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Influence of Starch Plus Gluten on the Nonenzymic Browning Reaction of the Glucose–Glutamic Acid System[1]

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The essence recovered from the heated (150 °C) model system comprised of glucose, glutamic acid, starch, and gluten was found to contain over 62 components, 47 of which were identified by combined gas chromatography–mass spectrometry. The addition of starch plus gluten to a glucose–glutamic acid system resulted in the formation of 13 new volatile components, namely pentan-2-one, hexan-1-al, pentan-1-ol, heptan-2-one, 2-*n*-pentylfuran, benzaldehyde, furfuryl alcohol, acetophenone, and five unknowns. The observations made in this study suggest the possible role of gluten in the development of crusty aroma through the nonenzymic browning reaction occurring during baking of the bakery goods.

A favorite way to investigate the nonenzymic browning reaction and the development of aroma in heated foods has been to use model systems in which sugars and amino acids react under simplified conditions. The aromas of the products of the reaction have been reported to vary with the amino acid (Kiely et al., 1960). The identity of the sugars influences the rate of reaction in addition to the aroma produced (Barnes and Kaufman, 1947). The amino acid valine, when reacted with glucose and xylose separately, has been reported to produce ryebread (Hertz and Shallenberger, 1960) and malt coffee (Rothe and Voigt, 1963) aromas, respectively. Food components other than sugars and amino acids may also influence the course of the browning reaction and the nature of aroma developed. A chocolate aroma could only be produced when valine and glucose were heated in the presence of epicatechine (Rohan, 1970). Besides the nature of reactants, temperature, time of heating, moisture content, and pH are also known to influence the Maillard reaction (Nursten, 1981).

The addition of starch plus gluten has been shown to markedly affect the aroma generated from glucose–amino acids reactions (Berry and Gramshaw, 1970). A bread crust odor, obtained by heating glucose and proline at 150 °C, was found to alter to mousey or cakelike when starch plus gluten were added to the reaction mixture. On the other hand, a mixture of glucose and glutamic acid on heating gave a caramel odor, which changed to a crusty, breadlike aroma when starch plus gluten were also present in the mixture (Berry and Gramshaw, 1970). The high-quality vital gluten is commonly used in the bakery trade to strengthen the dough (Dubois and Cottle, 1969). Gluten, in baked goods, creates a honeycomb structure in association with the starch gelatinized in situ. But how gluten influences the generation of volatile aroma compounds during baking of bakery products is not well understood.

This paper reports the changes in the volatile components brought out by the addition of starch plus gluten in the roasting rection mixture of glucose and glutamic acid.

EXPERIMENTAL SECTION

Materials. D-Glucose (AnalaR), L-glutamic acid (reagent grade), soluble starch (AnalaR), and wheat gluten were purified (Berry and Gramshaw, 1974) prior to use.

Recovery of Volatiles. A mixture of glucose (5 mmol), glutamic acid (5 mmol), starch (5 g), and gluten (2.5 g) was mixed in a mortar and placed in a round-bottom flask (100 mL), which was connected to two liquid-nitrogen-cooled receivers placed in series. The reaction mixture was heated at 150 °C (Woods metal bath) for 1 h. The reaction flask was then cooled, the solid residue was purged, and 0.5 mL of purified (extracted with *n*-pentane) distilled water was added. Heating and collection of volatiles was repeated. The entire process was immediately repeated with fresh reactants, the volatile products being collected in the same receivers. The combined condensate was extracted with redistilled diethyl ether (10 mL) and the aqueous phase separated and extracted three times with diethyl ether (5 mL). The combined ether extracts were dried over purified (extracted with diethyl ether) anhydrous $MgSO_4$, and the ether was distilled off through a 30-cm Vigreux column to leave ca. 10 μL of essence. All glass apparatus was used.

Gas Chromatography. The essence was examined by gas chromatography with a Pye 104 instrument equipped with heated FID. Three glass, porous layer open tubular capillary columns, each containing a different stationary phase, prepared according to Cronin (1970), were employed. Column A (37 m × 0.5 mm (i.d.)) containing Carbowax 1540 was used at an initial oven temperature of 50 °C for 4 min, rising by 2 °C/min to 110 °C. Column B (19 m × 0.5 mm (i.d.)) coated with Carbowax 20M was operated at an initial oven temperature of 75 °C for 4 min, rising by 2 °C/min to 200 °C when the analysis continued isothermally. Column C (20 m × 0.5 mm (i.d.)) contained Triton-X 305 and was operated under similar conditions as for column B except that the initial oven temperature was 60 °C.

Each column was used in conjunction with a precolumn (1 m × 0.5 mm (i.d.)) containing sodium carbonate to remove excess acids. For all three columns, the carrier gas used was helium at a flow rate of 3.75 mL/min. Injection and detector temperatures were 150 and 210 °C, respectively.

