Table III. Precision of the Method for Determination of Total Vitamin C and Total Isovitamin C in a Complete Meal

	within-assay precision		recovery test[a]	
	total C	total iso-C	total C	total iso-C
n	6	6	6	6
mean	91.7 μg/g	< 0.2 μg/g	97.1%	99.3%
SD	1.9 μg/g		4.7%	4.7%
CV	2.1%			

[a] The amounts added were 63 μg/g AA and 67 μg/g EA, respectively.

DISCUSSION

The method described in this paper allows a relative fast and sensitive simultaneous determination of total vitamin C and total isovitamin C in foodstuffs and beverages. Also, DHAA and DHEA can be determined simultaneously with the same procedure with omission of the oxidation by ascorbate oxidase. The method is easy to perform and is suited for large-scale routine analysis. The linearity range and sensitivity permit determinations of total vitamin C and total isovitamin C in a concentration as low as 0.2 μg/g. The precision of the method and the recoveries of AA and EA are good.

Keating and Haddad (1982) described a method for the simultaneous determination of AA and DHAA in foodstuffs by HPLC with UV measurement. The UV absorptivity of DHAA was enhanced by using the same derivatization procedure as described in this paper. We observed, however, that fluorometric detection of the derivative highly improved the sensitivity. Although Keating and Haddad also observed an increased sensitivity using fluorescence detection, this advantage was largely offset in their method by increased background signal due to fluorescent impurities that were not separated from DFQ by their HPLC system. The HPLC system with fluorescence detection incorporated in our method provided well-separated DFQ and IDFQ peaks.

Registry No. AA, 50-81-7; EA, 89-65-6; DHAA, 490-83-5; DHEA, 5959-82-0; ascorbate oxidase, 9029-44-1.

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Received for review July 15, 1983. Accepted October 3, 1983.

Oligosaccharide Content of Ten Varieties of Dark-Coated Soybeans

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Dry ripe beans of 10 varieties of black- or brown-coated soybeans [*Glycine max* (L.) Merril] were evaluated for oligosaccharide content. Oligosaccharides were extracted with 80% ethanol and analyzed qualitatively and quantitatively by thin-layer chromatography. An average of 3.88% sucrose, 0.79% raffinose, and 3.37% stachyose, on a dry weight basis, was found, with an approximate proportion of 5:1:4 for the three sugars. This ratio was of 4.5:1:4 for the yellow-coated soybeans, widely produced in this region and investigated here for comparison. On a dry weight basis, the sum of raffinose plus stachyose represented 15.84% of the carbohydrates present in dark-coated beans and 16.32% of those present in yellow-coated beans. Due to the similar content of nondigestible sugars, commonly accepted as the cause of flatulence, none of the varieties studied (black-, brown-, or yellow-coated beans) is preferable to any of the others.

The main oligosaccharides present in soybeans (*Glycine max*, L., Merril) are sucrose, raffinose, and stachyose. Verbascose has also been reported to be present, although in minimal amounts (Smith and Circle, 1972). These sugars represent approximately 15% of the dry bean weight and are important sources of energy for embryo development during germination and initial growth (Pazur et al., 1962). The level of oligosaccharides in legume seeds is known to differ among varieties and lines and also according to the degree of ripening (Hymowitz et al., 1972). Hardinge et al. (1965) stated that among the legume seeds studied by them (dry soybeans, dry black mung beans, dry green mung beans, cowpea, field bean, chickpea, horse gram, lentils, canned lima beans, and pigeon pea) soybeans had the highest level of raffinose (1.9%) and stachyose (5.2%). These sugars are even more abundant in ripe and dry beans, with sucrose, raffinose, and stachyose occurring at an approximate ratio of 4:1:2 (Pazur et al., 1962). Kawamura (1967) observed that more than 90% of the sugars present in ripe soybeans consist of sucrose, raffinose, and stachyose.

