Phytosterols and Fatty Acids in Fig (*Ficus carica*, var. Mission) Fruit and Tree Components

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ABSTRACT: The phytosterol compositions in unsaponifiables of fig (*Ficus carica*, var. Mission) fruit and 3 structural components of the branches; and the fatty acid composition of fig fruits were studied using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The phytosterols were determined from the trimethylsilyl ether (TMS) derivatives of the unsaponifiable samples. Fourteen compounds were separated from fig fruit; 13, 10, and 6 in bark, stem, and pith, respectively. Sitosterol was the most predominant sterol in all parts. Also detected were campesterol, stigmasterol, and fucosterol. Fatty acids in fig fruit, determined as their methyl esters, were myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids.

Keywords: *Ficus carica*; figs; phytosterols; sterols; fatty acids.

Introduction

FIGS (*FICUS CARICA*) ARE PERHAPS THE OLDEST OF ALL CULTIvated fruit species and are grown in numerous areas of the world with subtropical climates. In 1990, about 46,000 short tons of figs were produced in the United States. About 90% of the fig crop is utilized as dried figs. The Mission variety of fig is one of the 3 major types of dried figs available in the U.S. (Enswinger and others 1994). Significant amounts of fig tree branches are often pruned during harvesting and may contain nutraceuticals that have health benefits. In general, phytosterols (Figure 1) are formed in ornamental plants, medicinal herbs, edible plants, shrubs, and trees. They have been reported in seeds, seed oils, roots, stems and branches, and leaves and blossoms, though not in equal total amounts or equal proportions in various parts of a plant or a tree (Pollak 1985). It has been widely reported that phytosterols lower serum cholesterol level in animals and human (Sklan and others 1974; Bhattacharyya and Eggen 1984; Andriamiarina and others 1989; Laraki and others 1991; Howard and Kritchevsky 1997).

The hypocholesterolemic effect of phytosterols may be explained by 3 mechanisms, including inhibitions of (a) cholesterol absorption, (b) hepatic cholesterol esterase, and (c) HMG-CoA reductase (Howard and Kritchevsky 1997). Recent reports claim that phytosterols have effects in the treatment of benign prostatic hyperplasia, rheumatoid arthritis, allergies, and stress-related illness, and inhibit the development of colon cancer (Oomah and Mazza 1999). Phytosterols have been widely studied in vegetables, fruits, seeds, and nuts. A few studies have been conducted on the phytosterols and other chemical components in the unsaponifiables of figs and fig leaves. Weihrauch and Gardner (1978) reported the sterol content from a number of edible plants, but reported only 3 phytosterols in figs. Normen and others (1999) reported the plant sterol content in vegetables and fruits commonly consumed in Sweden. Ahmed and others (1988, 1990) studied the triterpenes from the leaves of fig. However, studies on unsaponifiables including phytosterols from other components of fig trees are scarce.

We studied the sterol profiles of various "parts" of fig tree branches; that is, structural components (bark, stem, pith) and dried figs, as well as the fatty acids composition of fig fruit using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques.

Materials and Methods

Materials

Fruits and branches of fig trees (*Ficus carica*, var. Mission) were obtained from the California Fig Advisory Board (Fresno, Calif., U.S.A.). Fruits were immediately freeze-dried and homogenized. Branches were first dried under room temperature, separated into bark, stem and pith, then freeze-dried and homogenized. Standards of campesterol, stigmasterol, and sitosterol were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). An oil reference standard (AOCA No. 5) and linolenic acid were also purchased from Sigma. Boron trifluoride/methanol reagent was purchased from Supelco, Inc. (Bellefont, Pa., U.S.A.).

