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Received for review August 20, 1974. Accepted February 3, 1975.

Organic Acids from Fresh California Strawberries

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The acids present in fresh California strawberries were isolated by solvent extraction. Analysis was by coupled gas chromatography-mass spectrometry of the methyl ester derivatives. A total of 33 acids were identified.

The organic acid content of fruit and changes occurring during ripening have been extensively studied (Hulme, 1971). Most of this work has been concerned with the nonvolatile acids. A number of investigators have studied the nonvolatile acids of strawberry (Hulme and Wooltorton, 1958; Johnston and Hammill, 1968; Sistrunk and Cash, 1973). Studies on the aroma constituents of strawberries, however, have revealed a number of volatile acids. These are presented in Table I. In addition, various authors have reported many of the esters of the more commonly occurring acids. The present work describes the isolation and characterization of the more volatile acids from fresh California strawberries. We are using the term "fresh" here only to indicate that the berries had not been frozen. It should be kept in mind that the elapsed time between harvest and analysis was probably about 10 days.

Table I. Volatile Acids Previously Identified in Strawberry

Acetic[a,b]	Tetradecenoic[c]
Butyric[b]	Pentadecanoic[c]
Isobutyric[b]	Palmitic[c]
Valeric[b]	Palmitoleic[c]
Hexanoic[a]	Heptadecanoic[c]
Pentenoic[c]	Stearic[c]
2-Hexenoic[c]	Oleic[c]
Octenoic[c]	Linoleic[c]
Nonanoic[c]	Linolenic[c]
Decenoic[c]	Nonadecenoic[c]
Dodecanoic[c]	Eicosanoic[c]
Tridecanoic[c]	Benzoic[c]
Myristic[c]	Cinnamic[c]

[a] Coppens and Hoejenbos (1939). [b] Dimick and Corse (1958). [c] Tressl et al. (1969).

EXPERIMENTAL SECTION

Extraction and Isolation of Acid Fraction. Two crates (24 pints) of fresh strawberries were obtained from California via National Produce (Long Branch, N.J.). The berries were of the Tioga variety and were harvested in July in Watsonville, Calif. Their organoleptic and visual qualities were good. The strawberries were stemmed, and each pint was ground in a glass Waring Blendor for 15 sec with 400 ml of distilled water. The slurry obtained from each pint was transferred to a 2500-ml glass funnel fitted with a coarse (145-175 μ) fritted disk (Ace Glass, Vineland, N.J.) and allowed to stand until the seeds and other particulate matter had risen to the top. The slurry was then filtered under nitrogen pressure. After the slurry from each pint was filtered, the funnel was back-flushed by forcing nitrogen up through the disk. The combined filtrates (about 8800 ml) were extracted in a separatory funnel in several portions. The initial extraction was done with a total of approximately 900 ml of distilled diethyl ether containing 10% methanol. The emulsion which formed was broken by filtration through absorbent cotton. The two succeeding extractions with about 900 ml each of diethyl ether were carried out with virtually no emulsion formation. The extract possessed a good, fresh strawberry aroma.

The acids were isolated by extraction of the ether phase with three 150-ml volumes of 5% sodium carbonate. The carbonate extract was acidified with 2 *N* hydrochloric acid and back extracted with approximately 300 ml of diethyl ether. The extract was dried over anhydrous sodium sulfate and concentrated by careful distillation in a Kuderna Danish concentrator (Kontes Glass Co., Vineland, N.J.). The last trace of solvent was driven off by a stream of nitrogen.

International Flavors and Fragrances, Inc., Union Beach, New Jersey 07735.

