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Confirmation of sugars and reductones in complex peanut flavor precursor extracts by ion chromatography and methylation analysis

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As confirmation of ion chromatographic determination of sugars and reductones in complex mixtures of peanut flavor precursors, extracts from defatted samples were derivatized as methylated alditol acetates to identify and semi-quantitate them by GC–MS. To obtain maximum information about a sugar/reductone sample, the ion chromatography/integrated pulsed amperometric detection (I.C.–I.P.A.D.) data from carbohydrate extracts (80% ethanol) of defatted raw peanuts were further elucidated by GC–MS chromatograms of the derivatives. The technique was tested on a set of samples from one crop year representing stages of peanut maturity and turnover of metabolites during postharvest maturation and curing. Although there are many interfering substances in the ion chromatography such as amino acids, peptides, tannin, and carboxylic acids, the GC–MS total ion chromatograms of the characteristic methylated derivatives confirm relative quantities as well as identities of the reductone/sugar components separated as ion chromatography peaks. The results indicated depletion of sugars and reductones from immature peanuts during the curing process and stabilization of mature peanuts at a much lower level of reactive reductone precursors. The present study should also be applicable in food chemistry to other flavor precursor studies involving mixtures of sugars and reductones.

INTRODUCTION

Recent developments in flavor chemistry have focused on the key role of the pyrolytic reactivity of matrix components of cereals, coffee and cocoa beans, chicory, etc. (Pazola & Cieslak, 1979) under roasting conditions to generate both olfactory and taste stimuli important to the overall flavor (Grosch & Schieberle, 1991). The contribution of thermal products of roasting to the unique flavor of the peanut have been studied from the point of view of their relationships to descriptive sensory properties, fate during storage, and origin of contributing aromas among precursor mixtures (Bett & Boylston, 1992; Crippen *et al.*, 1992; Vercellotti *et al.*, 1992*a,b*; Vercellotti *et al.*, 1993). Peanut proteins, polysaccharides, and other complex carbohydrates and lipids also involved in pyrolytic reactions of the roasting process have been extensively reviewed with respect to their contributions to flavor and physical properties of the roasted peanut (Ahmed & Young, 1982).

Sanders and coworkers published a communication on peanut curing which related continued maturation to slow curing processes (Sanders *et al.*, 1990). Curing refers to the process during which the high moisture content of the freshly dug peanuts (often >60%) is reduced to a final stable equilibrium level of the dormant seed (Young *et al.*, 1982). Little is understood, however, about the simultaneous biochemical processes which occur that result in hydrolytic or other bioenergetic reactions while residual water molecules are most efficiently ordered around the stabilized seed storage organelles, vesicles, and compartments. Maturity, curing, and the interaction between these two variables, have previously been shown to affect peanut flavor (Sanders, 1989; Sanders *et al.*, 1989; Pattee *et al.*, 1990).

Though documented in some detail in various papers and reviews (Tharanathan *et al.*,1975, 1976; Ahmed & Young, 1982; Shepherd & Rudolf, 1991; Basha *et al.*, 1991, 1992), reactive hydroxycarbonyl compounds have not been qualitatively or quantitatively defined as a metabolically changing set of precursors of roasted peanut flavor, principally for lack of suitable analytical methodology. In identifying and quantitating the complex mixture of metabolites from the peanut flavor pre-

cursor extract, it became apparent that further elucidation of ion chromatographic–pulsed amperometric detector composition profiles was necessary by GC–mass spectrometric methods to gain a maximum amount of information about this important flavor precursor system. The present study was undertaken, therefore, to make the reinforcing parallel methods available for flavor precursor researchers and to test the procedures on a well-chosen set of peanut samples in which some change was anticipated.

MATERIALS AND METHODS

General methods for determination of peanut matrix composition

Methods of the AOAC (1990) were consulted for proximate compositional analysis and other special techniques such as total dietary fiber determination (Prosky *et al.*, 1988). Florunner peanuts were selected from a 1991 crop year curing study with immature and mature samples separated by hull scrape classes (Williams & Drexler, 1981) and representative of degree of maturation (Sanders *et al.*, 1982; Sanders 1989; Sanders *et al.*, 1990). Samples were taken at time of digging (135 days after planting) as Day 0; after two days of windrow drying, Windrow Day 2; and after four windrow days drying and final commercial scale wagon drying for 21 hours, Windrow Day 4. Stackpole-cured peanuts were harvested also at 135 days of growth with samples taken after 12 days (Stack 12 or SP/12) and after 23 days (Stack 23 or SP/23). For windrow and stackpole samples, immature (orange hull-scraped maturity class) and mature (black hull-scraped maturity class) peanuts were usually hand selected. A sample of Florunner peanuts, previously obtained for full flavor quality, was used as a standard reference. After defatting according to Folch *et al.* (1957) with 2:1 methylene chloride–methanol (to include phospholipids and glycolipids as well as conjugated saponins) (Fig. 1), the soluble reductones and low molecular weight oligosaccharides were extracted with 80% aqueous ethanol and concentrated by rotary evaporation under mild conditions followed