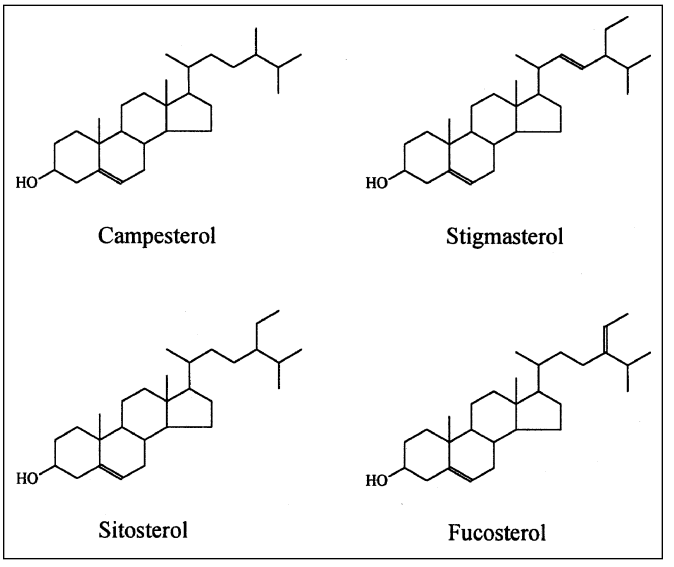

Figure 1—Structures of the representative phytosterols discussed in this work

Extraction of unsaponifiables

Saponification of the samples was by the method of Kovacs and others (1979). Ten g of homogenized sample were directly saponified in a round-bottom flask containing 25 mL 50% KOH and 100 mL 95% EtOH. The mixture was refluxed for 1 hr with moderate stirring, utilizing a heating mantle and a magnetic stirrer. After 1 hr, the mixture was cooled to room temperature and transferred to a separatory funnel with the aid of 30 mL 95% EtOH, 50 mL warm water, and 50 mL cold water. The unsaponifiables were extracted 6 times with 150-mL portions of petroleum ether. The combined petroleum ether extracts were washed with distilled water until soap-free and evaporated to dryness using a rotary evaporator and under N_2 at 40 °C. The weight of concentrate was recorded as total unsaponifiables.

Preparation of TMS derivatives

The unsaponifiables were dissolved in methylene chloride and 100 μL of the solution, with a known concentration, was placed in a capped test tube. Trimethylsilyl ether (TMS) reagent (200 μL) was added and the test tube heated at 60 °C for 20 min in a heating block after tightly capping (Rzama and others 1994). The reagent was removed under nitrogen gas, then dissolved in 250 μL methylene chloride and stored in a freezer for GC and GC-MS analysis.

Preparation of fatty acids

After extraction of the unsaponifiables mentioned above, the remaining aqueous phase was acidified with 6 M HCl until a strongly acidic condition was established using pH indicator paper, and then allowed to dissipate the heat of the neutralization reaction. The solution was extracted 3 times with 150-mL portions of hexane. The hexane extracts were combined and washed 2 times with 50-mL portions of distilled water, followed by drying over anhydrous sodium sulfate. The solvent was removed under reduced pressure in a rotary evaporator, followed by a gentle stream of N_2, and the residue was weighed as a total crude fatty acid. The residue was dissolved in 10 mL petroleum ether and an amount containing 25 mg of the solution was taken into a teflon-lined screw-capped vial. Two mL of boron trifluoride/methanol reagent was added and the vial heated in a heating block for 3 minutes at 80 to 100 °C. After cooling the vial to room temperature, 1 mL water was added and mixed thoroughly. The upper layer containing fatty acid methyl esters was taken, adjusted to 4 mL with petroleum ether, and stored in a freezer until ready for GC analysis.

GC/Mass spectrometry

The TMS derivatives of the unsaponifiables were analyzed by GC/MS using the Finnigan MAT Magnum ion trap mass spectrometer (Columbus, Ohio, U.S.A.) equipped with a DB-17 column (30 m x 0.25 mm i.d., J&W Scientific, Folsom, Calif., U.S.A.). The column temperature was programmed from 150 °C to 260 °C at 6 °C/min, then to 300 °C at 2.5 °C/min and held for 7 min. Helium was the carrier gas at a flow rate of 1 mL/min. The split/splitless injection port, transfer line, and manifold temperatures were 260 °C, 280 °C, and 220 °C, respectively. The mass spectra were obtained in the electron impact (EI) ionization mode at 70 eV and scanned in the range of 100 to 500 amu at 1 s/scan. Multiplier voltage and emission current were 1650 V and 11 μA, respectively.