Table II. Volatile Acids of Fresh California Strawberries

Acid[b,c]	I_E (methyl esters) Unknown	Known	Mass spectral data (methyl esters)[a]
* Propionic[T]	2.22	2.26	57, 29, *88*, 59, 89, 27
Butyric	3.30	3.34	43, 74, 71, 27, 41 . . . *102*
Isobutyric	2.56	2.50	43, 71, 41, 59, 27 . . . *102*
* 2-Methylbutyric	3.63	3.68	57, 88, 29, 27, 41 . . . *116*
Valeric	4.47	4.22	74, 29, 27, 57, 43 . . . *116*
* Isovaleric	3.76	3.79	74, 43, 41, 59, 29 . . . *116*
Hexanoic	5.57	5.53	74, 43, 27, 87, 29 . . . *130*
* Isohexanoic	5.03	4.63	43, 74, 57, 55, 27 . . . *130*
* Heptanoic	6.57	6.55	74, 43, 87, 41, 55 . . . *144*
* Isoheptanoic	6.09		74, 43, 41, 101, 59 . . . *144*
* Octanoic	7.64	7.56	74, 87, 43, 41, 29 . . . *158*
Nonanoic	8.66	8.58	74, 87, 43, 55, 41 . . . *172*
* Decanoic	9.60	9.58	74, 41, 43, 87, 29 . . . *186*
* Undecanoic[T]	10.56	10.61	74, 87, 41, 55, 29, . . . *200*
Dodecanoic	11.59	11.63	74, 41, 43, 87, 55 . . . *214*
Palmitic	15.83	15.52	74, 41, 43, 87, 55 . . . *270*
* 2-Methyl-2-butenoic	5.68	5.59	55, 83, 27, *114*, 29, 39
* Methylbutenedioic[T]	10.63		127, 59, 39, 29, 99 . . . *158*
* 2-Methyl-*cis*-3-pentenoic	5.24	5.28	69, 41, 39, *128*, 27, 113
* 2-Methyl-*trans*-3-pentenoic	5.39	5.40	69, 41, 39, *128*, 27, 113
* 2-Methyl-2-pentenoic	6.24	6.34	41, 69, 39, *128*, 27, 97
2-Hexenoic	6.62	6.61	55, 41, 39, 27, 68 . . . *128*
2-Octenoic	8.75		55, 29, 87, 27, 41 . . . *156*
* 3-Nonenoic	9.19		41, 55, 27, 29, 74 . . . *170*
* 3-Hydroxyhexanoic[T]	9.94		103, 74, 71, 43, 41 . . . *146*
* 3-Hydroxyoctanoic	12.03		103, 43, 71, 74, 29 . . . *174*
* Phenylacetic	11.12	11.23	91, *150*, 51, 65, 39, 92
* Phenylpropionic	11.97	12.00	104, 91, 105, *164*, 77, 51
Benzoic	9.86	10.00	105, 77, *136*, 51, 50, 29
* 4-Methylbenzoic	11.06	11.20	119, 91, *150*, 65, 39, 63
Cinnamic	14.37	14.27	131, 103, *162*, 77, 51, 101
* Furoic	9.37	9.38	95, 39, *126*, 38
Succinic	9.46	9.46	115, 55, 59, 114, 29 . . . *146*
* Glutaric	10.43	10.52	59, 100, 129, 42, 55 . . . *160*

[a] These data were taken from the mass spectra of known compounds. However, in every case the data for the unknown compound matched that for the known. The mass spectrometer used had a source temperature of 150°, an ionizing voltage of 70 eV, and an ionizing current of approximately 3.5 A. Numbers in italics are the molecular weights of the compounds. [b] An asterisk denotes first report in strawberry. [c] A T means mass spectral identification only tentative.

Derivatization and Analysis. The acids were esterified using the BF_3–methanol methylation procedure of Supelco, Inc. (Bulletin 721, 1972). Approximately 100 mg of the ether extract and 2.8 ml of the reagent were placed in a test tube and boiled for 2 min. The reaction mixture was transferred to a small separatory funnel containing 20 ml of distilled water and extracted with three 5-ml volumes of distilled Freon 11. The extract was washed with distilled water, and the solvent was removed under a stream of nitrogen.

The sample was analyzed on a Hitachi Model RMU-6E mass spectrometer coupled to a Hewlett-Packard 5750 gas chromatograph by means of a porous glass separator (Watson and Biemann, 1965). The column used was a 500 ft × 0.03 in. stainless steel, open tubular column coated with Carbowax 20M. The oven temperature was programmed from 70 to 190° at 2°/min, and the helium flow rate was 12 ml/min.

RESULTS AND DISCUSSION

Methanol was added to the ether used for the first extraction in order to decrease the stability of the resulting emulsion. Attempts to extract directly with ether resulted in the formation of extremely stable emulsions.

The 33 acids, identified as their methyl esters, are given in Table II. Identifications were accomplished by comparing the unknown spectra to those of known compounds and confirmed wherever possible by I_E values, that is, the retention indices relative to a series of ethyl esters of normal alkanoic acids (van den Dool and Kratz, 1963). Of the 33 acids identified, 22 are being reported for the first time as constituents of strawberry. These are indicated by an asterisk.