Gas Chromatography–Mass Spectrometry (GC–MS). Combined gas chromatography–mass spectrometry was carried out on a Philips PV 4000 gas chromatograph fitted with FID and linked to an Edward 60°, fast-scanning mass spectrometer having a 15-cm radius analyzer head and an adjustable single-stage jet separator (Cronin et al., 1972/1973). Operating conditions: ion source pressure, 3.0×10^{-6} Torr; connecting line, 180 °C; top hat, 160 °C; multiplier voltage, 3.0 kV; i_{beam}, 50 μA; i_{fil}, 2.4 A; i_{box}, 0.01 mA; scan, 3 s for m/e 16–300; UV recorder chart speed, 12.5 cm/s. The same GC conditions as described above were employed on Carbowax 1540 and Carbowax 20M columns.

Determination of Retention Index (RI). Standard compounds were analyzed by GC, under similar conditions, alone and mixed with one of the two mixtures of *n*-alkanes

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Table I. Compounds Identified in an Ether Extract of Volatiles from the Glucose, Glutamic Acid, Starch, and Gluten Browning System

S1 no.	component	approx rel amt (peak size)[e]	evidence, retention index A	evidence, retention index B	evidence, retention index C	MS[f]
1	acetaldehyde[a,b]	3	660			+
2	ethyl formate[a,b]	3	770			+
3	ethyl acetate[a,b]	2	835			+
4	methanol[a]	1	855			+
5	ethanol[a,b]	2	900			+
6	pentan-2-one[b]	1	930			+
7	hexan-1-al[b]	2	1035			+
8	pentan-1-ol[b]	1	1200			+
9	heptan-2-one[b]	1		1155	1085	+
10	2-*n*-pentylfuran[b]	1		1170		*c*
11	pyrazine[b]	1		1200	1065	+
12	unknown[a]	1		1220		+
13	2-methyltetrahydrofuran-3-one[a,b]	2		1220	1050	*c*
14	2-methylpyrazine[b]	1		1250	1125	+
15	3-hydroxybutan-2-one[a]	1		1260	1120	+
16	1-hydroxypropan-2-one[a,b]	2		1270	1130	+
17	2,5-dimethylpyrazine[a,b]	1		1300	1195	+
18	2-ethyl-6- or -5-methylpyrazine[a,b]	1		1385	1255	+
19	acetic acid[a,b]	2		1390	1255	+
20	2-furfural[a,b]	5		1425	1270	+
21	2-acetylfuran[a,b]	3		1470	1315	+
22	furfuryl acetate[a]	1		1480	1355	+
23	benzaldehyde[b]	3		1490	1335	+
24	5-methyl-2-furfural[a,b]	6		1535	1375	+
25	5-methyl-2-acetylfuran[a]	1		1580	1420	+
26	γ-butyrolactone[a,b]	4		1590	1400	+
27	2-furfuryl alcohol[b]	3		1600	1440	+
28	acetophenone	1		1610	1440	+
29	2-(5-methyl-2-furfuryl)furan[a]	1		1617	1500	+
30	4-methylbut-2-enoic acid γ-lactone[a,b]	3		1645	1450	+
31	unknown[a]	2		1650	1470	
32	2-methyl-5-(5-methyl-2-furfuryl)furan[a]	1		1690	1580	+
33	unknown	2		1690		
34	unknown[a]	2		1690	1500	
35	unknown	1		1700	1500	
36	but-2-enoic acid γ-lactone[a,b]			1715	1575	+
37	1-(2-furyl)propane-1,2-dione[a,b]	1		1725	1555	+
38	5-formyl-2-methylfuroate[a]	2		1725	1580	*d*
39	decadien-2,4-al[a,b]	2		1770	1640	+
40	hexanoic acid[a,b]	2		1780	1650	+
41	1-oxo-3-methylcyclopent-2-en-2-ol[a,b]	2		1800	1600	+
42	1-(5-methyl-2-furyl)-propane-1,2-dione[a,b]	2		1810	1640	+
43	unknown[a]	1		1820	1610	
44	2-*n*-pentylfuroate[a]	1		1845	1720	+
45	unknown	2			1660	
46	benzothiazole[a,b]	1		1900	1700	+
47	2-acetylpyrrole[a,b]	3		1905	1705	+
48	2-(2-furyl)pyrazine[a,b]	2		1945	1740	+
49	2-formylpyrrole[a,b]	2		1950	1735	+
50	unknown	2		1950		
51	γ-nonalactone[a,b]	1		1970	1800	+
52	2-(2-furyl)-5- or -6-methylpyrazine[a,b]	1		2010	1790	+
53	unknown[a]	1		2020		
54	2-formyl-5-methyl-pyrrole[a,b]	3		2035	1825	+
55	5-[(formyloxy)methyl]-2-furfural[a]	2		2120	1880	+
56	5-(acetoxymethyl)-2-furfural[a]	3		2120	1900	+
57	unknown	1		2145		
58	2-formylbenzothiazole[a]	1		2210	1990	*c*
59	5-(2-furfuryl)-2-furfural[a]	1		2245	2025	+
60	5-(5-methyl-2-furfuryl)-2-furfural[a]	1		2295	2075	+
61	5-(hydroxymethyl)-2-furfural[a,b]	4		2410	2135	+
62	unknown[a]	3		2410	2200	

