LA-UR- 02-5765

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Submitted to: Proceedings of Super Green 2002
Suwon, South Korea; November 3-6, 2002

Super Green 2002
Kyung Hee University, Korea, 2002

Extraction of lipid components from Hibiscus seeds by supercritical carbon dioxide and ethanol mixtures

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Abstract

The genus *Hibiscus* exhibits great diversity in the production of natural materials with edible and industrial applications. The seeds of twelve varieties of *Hibiscus* were extracted as a source for triglycerides and phospholipids that could be used in functional foods. The total lipids extracted from the hibiscus species ranged from 8.5% for a variety indigenous to Madagascar (*H. calyphyllus*) to 20% for a hybrid species (*H. Georgia Rose*). The other varieties tested contained between 10% and 15% oil. The fatty acid methyl ester (FAME) analysis indicated the presence of predominately unsaturated fatty acids (75 – 83%). Oleic, linoleic, and linolenic fatty acids were the major unsaturated fatty acids in the extracts which also contained minor amounts of C14, C18, and C20 saturated fatty acids with palmitic acid as the predominate saturated fatty acid. The distributions of the major phospholipids in the CO_2/ethanol extracts were found to vary significantly. Phosphatidylcholine and lysophosphatidylcholine were the predominate phospholipids in these extracts comprising between 50% and 95% of the total phospholipids present. These results show the capability of supercritical fluid extraction techniques to provide a rapid method to recover both polar and nonpolar lipids from *Hibiscus* seeds with a minimum amount of processing. The solvent-free extracts obtained are suitable for use in functional foods and other edible product formulations.

1. Introduction

The genus *Hibiscus* exhibits great diversity in the production of natural materials with edible and industrial applications. Components such as flowers and green tissues may be consumed as specialty foods while the bast fibers and seed oils are a renewable source of industrial materials. The introduction of a new crop is supported by the sale of products that provide an economic incentive for growers. A crop that offers several marketable products provides the grower with protection from overproduction and market saturation.

The seeds of several varieties of Hibiscus were investigated as a source of novel fatty acids. Seeds contain a variety of lipid compounds, e. g. triglycerides, phospholipids, and sterols, that may be conventionally extracted by organic solvents and subsequently isolated for use in functional foods and specialty products. The extraction and isolation of such natural products without the use of organic solvents would improve the marketability of these compounds and promote the development of native *Hibiscus* as an alternative crop.

Pressurized fluid extraction techniques have demonstrated promise for the separation of lipophilic compounds from oilseeds and other natural products (Reverchon, 1997). This initial study examined the major lipid components obtained by the extraction of whole ground seed with supercritical carbon dioxide and ethanol.

2. Experiments

2.1 Seed preparation

Whole seeds were obtained from hibiscus pods harvested in the summer and early fall of 2001. Seeds were separated by hand from the pods and mechanically ground in a Wiley mill to a nominal particle diameter of 0.1 mm. Ground seed samples were stored at -20°C until extractions were performed.

2.2 Extraction.

Lipid components were extracted from samples of ground seed with supercritical carbon dioxide and then a mixture of supercritical carbon dioxide modified with ethanol (Montanari et al., 1999). Extractions were performed with an ISCO model 3560 supercritical fluid extractor on 5-g samples of ground seed. The instrument was programmed to perform two sequential extractions of the ground seeds at 80° C and 53.7 MPa with a fluid flow rate of 2.0 ml/min. An initial static hold of one minute was followed by a dynamic extraction for 50 minutes using carbon dioxide. The second extraction was performed at the same conditions with a mixture of carbon dioxide and 10% ethanol by volume. The collection vials were maintained at 20° C for the first extraction and 10° C for the second extraction. The collected fractions were stored under nitrogen at –20° C prior to analyses.

2.3 Analysis of lipid fractions

High performance liquid chromatography (HPLC) was used to analyze extracts for major neutral and polar lipid components. An Alltech 5-um silica column, 250mm x 4.6mm, (Deerfield, IL) was used with a SpectraPhysics model SP8800 ternary pump system (San Jose, CA, USA), and a Thermo Separation Products model AS3000 autosampler. A 20 µl loop was installed on the autosampler and full loop injections were made. Samples were eluted with a gradient of solvent A: hexane/tetrahydrofuran, 99/1 (vol/vol), solvent B: isopropanol, and solvent C:water (Moreau et al., 1990). Detection of compounds was achieved with a Thermo Separation Products model SP8490 ultraviolet (UV) detector monitoring 205 nm, and the Alltech model 500 ELSD evaporative light scattering detector. Peak identification was achieved by comparison of retention time to commercial standards (Avanti Polar Lipids, Alabaster, AL).

