W.W. (ed); Oily Press, Dundee, (1993); pp. 69–111.
4. Rao, M.S., Hidajat, K., Ching, C.B.; Reversed-phase HPLC: The separation method for the characterization and purification of long chain polyunsaturated fatty acids—A review; *Journal of Chromatographic Science*, (1995);33: 9–21.
5. Fallon, A., Booth, R.F.G., Bell, L.D.; Applications of HPLC in biochemistry: Lipids. In *Laboratory techniques in biochemistry and molecular biology, Volume 17*. Burdon, R.H., van Knippenberg, P.H. (eds). Elsevier Science, Amsterdam, The Netherlands, (1987); pp. 193–212.
6. Emrani, J.; Lipid analysis by HPLC. In *Encyclopedia of chromatography*. Cazes, J. (ed). Marcel Dekker, Boca Raton, FL; (2002); pp. 476–480.

7. Christie, W.W.; Analysis of fatty acids by high-performance liquid chromatography; *Lipid Technology*, (1997); 9: 124–126.
8. Durst, H.D., Milano, M., Kikta, E.J., Connelly, S.A., Grushka, E.; Phenacyl esters of fatty acids via crown ester catalysts for enhanced ultraviolet detection in liquid chromatography; *Analytical Chemistry*, (1975); 47(11): 1797–1801.
9. Bligh, E.G., Dyer, W.J.; A rapid method of total lipid extraction and purification; *Canadian Journal of Biochemistry and Physiology*, (1959); 37: 911–917.
10. Slaughter, J.C., Minabe, M.; Fatty acid-containing lipids of yeast *Saccharomyces cerevisiae* during post-fermentation decline in viability; *Journal of the Science of Food and Agriculture*, (1994); 65: 497–501.
11. Borch, R.F.; Separation of long chain fatty acids as phenacyl esters by high pressure liquid chromatography; *Analytical Chemistry*, (1975); 47(4): 2437–2439.
12. Engelhardt, H., Elgass, H.; Optimization of gradient elution separation of fatty acid phenacyl esters; *Journal of Chromatography*, (1978); 158: 249–259.
13. Wood, R., Lee, T.; High performance liquid chromatography of fatty acids: Quantitative analysis of saturated, monoenoic, polyenoic, and geometrical isomers; *Journal of Chromatography*, (1983); 254: 237–246.
14. Hanis, T., Smrz, M., Klir, P., Macek, K.; Determination of fatty acids as phenacyl esters in rat adipose tissue and blood vessel walls by high-performance liquid chromatography; *Journal of Chromatography*, (1988); 452: 443–457.
15. Mulcock, A.P., Barnes, F., Hickey, B.; The analysis of sugars in beet. Part III. HPLC; *International Sugar Journal*, (1985); 87: 188–194.
16. Speer, K., Kölling-Speer, I.; Lipids. In *Coffee—Recent developments*. Clarke, R.J., Vitzthum, O.G. (eds). Blackwell Science, Ltd., London, UK, (2001); pp. 33–49
17. Folstar, P.; Lipids. In *Coffee: Chemistry, Volume 1*. Clarke, R.J., Macrae, R. (eds). Elsevier Applied Science, London, UK, (1985); pp. 203–222.
18. Braverman, J.B.S.; Some minor constituents of citrus juices. In *Citrus products: Chemical composition and chemical technology*. Interscience Publishers, Inc., New York, NY, (1949); pp. 122–135.
19. Rencken, I., Fleming, V., Meijering, I., Axcell, B.; Determination of selected sterols and fatty acids in yeast; *Journal of Chromatographic Science*, (1995);33: 525–530.
20. You, K.M., Rosenfield, C., Knipple, D.C.; Ethanol tolerance in yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid; *Applied and Environmental Microbiology*, (2003); 69(3): 1499–1503.
21. Waite, A.E., Pearson, A.G.M.; Investigation of the biochemical content of stillage waste from the rum industry. Proceedings of the 5th Annual National Conference on Science and Technology: Science and Technology in Industry. Scientific Research Council, Kingston, Jamaica, (1991); pp. 146–154.