Soluble Sugar Composition of Peanut Seed

Sheikh M. Basha

Biotechnology Laboratory, Division of Agricultural Sciences, Florida A&M University, Tallahassee, Florida 32307

To determine the soluble sugar composition of raw peanut (*Arachis hypogaea* L.) seed, sugars were extracted from defatted flours prepared from freeze-dried and cold-stored samples using 80% methanol and fractionated by HPLC. The results showed that except for Altika, all 20 peanut cultivars examined contained primarily sucrose followed by glucosamine (tentative), stachyose, and raffinose. During sugar extraction, exposure of samples to heat alone did not cause oligosaccharide breakdown but exposure to acidic solutions increased oligosaccharide breakdown into glucose and fructose. In addition, short-term refrigerated or frozen storage appeared to cause no major changes in soluble sugar composition of peanut seed. Results of this study indicated that the soluble sugar constituents of peanut seed include primarily sucrose, glucosamine (tentative), raffinose, and stachyose and that other monosaccharides such as glucose and fructose arise as a result of oligosaccharide breakdown during sample processing and analysis.

INTRODUCTION

Free sugars and amino acids have been found to be the major flavor precursors in roasted peanuts (Newell et al., 1967) and give rise to pyrazine and carbonyl compounds (Mason et al., 1966, 1967; Johnson et al., 1971a,b; Shu and Waller, 1971; Walradt et al., 1971). Previous workers (Holley and Hammons, 1968; Cobb and Swaisgood, 1971; Mason et al., 1969; Newell et al., 1967; Oupadissakoon et al., 1980; Tharanathan et al., 1975, 1976) have reported the occurrence of sucrose, glucose, and fructose in peanut seed, with sucrose being the major sugar. Tharanathan et al. (1975, 1976) reported the presence of glucose, fructose, sucrose, raffinose, stachyose, verbascose, and ajugose, while Oupadissakoon et al. (1980) reported the occurrence of fructose, glucose, inositol, sucrose, raffinose, and stachyose in the soluble fraction of peanut seed. In addition to qualitative differences, the amounts reported for individual sugars varied significantly. For example, Tharanathan et al. (1976) reported the percentage of glucose, fructose, and sucrose in defatted peanut flour as 2.89, 2.19, and 0.91%, while Oupadissakoon et al. (1980) reported them to be 0.01, 0.02, and 2.9%, respectively. Likewise, Mason et al. (1969) reported the amounts of fructose, glucose, and sucrose in raw Spanish peanuts as 0.27, 1.9, and 14.9%, respectively. The quantitative and qualitative differences in the soluble sugar composition of peanut observed by these workers may probably be due to sample preparation methods and analytical techniques employed by them. Of the analytical techniques employed for sugar determination, gas chromatography has been widely used. However, this technique (Pierce Method 16) involves a derivatization step which exposes samples to heat (70 °C) and acid (trifluoroacetic acid), resulting in partial breakdown of oligosaccharides. Hence, the free sugar composition reported for peanuts may not represent the true nature of the free sugars present in raw peanut seed. In view of the importance of free sugars as precursors of roasted flavor, it is essential to know the true nature of free sugars in raw peanut seed. This paper reports the content and composition of free sugars in freeze-dried raw peanut seed samples detected following HPLC analysis. In addition, it will also address the effects of heat, acid, and storage temperature on sugar composition of peanut seed.

MATERIALS AND METHODS

Materials. Twenty peanut (*Arachis hypogaea* L.) cultivars and breeding lines were obtained from Drs. A. J. Nordon and D. W. Gorbet of the Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL. Seed samples were freeze-dried and stored at −20 °C until the analysis. These were Altika, UF77318, Jenkins Jumbo, Early Bunch, Florunner, 439-16-10-3, Florigiant, UF75102, 439-16-10-3-1, 439-16-10-1-1, Southern Runner, UF81206-1, UF81206-2, NC6, 79x4-6-2-1-4-*b3*-B, NC 15745, UF79308-1, NC10247, 73x93-10-2-1-B-pk-*b3*-B, and 72x41A-6-1-2-2-*b3*-B.