[a] Previously identified in the glucose–glutamic acid system (Berry and Gramshaw, 1986). [b] Identified in white bread crust also (Folkes and Gramshaw, 1981). [c] Tentative identification; authentic sample not available for comparison. [d] Very tentative identification; MS data for comparison not available. [e] Key: 1, trace; 2, small; 3, moderate; 4, large; 5, very large; 6, extra large. [f] + = positive identication; MS and retention index compared with that of authentic, synthesized sample. A–C, three columns; see the Experimental Section for details.

Table II. Mass Spectral Data of Unknown Compounds

unknown component no.[b]	*M*/*e* (relative %)[a]
12	81 (100), 43 (37), 27 (33), 82 (30), 126 (30), 97 (17), 83 (11), 98 (10), 54 (9), 52 (4)
31	86 (100), 95 (60), 81 (55), 52 (53), 80 (36), 73 (33), 68 (22), 39 (21), 140 (19), 112 (16)
33	86 (100), 47 (97), 30 (93), 57 (93), 56 (85), 26 (76), 85 (71), 75 (65), 27 (61), 31 (61)
34	85 (100), 56 (96), 58 (88), 29 (74), 57 (74), 27 (65), 86 (48), 47 (29), 55 (29)8, 30 (27)
35	55 (100), 112 (52), 27 (48), 45 (24), 83 (19), 82 (15), 29 (14), 54 (10)
43	68 (100), 40 (51), 39 (28), 98 (28), 27 (24), 41 (23), 42 (19), 43 (19), 69 (12), 136 (6)
45	111 (off scale), 53 (100), 109 (80), 81 (54), 27 (52), 52 (46), 55 (44), 29 (22), 140 (36), 39 (40)
50	85 (100), 29 (49), 58 (40), 56 (34), 43 (24), 41 (23), 71 (23), 27 (21), 55 (9), 57 (7)
53	85 (100), 29 (97), 43 (97), 27 (79), 55 (61), 84 (51), 95 (51), 45 (45), 57 (36), 42 (30)
57	81 (100), 97 (72), 85 (64), 39 (32), 57 (32), 27 (28), 87 (20), 26 (12), 29 (12), 45 (12), 175 (10)
62	51 (100), 105 (92), 106 (54), 175 (37), 50 (32), 77 (31), 78 (22), 52.5 (22), 66.5 (13), 63 (13), 148 (10), 176 (5)

[a] Percent of base peak. [b] From Table I.

(C_5–C_{12} or C_7–C_{25}) depending upon the retention times (RT) encountered. The RT of the alkanes were plotted against 100 × their carbon numbers, and the resultant graph was used to convert the RT of standard compounds to RI. The essence was similarly analyzed, and RI were recorded from the graph.

The identities of the components in the essence were established by comparing their RI and the MS with those of the authentic and synthesized samples. Syntheses of the compounds not available from the chemical houses were carried out according to the standard methods reported in the literature.

RESULTS AND DISCUSSION

The combined GC–MS analysis of the essence recovered from the heated mixture of glucose, glutamic acid, starch, and gluten showed the presence of over 62 components, 47 of which were positively identified by comparing their MS and RI with those of the authentic and synthesized compounds, with a further four components partially or tentatively characterized (Table I). The compounds identified cover a range of compound types, including acids, alcohols, aldehydes, ketones, esters, lactones, furans, pyrazines, pyrroles, and thiazoles (artifacts). The odor characteristics of some of these components have previously been described (Berry and Gramshaw, 1986).