Gas chromatography

Table 1—Total unsaponifiables in dried fig fruit and various "parts" of the dried fig tree

Part	Content (mg/100g dry basis)
Fruit	433
Bark	1290
Stem[a]	202
Pith	702

[a] Stem. The woody material between the bark and the pith.

Fatty acid methyl esters were analyzed with a Varian 3400 gas chromatograph (Sugarland, Tex., U.S.A.) equipped with flame ionization detector (FID) and Supelco OMEGAWAX 250 capillary column (30 m x 0.25 mm i.d.). Column temperature was programmed as follows: 60 °C for 2 min, then increased to 220 °C by 4 °C/min and held for 30 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injector and detector temperatures were 250 °C and 260 °C, respectively. A standard fatty acid methyl ester mixture and linolenic acid were used for qualitative and quantitative analysis of sample peaks. Fatty acid contents were calculated on the basis of peak areas of the standards.

Identification of sterols

The identification of trimethylsilyl derivatives from the unsaponifiables was performed by comparison of relative retention times and mass spectra of samples with those of authentic standards (campestrol, stigmasterol, and sitosterol), INCOS data system with Wiley library, NIST data bank, and the literature (Rahier and Benveniste 1989; Abou Hadeed and others 1990; Kamal-Eldin and others 1992; Akihisa and others 1994; Casabuono and Pomilio 1997; Maatta and others 1999).

Results and Discussion

Content of unsaponifiables

The unsaponifiables were obtained from the direct saponification of dried samples. The content of the unsaponifiables from different parts of the fig tree is shown in Table 1. All parts were found to contain a high level of unsaponifiables. The bark, with the growing layers sometimes adhering, contains the highest amount (1290 mg/100 g dry weight) of unsaponifiables representing more than 1% of the dry weight. Jeong and others (1974) reported the content of unsaponifiables in the oils from various dried seeds and nuts with the range of 0.3 to 1.1%. Sesame oil has been reported to contain 1.1-1.3 % of unsaponifiables (Kamal-Eldin and others 1992). Therefore, it is surprising that the amount of unsaponifiables in bark, consisting mainly of fiber and lignin, is high. The pith of fig branches contains 702 mg of unsaponifiables in 100 g of dried sample although it represents a relatively low proportion of the branch. The fruit also has an elevated level of unsaponifiables (433 mg/100 g dry weight of fruit). The dried fruit of fig contains considerable amounts of small seeds rich in oil (Kolesnik aand others 1987), which may explain this high level of unsaponifiables in the fig fruits. Stem material without bark and pith contains the lowest level of unsaponifiables (202 mg/100 g dry weight). Some portions of the value for stem may come from residual pith adhering to stem due to the difficulty of removing it completely.

Composition of unsaponifiables

The derivatives of unsaponifiable matters from different "parts" of the fig tree were analyzed by GC/MS on a DB-17

Table 2—Relative retention times and fragmentation patterns of compounds in unsaponifiables of dried fig fruit and branch

