

Effect of Temperature and pH on the Generation of Flavor Volatiles in Extrusion Cooking of Wheat Flour

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Extrusion temperature (120, 135, and 150 °C) and quantity of added sodium hydroxide (0, 3, and 6 g/kg feedstock) were used as variables to study flavor generation in extrusion cooking of wheat flour. In total, 127 volatile components were identified in the extrudates, of which 51 contained sulfur. The levels of pyrroles, thiophenes, thiophenones, thiapyrans, and thiazolines increased at higher extrusion temperatures, whereas furans and aldehydes decreased. The addition of sodium hydroxide also affected the formation of volatile compounds. However, thiophenes, thiophenones, polythiacycloalkanes, thiazoles, thiazolines, pyrroles, and some pyrazines tended to increase with the more alkaline extrusion conditions. Some compounds from lipid–Maillard interactions were identified in the extrudates. Analysis of the volatile components by gas chromatography–olfactometry showed sulfur- and nitrogen-sulfur-containing heterocycles as possible contributors to the sulfury and rubbery odors observed in extrudates produced at the higher temperature and more alkaline conditions.

KEYWORDS: Cereal; wheat flour; extrusion processing; aroma volatiles; Maillard reaction; lipid degradation

INTRODUCTION

Flavor generation in direct extrusion cooking of cereals involves a complex network of reactions involving the Maillard reaction and the thermal degradation of lipids and other components. These reactions occur also in traditionally baked cereals, but flavor generation in extrusion cooking takes place over a relatively short time (1). Several studies have addressed the control and generation of flavor in cereal extrusion, e.g., by modification of process conditions (2) and addition of precursors to the cereal feedstock (3–5). The subject has been reviewed by Riha and Ho (6).

Another way to manipulate flavor generation in cereal extrusion may be the modification of the feedstock pH. Several studies on Maillard reaction model systems have demonstrated that the pH of the reaction medium results in qualitative and quantitative changes in volatile and colored products (7–10). In extrusion cooking of starch–glucose–glycine feedstock, increasing the pH from 3 to 7 gave a higher degree of browning in the extrudate (11). In another study with starch–glucose–glycine extrusion systems, mainly nitrogen-containing volatile compounds were generated at a feed pH of 7.7. Much lower

amounts of volatile compounds were generated at feedstock pHs of 5.0 and lower (12).

The Maillard reaction and other reactions involving carbonyl-amino condensations require the presence of the free amino group. Thus, alkaline conditions will favor these reactions. As a consequence more nitrogen-containing volatiles are produced in Maillard reactions carried out at higher pH, and increased quantities of melanoidin pigments are also formed. Other reactions associated with the Maillard reaction may also be base-catalyzed. On the other hand, some sulfur-containing volatiles derived from cysteine degradation may be favored by lower pH (13, 14).

In a previous study, we showed how flavor generation in extrusion cooking of maize flour was affected by extrusion temperature and moisture content (15). Higher temperatures and lower moisture levels stimulated generation of volatile aroma compounds. Some sulfur- and nitrogen-containing heterocyclic compounds were considered to be important, especially for the aroma of these extrudates. This present study aimed to characterize the volatile components in wheat flour extrudates in relation to extrusion temperature and pH of the cereal feedstock.

EXPERIMENTAL PROCEDURES

Flour. The flour was milled from a mixed grist of English soft wheats (Smith Flour Milling, Worksop, U.K.). The flour had a moisture content of 12.5% w/w and contained 9.5% protein and 1.5% lipids. It had a particle size of <100 μm with 70% less than 70 μm and was

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Table 1. Extrusion Processing Parameters Used for the Wheat Flour Extrudates

sample code	temp ^a (°C)	NaOH (g/kg)	extrudate pH ^b	SME ^c (kJ/kg)	die pressure (Mpa)
120/0	123	0	6.8	707	3.6
120/3	114	3	8.0	512	3.8
120/6	117	6	8.8	592	3.5
135/0	132	0	6.8	603	3.4
135/3	136	3	8.1	571	3.3
135/6	141	6	8.8	549	3.1
150/0	152	0	6.8	538	3.1
150/3	152	3	8.1	537	3.1
150/6	152	6	8.8	529	3.0

^a Temperature was measured behind the die channel and in good contact with the melt fluid. Other extrusion variables were kept constant, i.e., moisture level of 18%, screw rotation at 350 rpm, and residence time of 35 ± 2 s at a feedrate of 800 g/min. ^b pH measured in the extrudate. Standard deviations less than 0.2 pH units. ^c Specific mechanical energy.

fortified with nutrients to levels required by U.K. regulations, i.e., 2.4 mg/kg thiamin, 16.5 mg/kg iron, 16 mg/kg niacin, and between 2.35 and 3.90 g/kg calcium carbonate.

Extrusion Processing. The wheat flour was processed using an APV Baker MPF 50D co-rotating twin-screw extruder. Nine extrusion conditions were selected using the temperature and sodium hydroxide addition as variables, each at three levels (**Table 1**). The screw configuration gave a residence time of 35 ± 2 s at a feed rate of 800 g/min, as described previously (2). Twin circular dies with a diameter of 4 mm were utilized. The screw rotation was held constant at 350 rpm. The mass temperature along the barrel was adjusted to provide a constant temperature along the screws. Motor torque and die pressure were kept constant within each run. Modifications to the feedstock pH were obtained by pumping aqueous solutions of sodium hydroxide into the extruder to give a continuous alkali addition of either 3 or 6 g/kg feed while maintaining a moisture level of 18%. Control extrudates with no addition of sodium hydroxide were also prepared. Three product temperatures for the extrusions were used: 120, 135, and 150 °C. Extrudates were cut at a high rate, collected, milled, packed into plastic polyamide-polythene laminated bags and stored at -20 °C until analysis. The aromas of the extrudates were described by six experienced assessors. The ground extrudates were completely wetted with water and presented at room temperature to the assessors in screw-capped amber glass bottles as described previously (2).