Sample Preparation. After the peanut skins were removed, the cotyledons were ground into a meal using a mortar and pestle and defatted with hexane (Basha et al., 1976). A portion (100 mg) of the defatted meal was extracted twice with 5-mL portions of 80% methanol using a Polytron homogenizer. The homogenate was centrifuged, and the supernatant was dried using a Speedvac (Savant, Farmingdale, NY). The residue was dissolved in 5 mL of water and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA). The eluate was used for sugar analysis. For acid hydrolysis, defatted flour (100 mg) or dried methanolic extract (made from 100 mg of defatted meal) was taken up in H_2SO_4 solutions of various concentrations (0.0005–1 M) and heated in a boiling water bath for 40 min. After boiling, the samples were neutralized, filtered, and passed through Sep-Pak cartridges as described above. The eluates were used for sugar analysis by HPLC. Preliminary studies using 80% ethanol, 80% methanol, and methanol/chloroform/water (60:25:15 v/v/v) to extract the soluble sugars from defatted flours showed no qualitative and quantitative differences in sugar composition; hence, 80% methanol was used as an extraction media throughout the study.

Sugar Analysis. The HPLC system consisted of a refractive index detector, Model 510 pump, Sugar-Pak column, and a Model 820 data station (Waters). Twenty microliters of the extract was injected, and the column was eluted with water (90 °C) at 0.5 mL/min. Quantitation of individual sugars was made from a standard curve derived using the peak areas of sugar standards.

RESULTS AND DISCUSSION

Sugar Composition. Soluble sugar composition of peanut seed was determined by analyzing the 80% methanolic extracts, and the resulting methanolic residue was hydrolyzed with H_2SO_4 for 40 min and used for the determination of insoluble sugars. Figure 1 shows the soluble and insoluble sugar composition of Florunner peanut seed as determined by HPLC. As seen in the figure, the soluble

0021-8561/92/1440-0780$03.00/0 © 1992 American Chemical Society

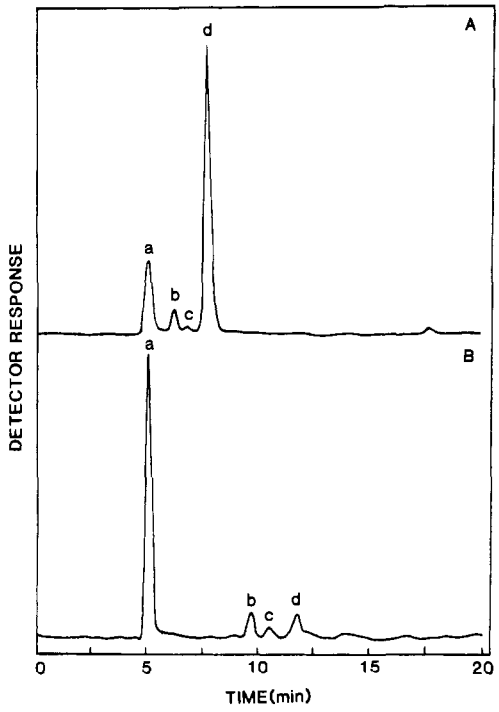

Figure 1. Soluble (A) and insoluble (B) sugar composition of Florunner peanut seed: (A) a, glucosamine; b, stachyose; c, raffinose; d, sucrose. (B) a, glucosamine; b, glucose; c, rhamnose; d, arabinose.