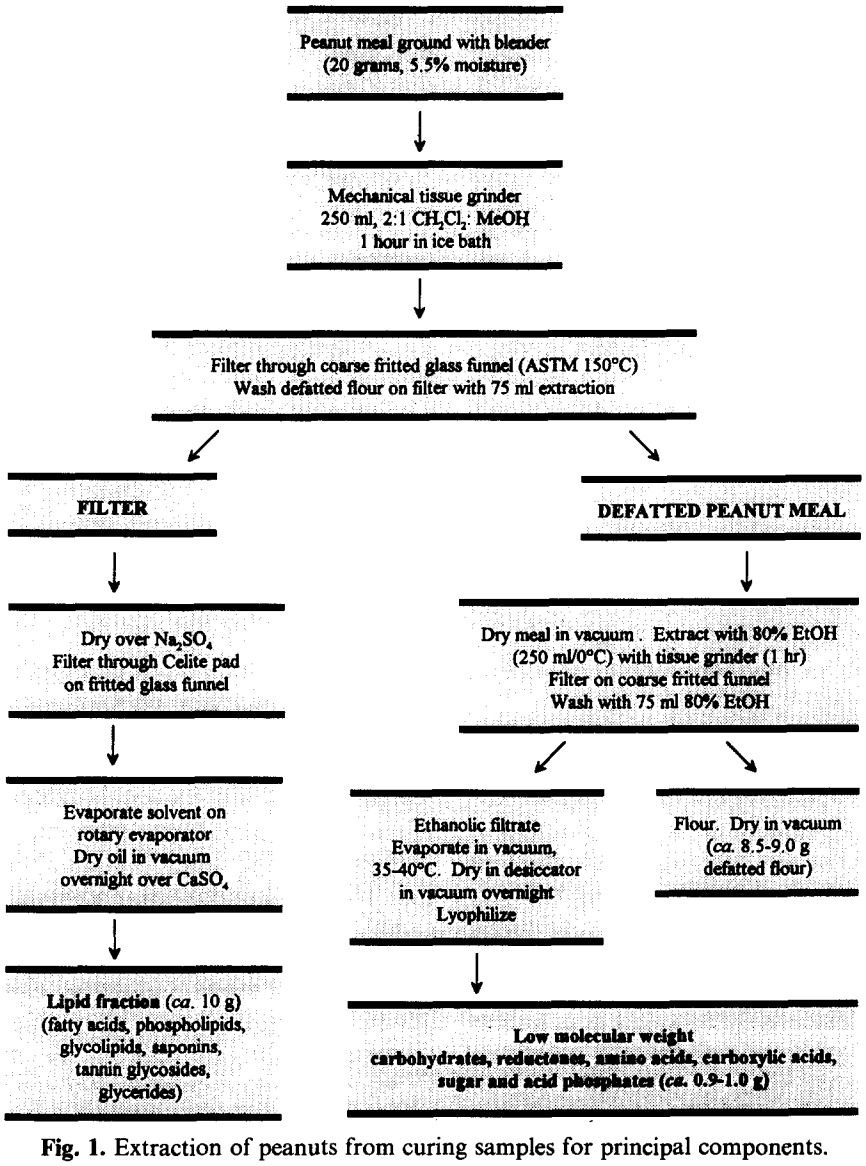

Fig. 1. Extraction of peanuts from curing samples for principal components.

by lyophilization to assure no condensation or Maillard-type reactions. After dissolving in sufficient water to solubilize the foamy solid, the sample was filtered through a Swinney adapter with a 0.45 μM nylon filter covered with a spun glass filter disk and the concentration brought to 4180 parts per million (0.418% (w/v)) for use in chromatographic and colorimetric experiments.

Chromatographic and colorimetric techniques

Thin layer chromatography was carried out on the syrupy 80% ethanol extract on Kieselgel 60 F-254 glass plates coated with 0.25-mm thickness of silica gel (Merck, Darmstadt). The plates were developed with ethyl acetate: acetic acid: methanol: water (5:3:3:2) and zones detected with ultraviolet light or by spraying with 5% sulfuric acid in ethanol, followed by heating at 110°C to char the zones. Protein was determined by the method of Lowry *et al.* (1951), as adapted by Smith *et al.* (1985) using bicinchoninic acid as copper (I) complexing agent (Pierce Chemical, Inc., Rockford, IL, Bulletin 23225). Estimation of free amino acids and peptides was done by quantitative ninhydrin assay according to Moore and Stein (1948) as adapted by Yemm and Cocking (1955). Total carbohydrate, both free and hydrolyzable to reducing sugar, was determined by the phenol–sulfuric acid method of Dubois *et al.* (1956). Uronic acids and pectin were quantitated by the *m*-hydroxybiphenyl determination of Blumenkrantz and Asboe-Hansen (1973). Glycosidic hydrolysis was done by the procedure of Albersheim with trifluoroacetic acid (Albersheim *et al.*, 1967) or by the primary and secondary sulfuric acid hydrolyses reported by Englyst and Cummings (1988). Component sugars were determined as alditol acetates, prepared by the procedure of Metz *et al.* (1970), and Li *et al.* (1985), using the gas chromatographic equipment described below for the methylation analyses.