The unsaturated acids may be postulated to arise from the hydroxy compounds by dehydration. This type of reaction may occur during the isolation or analysis. Since some of the hydroxy acids have been identified intact, it seems likely that these compounds exist in the fruit. Furthermore, it is well documented that the β-oxidation of carboxylic acids to methyl ketones involves both the unsaturated and the β-hydroxy acids (Thaler and Geist, 1939). Therefore, both types of compounds are likely to exist in the fruit. Unsaturated and hydroxy esters have been found in pineapple (Näf-Müller and Willhalm, 1971), pear (Heinz and Jennings, 1966), passion fruit (Winter and Klöti, 1972), grape (Stern et al., 1967), etc.

ACKNOWLEDGMENT

The authors thank Ms. Anne Sanderson for assistance in obtaining and interpreting mass spectra and Dr. Manfred H. Vock for helpful discussions and encouragement.

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Received for review August 30, 1974. Accepted January 30, 1975.

The Effect of the Selective Removal of Hemagglutinins on the Nutritive Value of Soybeans

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The hemagglutinating activity of a crude extract of unheated soybean flour was removed by passage through a column of Sepharose-bound concanavalin A. The trypsin inhibiting activity of the extract was not affected by this treatment. The hemagglutinin-free extract when fed to young rats produced a rate of growth and had a protein efficiency ratio which was not significantly different from that obtained with the original extract from which the hemagglutinin had not been removed. Heat treatment of the crude extract effected a marked improvement in growth response similar to that produced by heating the raw soybean flour itself. It was concluded that the soybean hemagglutinin plays a relatively minor role in the deleterious effects of unheated soybean flour.

The poor nutritive value of raw soybeans has been generally attributed to the deleterious effect of trypsin inhibitors and other heat-labile components (Liener, 1972; Rackis, 1972). Kakade et al. (1973) have recently reported that approximately 40% of the growth-depressing effect of unheated soybeans fed to rats could be accounted for by the trypsin inhibitors. The remainder of the growth depression was attributed to the poor digestibility of the native, undenatured protein and possibly to other growth inhibitors. In the latter instance, serious consideration should be given to a class of substances, known as phytohemagglutinins or lectins, which are widely distributed in the plant kingdom, particularly among the legumes (Sharon and Lis, 1972). Although their role as growth inhibitors in beans belonging to the genus *Phaseolus* would appear to be well established (Jaffé, 1969; Liener, 1974), their nutritional significance in the soybean is still uncertain. The soybean hemagglutinin (SBH) was first isolated by Liener (1951) and later more fully characterized by Sharon and his group (Lis et al., 1964; Lotan et al., 1974). Liener (1953) had shown that when purified SBH was added to a diet containing heated soybean meal, at a level approximating its occurrence in the raw meal, the growth of rats was significantly retarded. Growth inhibition, however, was not observed when the food intake of the control diet containing heated soybean meal was restricted to the food intake of the same diet containing SBH. Birk and Gertler (1961) reported poor correlation between the hemagglutinating activity and growth-depressing activity of various fractions of soybeans, and hence concluded that SBH must play a minor role in the detrimental effect of raw soybean meal.

In order to resolve this issue, a more rigorous approach to the problem was undertaken, one which involved the selective removal of SBH from a crude soybean extract. This approach, which is similar to the one which was employed for assessing the contribution of the trypsin inhibitors to deleterious effects of raw soybeans (Kakade et al., 1973), takes advantage of the fact that hemagglutinins strongly bind specific sugars and glycoproteins containing these sugars (Sharon and Lis, 1972). In the case of SBH, Sepharose-bound concanavalin A, which is itself a phytohemagglutinin, can be used to bind SBH (Bessler and Goldstein, 1973) since the latter is a glycoprotein containing mannose for which concanavalin A is specific (Goldstein et al., 1965). Thus, by comparing the growth response of rats to diets containing SBH-free soybean protein extract with the untreated extract it becomes possible to evaluate directly the role of SBH as a factor contributing to the poor nutritive value of unheated soybean protein.

MATERIALS AND METHODS

Preparation of Soybean Extracts. Fifty grams of defatted soybean flour (Central Soya, Chicago, Ill.) was suspended in 1 l. of 0.05 *M* phosphate buffer (pH 7.6) containing a 10^{-4} *M* concentration of each of the following salts: $CaCl_2$, $MgCl_2$, and $MnCl_2$. The suspension was stirred for 1 hr at room temperature and then centrifuged at 5000 rpm for 10 min. The supernatant which contained 85 to 90% of the protein of the flour was divided into two equal portions. One portion was immediately frozen for later use as the control, to be referred to as "extract (+SBH)," and the other half of the extract was rendered free of SBH by the procedure described below.

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