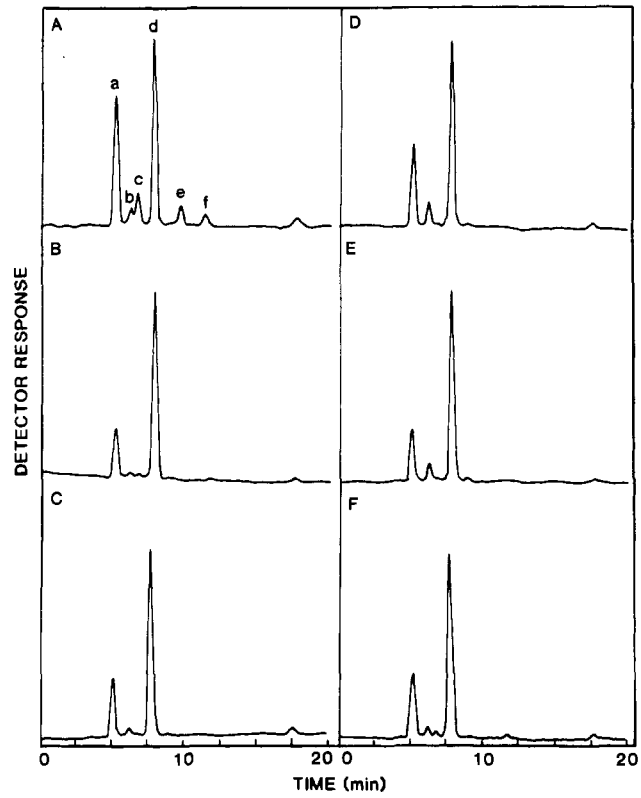

Figure 2. Soluble sugar composition of different peanut cultivars: A, Altika; B, UF 77318; C, Jenkins Jumbo; D, Early Bunch; E, Florunner; F, 439-16-10-3. a, glucosamine; b, stachyose; c, raffinose; d, sucrose; e, glucose; f, fructose.

sugar fraction consisted mainly of sucrose (5.4%), glucosamine (4.76%), stachyose (0.5%), and raffinose (0.03%), while the insoluble sugar fraction contained predominantly glucosamine (21%), arabinose (0.6%), and trace levels of glucose (0.05%) and rhamnose (0.09%). These values are slightly higher than those reported by Oupadissakoon et al. (1980) and agree with the findings of Cegla et al. (1977) and Holley and Hammons (1968). Except for those four soluble sugars, no other sugars were detected in Florunner, even after a 10-fold increase in sample loading size. Previous workers using gas chromatography (Mason et al., 1969; Newell et al., 1967; Oupadissakoon et al., 1980; Tharanathan et al., 1976), paper chromatography (Tharanathan et al., 1975), and column chromatography (Amaya-F et al., 1978) have reported the presence of additional sugars: glucose, fructose, rhamnose, galactose, inositol, verbascose, and ajugose in the soluble fraction and galactosamine, galactose, xylose, and arabinose in the insoluble fraction. To further verify our observation in Florunner, several peanut cultivars were analyzed for their soluble sugar composition. Figure 2 shows the soluble sugar composition of selected peanut cultivars. All of the other 14 cultivars (data not shown) had a similar sugar composition. The data showed (Figure 2) that sucrose was the major sugar in all of the cultivars, followed by glucosamine, stachyose, and raffinose. Unlike other cultivars, Altika (Figure 2A) contained relatively large amounts of raffinose, glucose, and fructose. This may be attributed to genetic variation or to the lower maturity level of Altika compared with that of the other cultivars. Besides these, none of the other sugars reported by previous workers were found in these cultivars. This discrepancy may be due to the thermal or enzymatic breakdown of oligosaccharides during sample processing and analytical methods employed by the previous workers. To reduce thermal and enzymatic breakdown of sugars, our samples were freeze-dried after harvest, defatted at 4 °C, stored at −20 °C, and the rest of the sample preparation steps were carried out between 10 and 20 °C. Unlike the gas and paper chromatographic procedures which require derivatization and color development, the HPLC system is gentler, requiring no acid and heat treatment of samples, and the detection is based on the refractive index of sugars. This is the first study indicating the presence of glucosamine in peanut seed. Hence, glucosamine identity is tentative pending on its confirmation by HPLC column of different chemistry. Occurrence of amino sugars in legume seeds is not new but has been reported extensively by previous workers (Pusztai, 1964, 1965; Koshiyama, 1966; Racusen and Foote, 1971; Ericson and Chrispeels, 1973; Basha and Beevers, 1976) in soybean, kidney beans, and peas.