Liquid chromatographic analyses of the soluble extract

Gel-filtration was effected on a Waters HPLC system with multiple-wavelength ultraviolet detector. Dupont (Wilmington, DE) GF-250 and GF-450 columns coupled consecutively were employed with 0.2 M, pH 7 potassium phosphate buffer with molecular weight calibration through appropriate peptide and protein standards (Sigma Chemical, St. Louis, MO). Sugars in the 80% ethanol soluble fraction described above were also determined by HPLC–ion chromatography with a Dionex BioLC instrument using a Dionex CarboPac PA1 column and integrated pulsed amperometric detection (H.P.L.C.–I.C.–I.P.A.D.). Improved statistical comparison for multiple runs with autoinjection over several days could be achieved by switching the I.P.A.D. to the Ag/AgCl reference electrode alone and omitting any post-column addition of base. With the Ag/AgCl reference alone and no post-column base addition, separations and recoveries were within acceptable ranges for quantitation on a CarboPac PA1 column (LaCourse & Johnson, 1993). Using the Dionex 8880 autoinjector and AI-450 chromatography software, multiple runs were accumulated to make standard response curves for glucosamine (internal standard), glucose, fructose, sucrose, raffinose, and

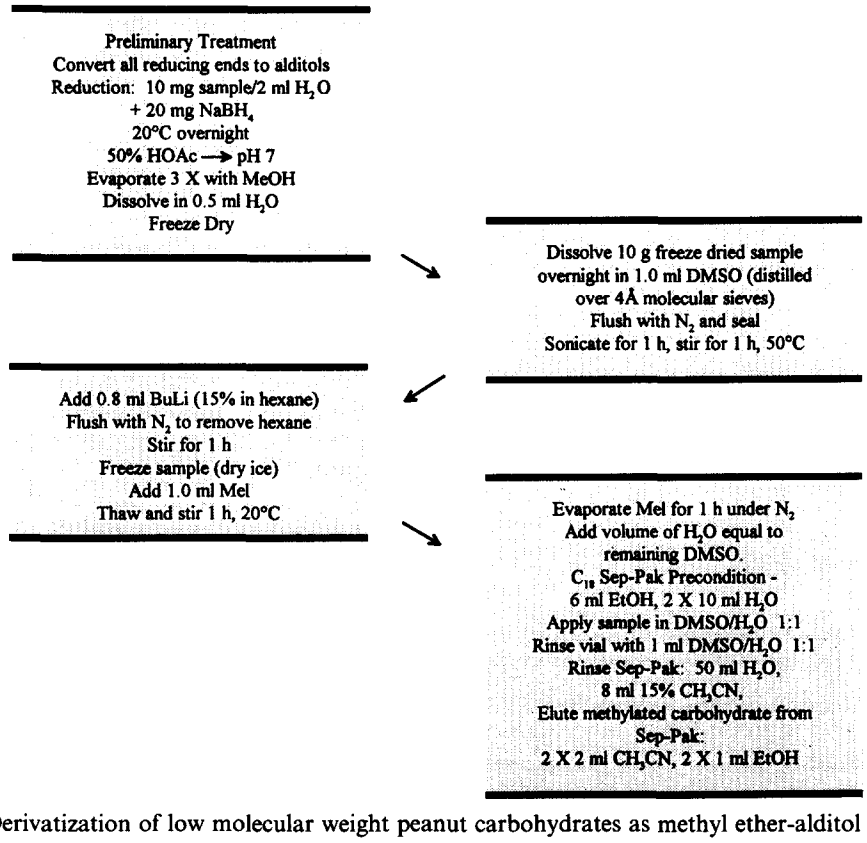

Fig. 2. Derivatization of low molecular weight peanut carbohydrates as methyl ether-alditol acetates.

stachyose using the following column eluent conditions: 16 mM NaOH isocratic (inject; 0–2.0 min), a gradient of 16 mM–160 mM (2.1–20 min), followed by isocratic 160 mM NaOH (20.1–30 min), 200 mM NaOH (30.1–40 min), and return to 16 mM NaOH (40.1–55 min) for a total run time of 55 min. The I.P.A.D. waveform settings found most stable (<1.0% drift overnight with autozeroing between runs) are 0.00 s=+0.05 V; 0.42 s=+0.05 V; 0.43 s=+0.75 V; 0.60 s=+0.75 V; 0.61 s=–0.15 V; and 0.96 s=–0.15 V. The duration of the I.P.A.D. integration interval was set at 0.2–0.4 s. Standard curves were generated from 0.5 to 1000 ppm for the sugars to test linearity in multiple runs and generate area response factors. Statistical analysis was run on triplicates of all samples to obtain regression curve fitting and percent standard deviation. Complex mixtures of peanut flavor precursors were quantitated using these parameters. Response factors were generated for each of the sugars and for the peanut extract reductones and sugars.