Cmpd code	Compounds (systematic name)	RRT[a]	Major fragmentation ions, m/e
1	Campesterol (ergost-5-en-3β-ol)	0.94	472 (M^+), 457 (M-Me), 382 (M-Me_3SiOH), 367 (M-Me_3SiOH -Me), 343 (M-$C_6H_{13}Si$), 255, 145, 129 ($C_6H_{13}OSi$, Me_3SiOH + part of ring A)[b]
2	Stigmasterol (stigmasta-5,22-dien-3β-ol)	0.95	484 (M+), 469 (M-Me), 394 (M-Me_3SiOH), 379 (M-Me_3SiOH-Me), 355 (M-$C_6H_{13}Si$), 255, 129 ($C_6H_{13}OSi$)[b]
3	Unknown	0.96	496 (M^+)[b] , 481 (M-Me), 439, 391 (M-Me_3SiOH-Me), 255, 145
4	Likely obtusifoliol	0.99	498 (M^+), 483 (M-Me), 393 (M-Me_3SiOH-Me)[b], 255, 241, 229, 189, 109
5	Sitosterol (stigmast-5-en-3β-ol)	1.00	486 (M^+), 471 (M-Me), 396 (M-Me_3SiOH), 381 (M-Me_3SiOH-Me), 357, 255, 129 ($C_6H_{13}OSi$)[b]
6	Likely fucosterol (stigmast-5,24(28)-dien-3β-ol)	1.04	484 (M^+), 469 (M-Me), 386[b] , 371, 296, 281, 255, 211, 129
7	Likely parkeol (lanosta-9(11),24-dien-3β-ol)	1.05	498 (M^+), 483 (M-Me), 439, 393 (M-Me_3SiOH-Me)[b], 281, 255, 241
8	Likely β-amyrin (olean-12-en-3β-ol)	1.06	498 (M^+), 483 (M-Me), 393 (M-Me_3SiOH-Me), 279, 257, 218 (rings A and B + part of ring C)[b] , 203 (218-Me), 189
9	Unknown	1.08	498 (M^+), 483 (M-Me), 408 (M-Me_3SiOH), 393 (M-Me_3SiOH-Me), 319, 241, 229[b]
10	Likely parkeol isomer	1.09	498 (M^+), 483 (M-Me), 393 (M-Me_3SiOH-Me)[b], 255, 241
11	α-Amyrin (urs-12-en-3β-ol)	1.11	498 (M^+), 483 (M-Me), 408 (M-Me_3SiOH), 393 (M-Me_3SiOH-Me), 279, 257, 218 (rings A and B + part of ring C)[b], 203, 189
12	Likely psi-taraxasterol (urs-20-en-3β-ol)	1.12	498 (M^+), 483 (M-Me), 408 (M-e_3SiOH), 393, 369, 218, 203, 189[b]
13	Likely psi-taraxasterol isomer	1.23	498 (M^+), 483 (M-Me), 439, 408 (M-Me_3SiOH), 393 (M-Me_3SiOH -Me), 369, 218, 203, 189[b]
14	Likely psi-taraxasterol isomer	1.25	498 (M^+), 483 (M-Me), 439, 408 (M-Me_3SiOH), 393 (M-Me_3SiOH-Me), 369, 339, 218, 203, 189[b]
15	Likely psi-taraxasterol isomer	1.28	498 (M^+), 483 (M-Me), 439, 408 (M-Me_3SiOH), 393 (M-Me_3SiOH-Me), 369, 339, 218, 203, 189[b], 121

[a] RRT, relative retention times to sitosterol (18.18 min).
[b] Base peak.

Table 3—Composition (%) of unsaponifiables in dried fig fruit and branch

	Percent composition[a]														
Part	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fruit	1.3	3.2	1.0	1.8	20.8	5.0	2.2	17.1	9.4	7.9	5.7	19.4	2.4	2.7	Tr[c]
Bark	2.9	3.6	Tr	1.5	28.2	Nd[d]	1.4	14.3	6.6	8.4	2.9	19.2	6.4	2.5	2.1
Stem	8.4	8.1	Nd	Nd	60.3	Nd	0.7	5.6	1.7	3.5	1.4	8.0	2.2	Nd	Nd
Pith	5.4	7.8	Nd	Nd	82.5	Nd	Nd	1.9	Nd	Nd	Nd	2.4	Tr	Nd	Nd

[a] Means of duplicates
[b] Compound code numbers as mentioned in Table 2
[c] Tr, trace amount less than 1%
[d] Nd, not detected

capillary column. The principal constituents of the unsaponifiables from most vegetable oils are sterols (Kochhar 1983). In our research, the majority of the unsaponifiables from fig plant components were regarded as sterols and triterpene alcohols by GC chromatogram and GC/MS data. Table 2 shows relative retention times (RRT) and interpretation of fragmentation patterns of unsaponifiable compounds from different "parts" of fig plant components determined by the GC/MS technique. Retention times relative to sitosterol (18.18 min) on DB-17 column were estimated. Compositions of those compounds in of fig and different parts of the fig tree are listed in Table 3. Compositions were estimated on the basis of calculation of the GC peak areas in percent by setting the total peak areas to 100%. Table 4 demonstrates compositions of 4 phytosterols, including campesterol, stigamasterol, sitosterol, and fucosterol in fig tree components.