Effect of Heat and Acid Concentration. To determine the effect of heat and acidity on peanut sugar composition, defatted flour as well as the soluble sugar fraction (80% methanolic extract) was heated in a boiling water bath for 40 min, with various concentrations of acid (H_2SO_4), neutralized, and analyzed by HPLC. The data showed (Figure 3A) that heating alone did not change free sugar composition or cause breakdown of sucrose into glucose and fructose. However, boiling in acid solutions caused breakdown of oligosaccharides (stachyose, raffinose, and sucrose). The rate of oligosaccharide breakdown increased with increasing acid concentration (Figure 3B–F). Sulfuric acid at concentrations above 0.001 M caused a complete breakdown of sucrose. These data showed that during sample preparation exposure of samples to high temperatures alone did not cause oligosaccharide breakdown but that an acidic environment is needed to cause oligosaccharide breakdown. Unlike peanuts, exposure of samples to higher temperatures alone has been reported to cause degradation of sucrose, stachyose, and raffinose in root crops (Tamate and Bradbury, 1980) and alfalfa herbage (Raguse and Smith, 1965; Smith, 1973). Hydrolysis of whole defatted meal with various concentrations

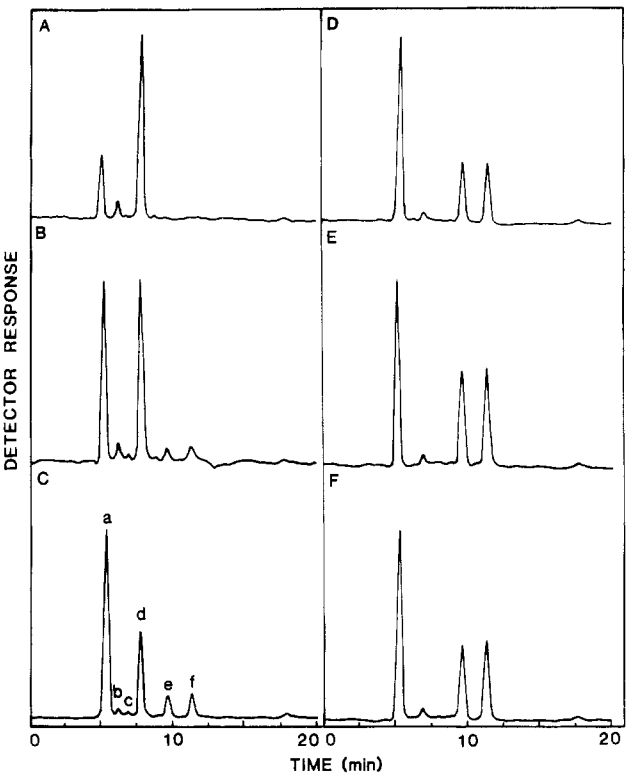

Figure 3. Effect of boiling in various concentrations of sulfuric acid on soluble sugar composition of peanut: A, water; B, 0.0005 M; C, 0.001 M; D, 0.005 M; E, 0.01 M; F, 0.1 M. a, glucosamine; b, stachyose; c, raffinose; d, sucrose; e, glucose; f, fructose.

(0.05, 0.5, and 1 M) of H_2SO_4 also showed (data not shown) the presence of only glucosamine, sucrose, glucose, and fructose, confirming that only these sugars are the primary soluble sugar constituents of peanut seed.