Methylation analysis and gas chromatography–mass spectrometry of derivatives

Methylation analysis was done according to combinations of the procedures of Kvernheim (1987), Hakomori (1964), and Bjorndal *et al.*, (1970). The flow chart for derivatization to methylated alditol acetates is shown in Fig. 2, with a reverse phase, C_{18}-SepPak (Waters Associates, Milford, MA) cleanup procedure added at the end of the sequence to best isolate the derivatives from the polysaccharide complex or glycoprotein of the peanut extract. Gas chromatography of the methylated alditol acetates was carried out with a DB-225 capillary column (0.33 mm × 30 m; J & W Scientific, Folsom, CA), instead of a packed OV-225 column as originally reported by Kvernheim (1987), using a Hewlett-Packard Model 5890 Series II gas chromatograph with flame ionization detector, computing integrator and HP 3359 laboratory automation system. Gas chromatography–mass spectroscopy was performed on an HP 5988A mass spectrometer equipped with an HP 59872C data system using a DB-225 column as on the screening gas chromatograph above, with GC–MS results reported as total ion currents of the peaks. Peak intensities of the methylated alditol acetate samples were recorded in mass spectral total ion currents and reproducibility calculated as percent standard deviation from triplicate injections.

RESULTS AND DISCUSSION

The previous two crop years, 1989 and 1990, have also been studied for these changes during curing, and the results of all three crop years will be published later in a consolidated study of the data. Plant physiological and seed developmental data on the role of maturation in quality of stackpole cured peanuts is currently in preparation (Sanders *et al.*, 1990, 1993). For purposes of establishing methods of ion chromatography correlated with methyl ether derivatization and gas chromatography–mass spectroscopy to profile flavor precursor reductones and reactive carbonyls in peanut curing, in this paper the results from the 1991 peanut curing study will be presented to illustrate the usefulness of those techniques.

Table 1. Composition of syrupy 80% ethanol-soluble defatted reference Florunner peanut fraction

Carbohydrate by phenol-sulfuric[a]	93·7%
Protein pos., (Lowry)	5·6%
Ninhydrin pos.,	8·2%
Ash	3·0%
Soluble sugars by HPLC–IC	
Reductones[b]	6·07%
Inositol	1·23%
Glucose	0·38%
Fructose	0·17%
Sucrose	80·14%
Raffinose	2·91%
Stachyose	9·10%

[a] Uronic acids, trace by *m*-hydroxybiphenyl reaction (Blumenkrantz & Asboe-Hansen, 1973).
[b] Calculated according to LC response factor for glucose.

The low molecular weight, 80%-ethanol soluble fraction (Fig. 1) was found to consist of sugars, peptides, amino acids, carboxylic acids, phenolic glycosides, etc. (Tables 1 and 2), and gave a gel filtration pattern with molecular weight <5000 D and a peak at 1300 D. Results reported in Tables 1 and 2 are consistent with previous compositional studies of carbohydrates in peanuts (Tharanathan *et al.*, 1975, 1976; Ross & Mixon, 1989; McMeans *et al.*, 1990; Basha, 1992; Basha & Young, 1992) except that no free 2-amino-2-deoxy-D-glucose was found in these samples as was reported by Basha (Basha, 1992). After concentration of the alcoholic solution followed by freeze-drying, the resulting syrup had a pleasant butterscotch aroma with notes of raw beany still present. Thin layer chromatography of the peanut extract syrup separated the major components into zones that were, by visual inspection, similar to ion chromatograms described below with R_fs of compounds identified in Tables 3 and 4 also found on the TLC plates corresponding to peanut syrup components. Since each syrup contained amino acids, carboxylic acids, carbohydrates, peptides, glycolipids, saponins, etc., strict quantitation was not attempted with HPLC response factors for all the species of components. However, since both amino acids and car-

Table 2. Mass balance of refrence Florunner peanut fractionation

Fraction	Description	% Mass
1	Oil	47·0
1a	Non-saponifiable material	2·0
2	Low MW, 80% ethanol soluble	5·7
3	Protein rich defatted peanut flour	37·3
4	Moisture	5·5
5	Salts, residue	2·5
	TOTAL	100·0

Table 3. High performance ion chromatographic analysis of sugars and reductones from immature peanut extract[a] (orange hull-scraped maturity stage) for windrow and stackpole curing treatments

Peanut samples	Carbohydrates (ppm)							
	Reductones[b]	Inositol	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Total carbohydrate
Reference Florunner	70·54	14·24	4·37	1·98	930·98	33·75	105·76	1 161·62
Day 0	131·07	11·04	8·62	1·49	1 472·05	58·28	236·86	1 919·42
Windrow 2	126·43	10·91	7·74	1·18	1 583·64	59·18	254·84	2 043·93
Windrow 4	98·69	14·29	8·12	0·86	1 692·78	73·27	179·27	2 067·28
Stack 12	83·81	11·10	8·67	0·79	1 524·95	55·81	161·64	1 846·77
Stack 23	81·82	12·23	8·49	0·60	1 712·21	45·91	142·12	2 003·38

[a] Integrated pulsed amperometric detector.
[b] Calculated according to response factor of glucose.

boxylic acids gave either much lower responses with the I.P.A.D. or retention times grouped outside of the ranges of the sugars, assignments of identities of the sugars are on a sound basis (retention time windows of the sugars have <2% relative standard deviation). Response factors for each sugar were carefully calibrated on the I.C.–I.P.A.D. with statistical analysis of precision for triplicate runs (<1 to 3% depending on the sugar). The relative quantities of each sugar component (Table 1) are defined and reported here for the soluble sugars and reductones as parts per million (ppm) (Tables 3 and 4). Values listed in Tables 3 and 4 resulted in adequate data for creating a model in turnover of the extractable 80% ethanol-soluble peanut carbon pool.