An aliquot of each collected triglyceride fraction was converted to the corresponding fatty acid methyl ester (FAME) by reaction with sodium methoxide. The esters were recovered in hexane, diluted and analyzed using a Hewlett-Packard 5890 gas chromatograph with a flame ionization detector. One µl injection volumes were used with a 100:1 split ratio. Inlet and detector temperatures were set to 250° C. An SP2380 column 30mx0.25mm id (Supelco, Bellefonte, PA) was used with He carrier gas. The oven was programmed initially to 100° C for 5 minutes, ramping to 190 ° C at 3° C/min and then to 250° C at 5° C/min.

3. Results

Analysis of the first and second fractions indicated that the addition of ethanol could extend the solvating power of SC-CO_2 to extract either predominately nonpolar components such as the triglycerides or moderately polar compounds such as the phospholipids.

The results presented in Table 1 show a high degree of unsaturation across all varieties examined. Oleic, linoleic, and linolenic fatty acids appear as predominate components with only minor amounts of the saturated fatty acids detected. Trace amounts of C14 and C20 typically appear also (not shown).

The oil yield was expressed as the weight percent of the total extracted lipids determined gravimetrically from the collected fractions on a dry basis of whole ground seed. These data are also presented in Table 1. The values range from a low of 8.5% for a variety indigenous to Madagascar (*H. calyphyllus*) to nearly 20% for a hybrid species (*Georgia Rose*). Excluding these two varieties the average oil yield was 11.4%.

The distributions of the major phospholipids were found to vary significantly. These data are reported in Table 2 expressed as the percent of total phospholipids. The' following abbreviations are used: phosphatidylethanolamine, PE; phosphatidic acid, PA; phosphatidylserine, PS; phosphatidylcholine, PC; lysophosphatidylcholine, LPC.

4. Discussion

The analysis of hibiscus seed extracts exhibited a large percentage of unsaturated fatty acids. The presence of the nutritionally essential fatty acids suggests potential applications in edible formulations. The oil yields, however, were relatively low compared to commodity oilseeds such as soybean. The variation in oil content shown by these species appears to offer the possibility to

improve oil yield through a program of selective breeding.

The analysis of the extracts for polar lipids suggests that the seed could be a significant source of phospholipids. These could be easily recovered by a selective extraction with carbon dioxide-ethanol mixtures. This would be appropriate for compounds destined for the edible or nutraceutical markets. Natural products recovered by such environmentally acceptable processes could be obtained in high purity and offer an advantage in the market. These products would be included in the natural or nutraceutical category to be marketed directly as supplements or formulated into functional foods (E. Koch, 2001).

5. Conclusion

These results demonstrate the extraction of polar and nonpolar compounds from hibiscus seeds by supercritical carbon dioxide and mixtures of supercritical carbon dioxide and ethanol. The seeds require a minimum of processing prior to extraction, i. e., size reduction, and the extracts obtained are solvent free and suitable for edible products.

References

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Table 1: Fatty acid distributions of SC-CO_2 extracts obtained from several varieties of *Hibiscus* seeds

C16	C18	C18:1	C18:2	% oil	Species
17.4	3.0	24.8	54.9	13.8	moscheutos "Caroline's pink"
18.2	3.4	26.0	52.5	12.7	moscheutos "Arkansas"
17.1	2.7	24.4	55.8	15.3	moscheutos "hairy"
19.0	3.6	29.0	48.5	36.5	moscheutos "smooth"
25.7	3.7	19.9	50.6	13.6	striatus lambertianus "Texas"
19.7	3.7	27.2	49.5	12.7	dasycalyx "big pod"
17.3	3.2	25.8	53.7	17.8	dasycalyx "hairy pod"
17.9	2.9	17.8	61.4	7.1	mutabilis "single pink"
18.3	2.1	15.6	64.0	11.1	mutabilis "short"
16.6		29.0	54.4	11.8	Nathan's Star
17.7		30.1	50.4	19.6	Georgia Rose
17.4	3.3	27.1	52.2	15.1	Grace Coolidge
23.5		18.4	55.3	8.5	calyphyllus

Table 2. Major phospholipids extracted from *Hibiscus* seeds by SC-CO2 modified with 15% ethanol

PE	PA	PS	PC	LPC	Species
3.3		15.5	36.9	44.3	moscheutos "Caroline's pink"
13.0		20.5	8.3	58.1	moscheutos "Arkansas"
4.6	15.6	23	24.6	32.2	moscheutos "hairy"
6.3	4.2	4.6	34.7	50.2	moscheutos "smooth"
4.6	2.3	5.8	47.4	40.0	striatus lambertianus "Texas"
5.1	3.5	2.9	59.4	29.1	dasycalyx "big pod"
7.6		7.0	52.3	33.2	dasycalyx "hairy pod"
4.4	2.7	9.2	17.6	66.2	mutabilis "single pink"
4.4			56.5	39.0	mutabilis "short"
	16.9	31.8	51.3		Nathan's Star
		5.6	44.0	50.5	Georgia Rose
		29.1	70.9		Grace Coolidge