Hydrolysis of 80% methanolic pellet (insoluble fraction) with various concentrations of H_2SO_4 consistently showed the presence of glucosamine (21%), glucose (0.05%), rhamnose (0.09%), and arabinose (0.6%) (Figure 4). In the hydrolysates, glucosamine concentration increased with increasing concentrations of acid, and also glucose, rhamnose, and arabinose were detected at acid concentrations above 0.1 M, indicating that these sugars are more tightly bound than the glucosamine. D-Glucosamine, in addition to being a constituent of reserve protein, has also been reported (Racusen and Foote, 1974; Roberts et al., 1972; Roberts and Pollard, 1975; Nagahashi et al., 1980) to be an important component of plant membrane structure. From these data it can be concluded that in the peanut glucosamine appears to be the major component of seed membrane structure and that other sugars such as arabinose, rhamnose, and glucose are present only in trace amounts.

Effect of Storage Temperature. To determine the effect of sample storage temperature on sugar composition of peanut, defatted peanut flours that were stored refrigerated (4 °C) or frozen (−20 °C) for about 6 months were analyzed for soluble sugar composition. The data showed (Figure 5) that storage temperature had no effect on soluble sugar composition of peanut seed. Thus, comparison of sugar profiles of Florunner peanut seeds, stored refrigerated or frozen, showed no major differences in their sugar composition, indicating frozen storage did not degrade oligosaccharides in peanut. Studies with other plant materials have shown degradation of oligosaccharides during frozen storage (Bieleski, 1982; Brown and Summers, 1985; Jordan, 1965) and freeze–thawing (Hendrix and Peelan, 1987).

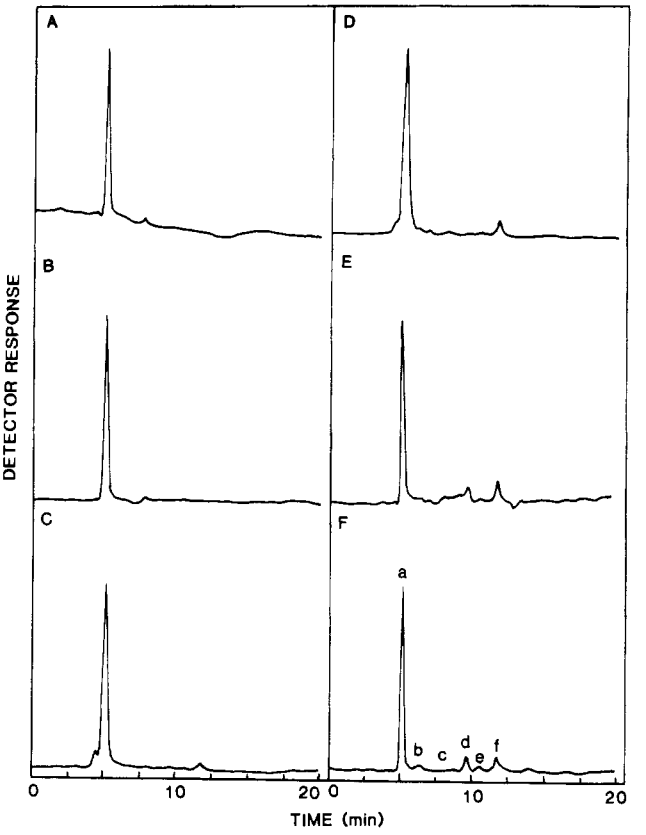

Figure 4. Sugar composition of 80% methanol insoluble residue after boiling for 60 min in various concentrations of sulfuric acid: A, 0.005 M; B, 0.01 M; C, 0.05 M; D, 0.1 M; E, 0.5 M; F, 1 M. a, glucosamine; b, stachyose; c, sucrose; d, glucose; e, unknown; f, arabinose.