In the present report the methodology was designed to define and characterize changes during curing in carbon pools or carbohydrate precursors of peanut flavor for mature (black hull-scrape class) *versus* immature peanuts (orange hull-scrape class) (Williams & Drexler, 1981). Comparison of ion chromatography of the low molecular weight sugar alcohols and reductones (first 5 min in Tables 3 and 4) and other sugars (identified by retention times) permitted formulation of a biochemical model of carbohydrate turnover in peanut curing. The profiles indicate that these metabolites decrease to a stable concentration by the time curing dehydration reaches equilibrium in both windrow/wagon-dried peanuts as well as in longer-term stackpole drying. The following conclusions were drawn from Tables 3 and 4 about the mass spectral ion chromatograms of the derivatives from the various maturity and curing stages of peanuts from which the 80% alcoholic extracts were taken:

—The immature (orange) peanuts have more low molecular weight reducing substances as well as oligosaccharides than the mature (black) peanuts at all post-harvest stages of curing.
—Immature (orange) peanuts decrease in polyhydroxy reductones from Day 0 to the final day of drying either as windrow/wagon dried peanuts or in stackpoles but still have higher final levels than the mature (blacks).
—The mature (black) peanuts at Day 0 are distinguishable by ion chromatography in some component peaks from blacks separated either from windrow or stackpole drying, including final samples, but change only slightly in most sugars and reductones during either kind of curing treatment.
—The high quality Florunner reference sample of mixed medium and jumbo commercial seed size possesses an ion chromatogram of syrupy sugar extract identical to the mature black samples from this 1991 crop year curing study.

In Tables 3 and 4 the relative quantities of 2, 3, 4, and 5 carbon pool reducing or hydroxylated fractions decreased in the immature samples as curing came to completion. The reference Florunner peanut was similar in species of sugars and reductones to the last of the longest cured, mature (black) samples from windrow/wagon drying (Day 4) and from SP/12 and SP/23, although relative quantities differ somewhat.

Table 4. High performance ion chromatographic analysis of sugars and reductones from mature peanut extract[a] (black hull-scraped maturity stage) for windrow and stackpole curing treatments

Peanut samples	Carbohydrates (ppm)							
	Reductones[b]	Inositol	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Total carbohydrate
Reference Florunner	70·54	14·24	4·37	1·98	930·98	33·75	105·76	1 161·62
Day 0	78·24	6·69	1·07	0·68	519·68	9·96	58·83	675·14
Windrow 2	97·35	7·64	1·27	0·71	888·06	16·78	121·08	1 132·90
Windrow 4	86·47	6·75	1·26	0·70	899·22	24·28	105·40	1 124·09
Stack 12	97·54	7·18	1·10	0·79	802·78	19·42	93·39	1 022·21
Stack 23	78·57	7·73	2·15	0·80	867·75	30·64	91·15	1 078·78

[a] Integrated pulsed amperometric detector.
[b] Calculated according to response factor of glucose.

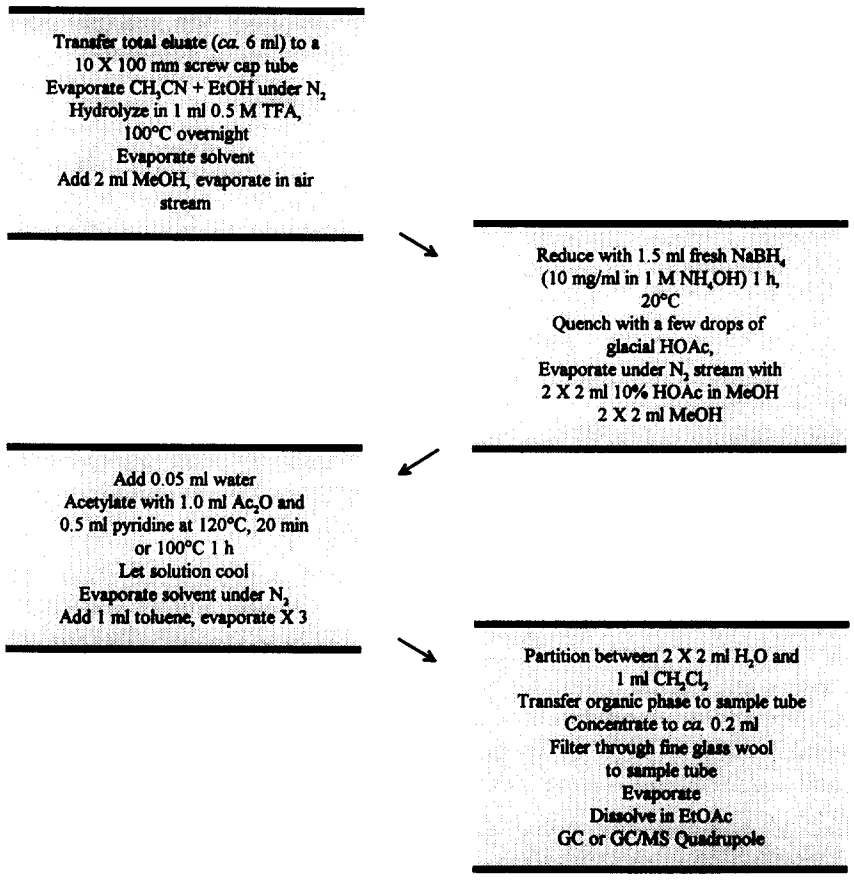

Fig. 3. Hydrolysis and sugar analysis of methyl ether-alditol acetates from peanut extracts. BuLi = butyllithium; Mel = methyl iodide; Ac_2O=acetic anhydride.