Soybean oligosaccharides are generally considered undesirable because they represent one of the causes of fla-

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0021-8561/84/1432-0355$01.50/0

Table I. Proximate Analysis of Ten Dark-Coated Soybean Varieties Compared to Yellow-Coated Soybeans

variety	color	moisture, %	g of constituent/100 g dry weight[a] protein	crude fiber	ash	lipids	carbohydrates
NC 55	black	9.02	41.97	5.97	4.39	20.85	26.82
PI 192874	black	11.30	46.07	6.03	4.55	17.19	26.16
Otootan	black	10.43	45.16	6.03	4.47	17.99	26.35
Chi Key No. 13	black	10.92	44.17	5.68	5.27	17.17	27.71
PI 265491	black	11.54	44.31	5.23	4.49	16.30	29.67
Aksarben 1 "S" Bl	black	10.89	44.20	5.10	4.69	19.47	26.54
Aksarben 1 "S" Br	brown	13.01	42.62	7.06	4.87	19.95	25.50
Gatan	brown	12.53	44.88	6.83	4.61	18.47	25.21
Chi Key No. 15	coffee-brown	10.40	41.36	6.25	5.02	21.30	26.07
Tanner	reddish brown	9.30	46.04	5.52	5.18	20.77	22.49
mean[b]		10.93	44.07 ± 1.62[b]	5.97 ± 0.63	4.75 ± 0.31	18.94 ± 1.76	26.25 ± 1.82
Viçoja	yellow	10.03	42.69	6.29	5.50	18.98	26.54
IAC-2	yellow	10.20	44.33	6.01	4.96	21.71	22.99
Santa Rosa	yellow	11.50	44.40	5.61	4.97	21.78	23.24
mean[b]		10.57	43.80 ± 0.96	5.97 ± 0.34	5.14 ± 0.30	20.82 ± 1.59	24.25 ± 1.98

[a] Mean of triplicate determinations. [b] Mean ± standard deviation.

Table II. Oligosaccharide Content of Ten Dark-Coated Soybean Varieties Compared to Yellow-Coated Varieties

variety	color	oligosaccharides, g/100 g dry weight[a] sucrose	raffinose	stachyose
NC 55	black	3.76	0.81	3.30
PI 192874	black	4.02	0.76	3.23
Otootan	black	3.93	0.77	3.09
Chi Key No. 13	black	3.94	0.79	3.07
PI 265491	black	3.66	0.79	2.75
Aksarben 1 "S" Bl	black	4.23	0.76	4.02
Aksarben 1 "S" Br	brown	3.59	0.79	3.17
Gatan	brown	3.73	0.81	3.72
Chi Key No. 15	coffee-brown	4.08	0.77	3.51
tanner	reddish brown	3.86	0.85	3.91
mean[b]		3.88 ± 0.19[b]	0.79 ± 0.02	3.37 ± 0.40
Viçoja	yellow	3.84	0.81	2.96
IAC-2	yellow	3.81	0.87	3.59
Santa Rosa	yellow	3.44	0.81	2.85
mean[b]		3.69 ± 0.22	0.83 ± 0.03	3.13 ± 0.39

[a] Mean of triplicate determinations. [b] Mean ± standard deviation.

Table III. Comparison of the Results Obtained by Several Investigators and in This Work in Terms of the Sucrose, Raffinose, and Stachyose Content of Soybean Seeds

soybeans analyzed	oligosaccharide content, g/100 g sucrose	raffinose	stachyose	authors
Hawkeye variety	8.0	1.9	5.2	Pazur et al. (1962)
U.S. varieties (mean for 6 varieties)	4.5	1.1	3.7	Kawamura (1967)
Japanese varieties (mean for 3 varieties)	5.7	1.1	4.1	Kawamura (1967)
Hardee variety	5.05	0.36	3.18	East et al. (1972)
mean for 20 lines of 00 and 0 ripeness	5.1	0.2	3.1	Hymowitz et al. (1972)
mean for 20 lines of I and II ripeness	4.8	0.5	2.6	Hymowitz et al. (1972)
mean for 20 lines of III and IV ripeness	5.4	0.5	2.8	Hymowitz et al. (1972)
Corsoy variety	6.44	0.36	2.10	Ku et al. (1976)
Wells variety	4.71	0.81	3.23	Chen and Luh (1976)
dehulled Ada variety	8.24	0.94	4.70	Cannela and Sodini (1977)
from U.S.A.	4.6	1.1	4.2	Schweizer et al. (1978)
from Canada	7.2	1.2	4.8	Schweizer et al. (1978)
from India	4.1	1.8	3.6	Schweizer et al. (1978)
from Malaysia	5.8	1.5	3.8	Schweizer et al. (1978)
SRF-200 variety	6.81	1.01	3.53	Wang et al. (1979)
mean for 10 dark-coated varieties	3.88	0.79	3.37	this work
Brazilian varieties (mean for 3 varieties)	3.69	0.83	3.13	this work

tulence. Few studies have been published on the content of oligosaccharides such as sucrose, raffinose, and stachyose of soybeans having dark pigmented coats. These varieties could be used to increase direct soybean consumption by the Brazilian population because their color is close to that of common beans (*Phaseolus vulgaris*, L.), which are widely accepted and consumed without any industrial processing. Therefore, the objective of the present study

was to provide information on the oligosaccharide content of 10 varieties of dark-coated soybean grown on experimental fields and to compare it to that of yellow-coated soybean seeds, widely produced in the agricultural region of Ribeirão Preto.