Addition of starch plus gluten to the heated glucose–glutamic acid system was found to alter both qualitative and quantitative composition of the volatiles generated. The relative amounts of some components, viz., acetaldehyde, ethyl acetate, ethanol, 2-methyltetrahydrofuran-3-one, 1-hydroxypropan-2-one, 2-ethyl-6- or 5-methylpyrazine, γ-butyrolactone, 2-butenoic acid γ-lactone, γ-nonalactone, 2-formyl-5-methylpyrrole, 5-[(formyloxy)methyl]-2-furfural, and 5-(acetoxymethyl)-2-furfural, were found to decrease whereas that of 5-formyl-2-methylfuroate, decadien-2,4-al, 1-(5-methyl-2-furyl)propane-1,2-dione, and 2-(2-furyl)pyrazine increased. Pyrazine and 2-methylpyrazine, which could not be detected in the essence from the glucose–glutamic acid system (Berry and Gramshaw, 1986), were found to occur in the essence derived from the system under discussion. A number of compounds, namely pentan-2-one, hexan-1-al, pentan-1-ol, heptan-2-one, 2-*n*-pentylfuran, benzaldehyde, furfuryl alcohol, acetophenone, and unknowns 33, 35, 45, 50, and 57, were formed only in the presence of starch plus gluten. The MS data of unknown compounds are given in Table II. Hexan-1-al has been reported (Okada, 1969) as the major carbonyl compound found in the steam distillate of wheat gluten. Heptan-2-one associated with heated fats (Parks, 1967; Scanlan et al., 1968), may have originated from traces of residual fat present in the gluten. Pentan-2-one, hexan-1-al, pentan-2-ol, heptan-2-one, 2-*n*-pentylfuran, benzaldehyde, and furfuryl alcohol have been identified in white bread crust (Folkes and Gramshaw, 1981). Starch roasted at 230 °C has been reported (Shimizu et al., 1970) to yield a wide variety of compounds; however, in this investigation only a little 5-(hydroxymethyl)-2-furfural was detected when starch and gluten were together roasted at 150 °C. Starch is known to stabilize gluten to thermal stress greater than glucose (Ziderman and Friedman, 1985).

Gluten may possibly influence the generation of volatile compounds either through the effect of S–H and S–S groups present or through the formation of a glucose–gluten complex as shown by Hlynka and Bass (1949). Thiol groups are known to influence sugar degradation (Ingles, 1963). The degradation mechanism of a glucose–gluten complex could be different from that of a glucose–glutamic acid complex, thus giving different volatile products.

These observations suggest that gluten not only offers textural characteristics to the baked goods but also influences the course of the nonenzymic browning reaction occurring between sugars and amino acids during the baking process.

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Registry No. Glucose, 50-99-7; glutamic acid, 56-86-0; starch, 9005-25-8; pentan-2-one, 107-87-9; hexanal, 66-25-1; 1-pentanol, 71-41-0; heptan-2-one, 110-43-0; 2-*n*-pentylfuran, 3777-69-3; benzaldehyde, 100-52-7; furfuryl alcohol, 98-00-0; acetophenone, 98-86-2; acetaldehyde, 75-07-0; ethyl formate, 109-94-4; ethyl acetate, 141-78-6; methanol, 67-56-1; ethanol, 64-17-5; pyrazine, 290-37-9; 2-methyltetrahydrofuran-3-one, 3188-00-9; 2-methylpyrazine, 109-08-0; 3-hydroxybutan-2-one, 513-86-0; 1-hydroxypropan-2-one, 116-09-6; 2,5-dimethylpyrazine, 123-32-0; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-5-methylpyrazine, 13360-64-0; acetic acid, 64-19-7; furfural, 98-01-1; 2-acetylfuran, 1192-62-7; furfuryl acetate, 623-17-6; 5-methyl-2-furfural, 620-02-0; 5-methyl-2-acetylfuran, 1193-79-9; γ-butyrolactone, 96-48-0; 2-(5-methyl-2-furfuryl)furan, 13678-51-8; 4-methylbut-2-enoic acid γ-lactone, 591-11-7; 2-methyl-5-(5-methyl-2-furfuryl)furan, 13679-43-1; but-2-enoic acid γ-lactone, 497-23-4; 1-(2-furyl)propane-1,2-dione, 1438-92-2; 5-formyl-2-methylfuroate, 1125-59-3; decadien-2,4-al, 2363-88-4; hexanoic acid, 142-62-1; 1-oxo-3-methylcyclopent-2-en-2-ol, 80-71-7; 1-(5-methyl-2-furyl)propane-1,2-dione, 1197-20-2; 2-*n*-pentylfuroate, 116303-23-2; benzothiazole, 95-16-9; 2-acetylpyrrole, 1072-83-9; 2-(2-furyl)pyrazine, 32736-95-1; 2-formylpyrrole, 1003-29-8; γ-nonalactone, 104-61-0; 2-(2-furyl)-5-methylpyrazine, 27610-38-4; 2-(2-furyl)-6-methylpyrazine, 32737-03-4; 2-formyl-5-methylpyrrole, 1192-79-6; 5-[(formyloxy)methyl]-2-furfural, 102390-86-3; 5-(acetoxymethyl)-2-furfural, 10551-58-3; 2-formylbenzothiazole, 6639-57-2; 5-(2-furfuryl)-2-furfural, 33488-56-1; 5-(5-methyl-2-furfuryl)-2-furfural, 34995-74-9; 5-(hydroxymethyl)-2-furfural, 67-47-0.

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