Thin layer chromatograms of the syrupy peanut extract, as described above, indicated densities of zone patterns which followed essentially the same conclusions as the HPLC data, namely, that as curing progressed the composition of the extract from the immature peanuts decreased quantitatively in complexity, whereas the extracts of the mature, black peanuts were essentially the same throughout the curing period. Oxidation processes are implicated in some of these changes and further investigation must be carried out to create a metabolic regulation model of the bioenergetics involved (Stadtman & Oliver, 1991; Kanner, 1992).

Methylation analysis of 80% ethanol extract of defatted peanut

Quantitative levels and mass balances of derivatized polyhydroxy aldehydes or ketones, and other reductones were also determined on these 80% alcohol extracts of raw peanuts using gas chromatographic methods as confirmation of the ion chromatographic data above. Modification of non-reducing sugars, invert, and lower molecular weight 2, 3, and 4 carbon reductones by borohydride treatment, methylation, hydrolysis followed by another reduction, acetylation, and GC–MS of the alditol acetate methyl ethers from the soluble carbon pool fragments provided a profile of these peanut flavor precursors in the concentrated syrupy extracts of the defatted peanut. Figures 2 and 3 are flow charts for the processes used for the alditol acetate, methyl ether derivatization of these sugars (Bjorndal *et al.*, 1970; Lindberg & Lonngren, 1978; Kvernheim, 1987) and presented in detail here to facilitate use of the present experience on other food-related extracts.

Peaks identified by the mass spectral total ion current (TIC) gas chromatogram of methylated alditol acetates from the 80% ethanol extract of the defatted Florunner reference peanut are shown in Table 5. The balanced flavor quality of this reference Florunner peanut has been well proven in sensory evaluation studies (Bett & Boylston, 1992; Ory *et al.*,1992). The derivatization method proceeded smoothly and did not result in large amounts of degradation products, especially since, as shown in Tables 1 and 2, there were many potential Maillard constituents present. It could be that some of these compounds were extracted out of the mixture during derivatization separations.

Tentative assignments (Table 5) could be made for only 35 out of 85 peaks in the chromatogram of the Florunner reference peanut. The chromatograms corresponding to Table 6 (but not presented in this text) for the maturity stages of various curing times in this study also show these unknown peaks. The most plentiful peaks in Tables 3 and 4 correspond to those methyl ether alditol acetates associated with glucose and fructose of sucrose and its homologous α-D-(1 $\rightarrow$ 6)-galactosyl substitution products, raffinose, stachyose, and verbascose (approximately 85% of the total ion current from the mass spectrometric chromatogram).

Table 5. Principal methylated alditol acetates of sugars identified by mass spectral interpretation from defatted reference Florunner peanut

Retention time (min)	Peak area[a]	Compounds with probable sources
3·27[b]	47·3	**Di-O-Me ethylene glycol (glycoaldehyde)**
3·74	69·8	**O-Me, O-Ac ethylene glycol (glycolic acid)**
3·93	30·3	Di-O-Ac ethylene glycol (glyoxyllic acid)
4·45	7·5	Tri-O-Me glycerol (glycerol)
5·45	38·4	**Di-O-Me, O-Ac glycerol (glyceric acid)**
6·06	42·6	**O-Me, Di-O-Ac glycerol (diglyceride)**
6·31	4·6	**Tri-O-Ac glycerol (triclyceride)**
6·42	4·6	Di-O-Me, di-O-Ac tetritol (tartaric acid)
6·68	4·0	Tri-O-Me, O-Ac tetritol
6·82	3·5	Di-O-Me, tri-O-Ac pentitol
6·95	4·2	Tri-O-Me, di-O-Ac pentitol
7·07	8·7	Tetra-O-Me, O-Ac pentitol
7·41	2960·0	**1,3,4,6-tetra-O-Me-2,5-di-O-Ac hexitol (fructose)**
8·33	3220·0	**2,3,4,6-tetra-O-Me-1,5-di-O-Ac hexitol (glucose)**
8·65	538·0	**2,3,4,6-tetra-O-Me-1,5-di-O-Ac hexitol (galactose)**
9·94	320·0	**3,4,6-tri-O-Me-1,2,5-tri-O-Ac hexitol (fructose)**
10·24	164·0	**2,3,6-tri-O-Me-1,4,5-tri-O-Ac hexitol (maltodextrin?)**
10·76	466·0	**2,3,4-tri-O-Me-1,5,6-tri-O-Ac hexitol (glucose)**
11·42	355·0	**2,3,4-tri-O-Me-1,5,6-tri-O-Ac hexitol (galactose)**
11·96	46·0	4,6-di-O-Me-1,2,3,5-tetra-O-Ac hexitol
12·73	20·0	2,3-di-O-Me-1,4,5,6-tetra-O-Ac hexitol
12·88	28·0	3,4-di-O-Me-1,2,5,6-tetra-O-Ac hexitol
13·20	14·0	6-O-Me-1,2,3,4,5-penta-O-Ac hexitol
13·54	7·0	2,4-di-O-Me-1,3,5,6-tetra-O-Ac hexitol
14·34	8·4	2-O-Me-1,3,4,5,6-penta-O-Ac hexitol
16·03	55·2	**1,2,3,4,5,6-hexa-O-Ac cyclohexitol (from phytate?)**
18·24	34·0	**Methylated, reduced and acetylated amino acid (aspartic?)**
19·62	24·1	**Sesquiterpene acetate**
19·96	66·6	**Methylated catechol**
20·50	69·9	**Methylated, acetylated reduced tannin**
20·77	55·0	**Acetate of a flavone**
22·87	51·1	Phenolic acetate
27·99	107·0	**Methylated, reduced, and acetylated phenolic acid**
29·83	29·5	Methylated, reduced, and acetylated aromatic amino acid
30·15	49·4	**Sterol acetate**