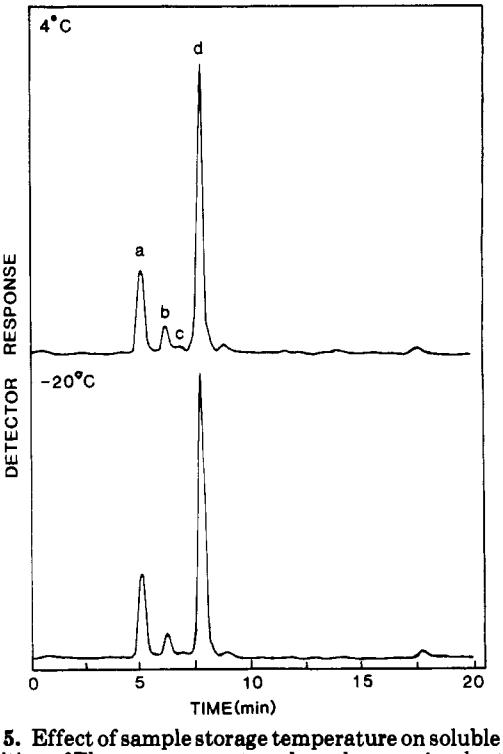

Figure 5. Effect of sample storage temperature on soluble sugar composition of Florunner peanut seed: a, glucosamine; b, stachyose; c, raffinose; d, sucrose.

Overall, these data indicated that peanut seed contained mainly sucrose, glucosamine, stachyose, and raffinose as free sugars and that glucose and fructose result primarily due to degradation of oligosaccharides during sample preparation and analysis.

ACKNOWLEDGMENT

This research was supported by a grant from the USDA-SEA/CSRS, Washington, DC.

LITERATURE CITED

Amaya-F, J.; Basha, S. M.; Young, C. T. Variation of total monosaccharides in developing peanuts (*Arachis hypogaea*). *Cienc. Cult.* **1978**, *30*, 79.

Basha, S. M.; Beevers, L. Glycoprotein metabolism in cotyledons of *Pisum sativum* L. during development and germination. *Plant Physiol.* **1976**, *57*, 93.

Basha, S. M.; Cherry, J. P.; Young, C. T. Changes in free amino acids, carbohydrates, and proteins of maturing seeds from various peanut (*Arachis hypogaea*) cultivars. *Cereal Chem.* **1976**, *53*, 586.

Bieleski, R. L. Intracellular carbohydrates. In *Plant Carbohydrates*; Loewus, F. A., Tanner, W., Eds.; Encyclopedia of Plant Physiology, NS; Springer-Verlag: New York, 1982; p 158.

Brown, A. C., Jr.; Summers, W. L. Change in carbohydrate concentration during watermelon (*Citrullus lanatus*) juice storage. *Hortic. Sci.* **1985**, *20*, 896.

Cegla, G. F.; Bell, K. R. High pressure liquid chromatography for the analysis of soluble carbohydrates in defatted oil seed flours. *J. Am. Oil Chem. Soc.* **1977**, *54*, 150.

Cobb, W. Y.; Swaisgood, H. E. Roasted peanut flavor and its relation to growth environment. *J. Food Sci.* **1971**, *36*, 538.

Ericson, M. E.; Chrispeels, M. J. Isolation and characterization of glucosamine containing storage glycoproteins from the cotyledons of *Phaseolus aureus*. *Plant Physiol.* **1973**, *52*, 98.

Hendrix, D. L.; Peelan, K. K. Artifacts in the analysis of plant tissues for soluble carbohydrates. *Crop Sci.* **1987**, *27*, 710.

Holley, K. T.; Hammons, R. O. Strain and seasonal effects on peanut characteristics. *Res. Bull.—Univ. Ga. Coll. Agric. Exp. Stn.* **1968**, *No. 32*.

Johnson, B. R.; Waller, G. R.; Burlingane, A. L. Volatile components of roasted peanuts: Basic fraction. *J. Agric. Food Chem.* **1971a**, *19*, 1020.

Johnson, B. R.; Waller, G. R.; Faltz, R. L. Volatile components of roasted peanuts. Neutral fraction. *J. Agric. Food Chem.* **1971b**, *19*, 1025.

Jordan, W. S. Chromatographic analysis of the alcohol-soluble sugars isolated from kernels of maize endosperm mutants. Ph.D. Thesis, The Pennsylvania State university, University Park, PA, 1965.