MATERIALS AND METHODS

Soybean Seeds. The 10 varieties of dark-coated soybeans (*G. max* (L.) Merril) were provided by the Faculdade de Ciencias Agrarias e Veterinaria de Jaboticabal, UNESP, Jaboticabal, Estado de São Paulo, while the three yellow-coated varieties, Santa Rosa, IAC-2, and Viçoja, were supplied by the Divisão Regional Agricola de Ribeirão Preto. The seeds were wrapped in plastic bags and stored at 4 °C until the time of analysis.

Proximate Analysis. Moisture, ash, crude fiber, and crude fat were determined by AOAC techniques (AOAC, 1975), and crude protein ($N \times 6.25$) was determined by the micro-Kjeldahl method. The total carbohydrate content was calculated by difference.

Isolation, Identification, and Quantitative Determination of Oligosaccharides. Whole soybean seeds were pulverized with a manual disk mill. Appropriate samples were refluxed with 100 mL of an aqueous solution of 80% ethanol for 1 h. After filtration, the residue was separated and heated again with fresh 80% ethanol for an additional 30 min, filtered, and washed with 200 mL of 80% ethanol. Extracts and wash fluids were pooled and treated as described in a previous paper (Silva and Braga, 1982) for oligosaccharide identification and quantitation by thin-layer chromatography. All measurements were carried out in triplicate.

RESULTS AND DISCUSSION

The results of proximate analysis of dark-coated and yellow-coated soybean seeds, the latter used as a comparison, are presented in Table I and expressed on a dry weight basis. A wide range of variation in fat content (16.30–21.30%) as well as in protein content (41.36–46.07 g/100 g) was observed among the dark-coated varieties.

The oligosaccharides sucrose, raffinose, and stachyose were identified in both dark-coated and yellow-coated bean extracts. Visual comparison of the color intensity of the stains obtained for all extracts revealed traces of a fourth sugar with a lower R_f than that of stachyose, which we assumed to be verbascose, for which we have no authentic standard. The results of quantitative determination of sucrose, raffinose, and stachyose in the 10 dark-coated and 3 yellow-coated soybean varieties are shown in Table II. In the dark-coated seeds, the mean values obtained for sucrose (3.88%), raffinose (0.79%), and stachyose (3.73%) on a dry weight basis showed a ratio of approximately 5:1:4 for the three sugars. The proportion observed for the yellow-coated seeds was of approximately 4.5:1:4. The sum of raffinose plus stachyose, sugars that are not metabolized by the human organism, represents 15.84% of the carbohydrates present in the dark-coated varieties and 16.32% of those present in the yellow-coated varieties, on a dry weight basis. Sucrose, raffinose, and stachyose represent 9.0% of the total solids present in dark-coated seeds and 8.5% of those present in the yellow-coated seeds. Variability in the content of individual sugars has been observed among cultivars and lines of soybean seeds. Table III compares the oligosaccharide content of soybeans seeds of different origins analyzed by other investigators with those obtained in the present study. It should be pointed out that, in addition to different seed origin, different samples and methods were used for the results reported in Table III. Recently Sosulki et al. (1982) determined the oligossaccharide content of 11 different legume seeds, but the results they obtained with soybeans have not been included in the table because a commercial flour rather than whole seeds was used in their study.

On the basis of our data, we conclude that none of the dark-coated or yellow-coated soybean varieties investigated in the present study is preferable to any other in terms of content of raffinose plus stachyose, considered to be the main sugars involved in the flatulence problem.

ACKNOWLEDGMENT

We are grateful to Dr. Alfredo Lam-Sanchez, Faculdade de Ciencias Agrarias e Veterinaria de Jaboticabal, UNESP, Jaboticabal, Sao Paulo, for his generous gift of dark-coated soybean seeds.

Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3.

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Received for review June 9, 1983. Accepted November 29, 1983. Presented by M.d.L.P.B. as part of the requirements of the Course of Specialization in Nutrition, Faculdade de Medicina de Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, SP, Brazil.