[a] Mass spectral total ion current (intensity counts × 10^{-4}) of gas chromatographic peaks detected after fragmentation of sugar alditol acetate methyl ethers.
[b] Entries in bold type are peaks consistently found in common with the other 1991 crop year curing study samples analyzed.

Mass spectral *m/e* ratios for the methylated alditol acetates as well as their characteristic fragmentation products are well known (Bjorndal *et al.*, 1970; Lindberg & Lonngren, 1978). Interpretation of the mass spectra listed in Table 3 was undertaken by following fragmentation pattern precedents of methylated alditol acetates. The lower molecular weight members of the compounds in Table 5 are derived from reduced methylated and/or acetylated reductones such as glycolaldehyde (e.g. to 1,2-di-*O*-methyl ethylene glycol, *m/e* 90); dihydroxyacetone, L-glyceraldehyde, erythritol, D-xylulose, (and corresponding mono- or diphosphates), etc., which participate in the carbon pool of carbohydrate metabolism. Other oxygenated species such as low molecular weight oxycarboxylic acids (e.g. pyruvic, lactic, tartaric, or malic acids) are probably among the syrupy extract pool of those compounds that went unidentified at this stage of the investigation.

As an example, permethylated sucrose upon hydrolysis yields 2,3,4,6-tetra-*O*-methyl-D-glucose and 1,3,4,6-tetra-*O*-methyl-D-fructose. After reduction and acetylation, the methylated alditol acetates derived from sucrose are identified on the mass spectrometer as 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-D-glucitol (*m/e* 322 with fragmentation in highest abundance at *m/e* 277, 205, 161, 117, 73, 72, 45, and 44) and 1,3,4,6-tetra-*O*-methyl-2,5-di-*O*-acetyl-D-glucitol/mannitol (*m/e* 322 with the similar fragments as the previous example except in differing abundances). The mannitol derivative is produced upon borohydride reduction of fructose in approximately equal proportions to its glucitol C-2 epimer. After derivatization, these epimeric isomers are then adequately separated on the DB-225 column for mass spectral identification. The homologous α-D-(1 → 6)-galactosides of sucrose, namely raffinose, stachyose, and verbascose, also produce rational fragmentations of the substitution patterns on the terminal *versus* internal methylated galactosides and were identifiable for Tables 5 and 6. Thus, the tetrasaccharide stachyose (mol. wt 666) is reconstructed after identification of derivatives in Table 5 from 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-D-galactitol (*m/e* 322); 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl-D-galactitol (*m/e* 350); 2,3,4-tri-*O*-1,5,6-tri-*O*-acetyl-D-glucitol (*m/e* 350); and 1,3,4,6-tetra-*O*-methyl-2,5-di-*O*-acetyl-D-glucitol/mannitol (*m/e* 322). Both raffinose and verbascose

Table 6. Total ion current mass spectral ratios of methylated alditol acetate peaks of the Florunner reference and 1991 peanut curing study samples[a]

Retention time (min)[b]	Total ion current (Abundance) (× 0·000 1)						
	Day 0	Immature SP/23	Windrow 4	Day 0	Mature SP/23	Windrow 4	Reference florunner[c]
3·27	20·0	21·0	30·0	48·0	8·0	40·0	47·3
3·74	15·0	20·0	21·0	0·0	1·1	30·0	69·8
5·45	21·0	2·0	42·0	30·0	5·0	32·5	38·4
6·06	20·0	4·0	0·0	5·0	8·0	25·0	42·6
6·31	0·0	0·0	0·0	4·0	11·0	8·0	4·6
7·07	12·0	3·0	41·0	0·0	0·0	41·0	8·8
7·41	485·0	585·0	1 200·0	82·0	430·0	825·0	2 960·0
8·33	820·0	1 200·0	1 400·0	275·0	590·0	1 630·0	3 220·0
8·65	175·0	165·0	420·0	52·0	90·0	225·0	538·0
9·94	60·0	95·0	150·0	125·0	60·0	65·0	320·0
10·76	110·0	120·0	320·0	75·0	80·0	180·0	466·0
11·42	95·0	85·0	280·0	60·0	40·0	170·0	355·0
16·03	16·5	29·5	16·0	250·0	8·2	20·0	55·2
18·24	8·0	5·0	16·0	30·0	5·0	12·0	34·0
19·62	17·0	0·0	11·0	0·0	1·0	6·0	24·1
19·96	18·5	17·8	33·5	0·0	15·2	21·0	66·6
20·50	16·0	22·0	42·0	0·0	21·0	22·0	69·9
20·77	15·5	16·8	40·0	0·0	17·0	25·0	55·0
22·87	15·0	6·5	15·0	41·0	6·5	8·0	51·1
27·99	9·5	24·0	26·0	172·0	9·5	11·2	107·0
30·15	16·2	9·0	30·0	0·0	9·0	12·5	49·4