All "parts" are found to have campesterol (1), stigmasterol (2), and sitosterol (5), which are the most common phytosterols in nature (Weihrauch and Gardner 1978). β-amyrin (8) and two unidentified compounds (12 and 13) are also present in all "parts." Sitosterol is present more frequently in plants than any other phytosterol, and it is usually present in larger quantity than other sterols (Pollak 1985). Sitosterol was the most predominant sterol in all "parts" of the fig tree, but the percent composition of the sterol varied with the "parts." Sitosterol amounts to 82.5% of total unsaponifiables in the pith; and 60.3, 28.2, and 20.8% in the stem, bark, and fruit, respectively.

Fourteen compounds were determined from the unsaponifiables of fig fruits (Table 3). Sitosterol was the most predominant component in fig fruit unsaponifiables, while two triterpene alcohols,including tentatively assigned pseudotaraxasterol and β-amyrin, were the next major components. These three groups occupy almost 60% of the unsaponifiable matter in fig fruit. Sitosterol amounts to almost 70% in total

Table 4—Phytosterol composition of fig tree components

	Percent composition			
Part	Campesterol	Stigmasterol	Sitosterol	Fucosterol
Fruit	4.3	10.6	68.6	16.5
Bark	8.4	11.2	81.3	—
Stem	10.9	10.5	78.5	—
Pith	5.6	8.2	86.2	—

Table 5—Fatty acids content of dried fig fruit

Fatty acid content[a] (mg/100 g of dried fruit)						
14:0	16:0	18:0	18:1	18:2	18:3n3	Total
0.4	25.3	3.5	18.0	38.6	97.2	183

[a]Means of duplicates

sterol content as shown in Table 4. Substantial amounts of other triterpene alcohols (compounds 9, 13, and 14) with molecular weight 426 (excluding derivatization) are also present in fig fruit. Compounds 13 to 15 have the most abundant ion at m/z 189, which typically appear in the mass spectra of pentacyclic triterpene alcohols such as pseudotaraxasterol or those with lupane or hopane skeletons (Casabuono and Pomilio 1997). Pseudotaraxasterol, β-amyrin, lupeol, 24-methylenecycloartanol, and baurenol have been reported present in the leaves of fig (Ahmed and others 1988).

Bark appears to have almost the same unsaponifiables profile as that of fruit, except for the absence of fucosterol, but the percent composition of unsaponifiables in bark is slightly different from that of fruit. Bark contains a higher percentage of sitosterol and campesterol than fruit.

The compositions of unsaponifiables in stem and pith are quite different from those of fruit and bark. Ten compounds are found in stem and only 5 in pith. Both stem and pith consist of very high level of sterols (76.8 and 95.7% in unsaponifiables, respectively) and low levels of triterpenes. These results indicate that prunings of the branches of fig tree, now regarded as waste products, might serve as a good source of phytosterols, the processing of which would need a study for economic viability.

Fatty acids in dried fig fruit

Fatty acids in dried fig fruit were determined as their methyl esters by gas chromatography. The composition is listed in Table 5. Myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids were detected. Linolenic acid was the most predominant fatty acid (53.1%) in dried fig fruit, followed by linoleic acid (21.1 %), palmitic acid (13.8%), and oleic acid (9.8%).

Unsaturated fatty acids account for 84% of the total fatty acids. Among these unsaturated fatty acids, oleic acid is reported to have plasma cholesterol-lowering activity, while linoleic and linolenic acid can convert to hormone-like substances called eicosanoids which affect physiological reactions ranging from blood clotting to immune response (Oomah and Mazza 1999). The content of linolenic (18:3) acid was detected at a higher level (53%) in our results than in those previously reported (about 40%, Kolesnik and others 1987). However, the overall composition of fatty acids and the ratio of unsaturated to saturated fatty acids were similar to those reported in the literature. Our results are in general agreement with Yarosh and Umarov (1971), who reported that fig seed oil is characterizd by the high content (49%) of linolenic acid.

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