Koshiyama, I. Carbohydrate component in 7S protein of soybean casein fraction. *Agric. Biol. Chem.* **1966**, *30*, 646.

Mason, M. E.; Johnson, B.; Hamming, M. Flavor components of roasted peanuts. Some low molecular weight pyrazines and a pyrrole. *J. Agric. Food Chem.* **1966**, *14*, 454.

Mason, M. E.; Newell, J. A.; Johnson, B. R.; Koehler, P. E.; Waller, G. R. Nonvolatile flavor components in peanuts. *J. Agric. Food Chem.* **1969**, *17*, 782.

Nagahashi, J.; Browder, S. K.; Beevers, L. Glycosylation of pea cotyledon membranes. *Plant Physiol.* **1980**, *65*, 648.

Newell, J. A.; Mason, M. E.; Matlock, R. S. Precursors of typical and atypical roasted peanut flavor. *J. Agric. Food Chem.* **1967**, *15*, 767.

Oupadissakoon, C.; Young, C. T.; Mozingo, R. W. Evaluation of free amino acid and free sugar contents in five lines of virginia-type peanuts at four locations. *Peanut Sci.* **1980**, *7*, 55.

Pusztai, A. Hexosamine in the seeds of higher plants (Spermatophytes). *Nature (London)* **1964**, *201*, 1328.

Pusztai, A. A study on the glucosamine containing constituents of the seeds of kidney beans (*Phaseolus vulgaris*). *Biochem. J.* **1965**, *94*, 604.

Racusen, D.; Foote, M. The major glycoprotein in germinating bean seeds. *Can. J. Bot.* **1971**, *49*, 2107.

Racusen, D.; Foote, M. The hexosamine content of leaves. *Can. J. Bot.* **1974**, *52*, 2111.

Raguse, C. A.; Smith, D. Carbohydrate content in alfalfa herbage as influenced by methods of drying. *J. Agric. Food Chem.* **1965**, *13*, 306.

Roberts, R. M.; Pollard, W. E. The incorporation of D-glucosamine into glycolipids and glycoproteins of membrane preparations from *Phaseolus aureus* hypocotyls. *Plant Physiol.* **1975**, *55*, 431.

Roberts, R. M.; Cetorelli, J. J.; Kirby, E. G.; Ericson, E. Location of glycoproteins that contain glucosamine in plant tissues. *Plant Physiol.* **1972**, *50*, 531.

Shu, C. K.; Waller, G. R. Volatile components of roasted peanuts: Comparative analysis of the basic fraction. *J. Food Sci.* **1971**, *36*, 579.

Smith, D. Influence of drying and storage conditions on non-structural carbohydrate analysis of herbage tissue—A review. *J. Br. Grassl. Soc.* **1973**, *28*, 129.

Tamate, J.; Bradbury, J. H. Determination of sugars in tropical root crops using carbon-13 NMR spectroscopy: Comparison with the high-performance liquid chromatography method. *J. Sci. Food Agric.* **1985**, *36*, 1291.

Tharanathan, R. N.; Wankhede, D. W.; Rao, M. R. R. Carbohydrate composition of groundnuts (*Arachis hypogaea*). *J. Sci. Food Agric.* **1975**, *26*, 749.

Tharanathan, R. N.; Wankhede, D. B.; Rao, M. R. R. Mono- and oligosaccharide composition of groundnut (*Arachis hypogaea*). *J. Food Sci.* **1976**, *41*, 715.

Walradt, J. P.; Pitter, A. O.; Kinlin, T. E.; Muralidhara, R.; Sanderson, A. Volatile components of roasted peanuts. *J. Agric. Food Chem.* **1971**, *19*, 972.

Received for review August 16, 1991. Revised manuscript received December 10, 1991. Accepted February 10, 1992.

Registry No. Sucrose, 57-50-1; glucosamine, 3416-24-8; stachyose, 470-55-3; raffinose, 512-69-6; glucose, 50-99-7; fructose, 57-48-7.