[a] See Fig. 2 for derivatization procedure.
[b] For compound identity, see Table 3. Immature refers to the orange hull-scraped maturity stage, and mature to the black.
[c] Commercial seed size to ride 18/64 inch screen, not sorted by maturity stages.

would contribute derivatized components to these peaks although verbascose would have one more (and raffinose, none) of the 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl-D-galactitol units than from stachyose. In this work, known standards of the variously substituted sugars were available and were compared where doubtful assignments were possible. With only a few exceptions, adequate separation occurred on the DB-225 capillary column for clear interpretation of the mass spectral patterns made by the derivatives. Undermethylation due to interference might explain some of the mono-, di-, and tri-*O*-methyl derivatives found. However, some of these could be sugar phosphates or esters of phenolic acids which were cleaved during hydrolysis or derivatization. These correspond to several of the mass spectral fragmentation patterns found in this work.

In Table 6, TIC mass spectral chromatograms for the derivatized extracts of the Day 0 immature peanuts are compared with the Day 0 mature peanuts. The peaks are relatable to Table 5 for the derivatized extract of the Florunner reference. This indicates that sugar and reductone compounds are diagnostic for precursors generated in the process of maturation. On the other hand, using simple pattern recognition, the immature sample has much higher responses for most components in the TIC than the mature, indicating that the mature peanut has less of these carbon-pool precursors even at Day 0. The Day 0 immature peanuts also have a number of additional peaks, which are not present in the mature ones at any point in the curing treatment. It is interesting, however, that each maturity class possesses essentially the same key compounds, except that the relative quantities are less in all the mature samples. This pattern of immature peanuts possessing more of the low molecular weight reductones than the mature ones continues in the representative mature and immature samples of windrow Day 4 samples as well as the Stack 23 samples.

The significance of the above analyses lies in the fact that thermal cleavage of glycosidic linkages in glycoproteins and cell-wall polysaccharides also generate sweet aromatic attributes *per se*, or other reductones (Theander, 1987; Bailey & Um, 1992; Kanner, 1992; Vernin *et al.*, 1992) that are quite reactive in heterocyclic ring closures leading to the myriad roasted peanutty flavors (Bett & Boylston, 1992; Vercellotti *et al.*, 1992*a,b*, 1993; Crippen *et al.*, 1992). These same reductones, such as the hydroxyfuranones or furaneols, contribute powerful fruity/fermented off-flavors or are principal characteristic fruit flavors in themselves (e.g. the hydroxyfuranones are important contributors to pineapple, strawberry, passion fruit, apricot, etc., flavors). Damage to peanut cell walls in pre- or post-harvest treatments results in irretrievable losses due to off-flavors such as fruity/fermented that permeate improperly dried or freeze damaged peanuts (Pattee *et al.*, 1982; Sanders *et al.*, 1989; Ory *et al.*, 1992). In addition, many lipid oxidation products are linked glycosidically or through hemiacetal or ketal linkages and are released from polysaccharides during the roasting process. The fuller roasted peanut flavor from mature peanuts, and the poorer potential for production of the

delicate balance of high-impact flavor components in immature peanuts has been demonstrated (Sanders *et al.*, 1989; Crippen *et al.*, 1992; Ory *et al.*, 1992). Proper harvest and curing techniques will result in fewer immature peanuts in various commercial seed sizes and should result in a higher quality product because the off-flavor and low-impact notes from the immature peanuts will be far less concentrated (Johnsen *et al.*, 1988; Sanders, 1989; Sanders *et al.*, 1989).

In conclusion, a method was developed to confirm the constituent sugars and reductones in complex mixtures of peanut flavor precursors extracted with 80% ethanol from defatted samples. The technique was tested on a set of selected samples from one crop year representing stages of peanut maturity and turnover of metabolites during post-harvest maturation and curing. Results of ion chromatography–integrated pulsed amperometric detection were compared by GC–MS for methylated alditol acetate derivatives of the same peanut extracts. Although there are many interfering substances in the ion chromatography such as amino acids, peptides, tannin, and carboxylic acids, the total ion chromatograms of the characteristic methylated derivatives confirm relative quantities as well as identities of the reductone/sugar components separated as ion chromatography peaks. Immature peanuts were demonstrated to have much higher levels of sugars and reductones than mature peanuts. The combined ion chromatographic-methylated alditol acetate/GC–MS approach is recommended to the food chemist as a useful confirmation of reductone/sugar food flavor precursors applicable to a broad spectrum of food materials.

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