Extrudate pH Measurement. The pH of each extrudate was measured by soaking 5.0 g of sample in 20 mL of distilled water (pH 7.3). The suspension was left for 30 min and mixed just before inserting the pH electrode. The addition of sodium hydroxide to the extruder feedstock increased the extrudate pH from 6.8 (no addition) to 8.1 with the addition of 3 g/kg NaOH, and to 8.8 for the 6 g/kg addition (**Table 1**).

Dynamic Headspace Collection. Each extrudate (10 g) was placed in a 250-mL conical flask fitted with a Drechsel head. Distilled water (20 mL) was added, and the mixture was shaken until the water was completely absorbed. Oxygen-free nitrogen was passed over the sample for 1 h at a rate of 40 mL/min. The volatiles were swept onto a preconditioned glass-lined trap, packed with Tenax GC (Scientific Glass Engineering Ltd, Ringwood, Australia). Throughout the collection the sample was maintained at 37 °C. The trap was then connected directly to the nitrogen supply for 5 min to remove excess moisture. A 1,2-dichlorobenzene internal standard (1 µL) in redistilled diethyl ether (130 mg/L) was then injected onto the trap, which was maintained under slight vacuum.

Separation and Identification of Volatiles. A Hewlett-Packard 5890/5988A GC-MS system equipped with a modified Unijector (SGE Ltd.) connected to a 50 m × 0.32 mm i.d. fused silica capillary column, coated with BPX-5 (SGE Ltd.) at 0.5 µm film thickness, was used to analyze the volatile compounds collected on the trap. The compounds were thermally desorbed at 250 °C in the Unijector and cryofocused on the GC column while the oven was held at 0 °C for 5 min. The

oven temperature was then raised to 60 °C over 1 min and held for 5 min, before programming to 250 °C at a rate of 4 °C/min and a final hold for 20 min. Mass spectra were recorded in the electron impact mode at an ionization voltage of 70 eV and a source temperature of 200 °C. A scan range of 29–400 amu with a scan time of 0.69 s was used. Each extrudate was analyzed in duplicate.

Identifications were made by comparison of mass spectra with spectra from authentic compounds, those in reference collections, or other published spectra. Linear retention indices (LRI) for temperature-programmed GC were calculated using a homologous series of C₆ to C₂₂ *n*-alkanes. Mass spectral identifications were confirmed by comparison of the LRI with those from authentic compounds run under similar GC-MS conditions. Where reference spectra were not available, identifications were considered tentative.

The approximate quantity of each compound was estimated by comparing the peak area in the total ion chromatogram with that of the internal standard, using a response factor of 1.

Gas Chromatography-Olfactometry. Volatile compounds collected as described above were thermally desorbed under similar GC conditions on a HP 5890 GC in which the column effluent was split equally between a flame ionization detector and an odor ("sniffing") port. The odors from headspace volatile collections from extrudates were evaluated (6 replicates of each) by a single trained assessor. As compounds eluted from the GC column the odor descriptions, retention time, and duration of the odor were recorded. Another assessor was used to confirm important odors in the GC effluent.

Data Analysis. The effects of extrusion cooking on the generation of volatile compounds (**Table 2**) were summarized by principal component analysis (PCA) using the Unscrambler software (CAMO ASA, Trondheim, Norway). In the PCA, quantities for each compound were weighted according to their standard deviation for the samples. Full cross-validation was used as validation method and Jack-knifing was used to remove outliers.

RESULTS AND DISCUSSION

A total of 127 volatile components was identified in the nine wheat flour extrudates (**Table 2**). The majority (82) of these compounds were heterocyclic, 51 contained sulfur, and eleven of these contained sulfur and nitrogen. Twenty compounds had only nitrogen as the heteroatom.

Volatile compounds not reported previously in cooked cereals included 2-ethyl-(2*H*)-thiapyran and 2-butyl-(2*H*)-thiapyran. Several 2-alkyl-(2*H*)-thiapyrans, including the ethyl and butyl homologues, have recently been reported in the volatiles of cooked lamb and beef, and their identities were confirmed by synthesis (16). The most likely route for the formation of these compounds is the reaction of 2,4-alkadienals, from lipid oxidation, with hydrogen sulfide from the degradation of cysteine. Alkylthiophenes, such as 2-propyl-, 2-butyl-, and 2-pentylthiophene can also be formed in such reactions and were also present in the headspace volatiles of the extruded wheat.

Four alkylthiazolines were tentatively identified in the wheat extrudates, including 4-ethyl-2-methyl-3-thiazoline which has not been reported previously as a food volatile. It was tentatively identified by comparison of its mass spectrum with those of related methyl-substituted thiazolines (**Figure 1**). The base peak results from the cleavage of the ring in the 2,3 and 4,5 positions giving a C₄H₈S⁺ ion (*m/z* 88) from a 5-methylthiazoline, but C₃H₆S⁺ (*m/z* 74) from a thiazoline with no substituent in the 5-position. Another major fragment corresponds to the loss of the methyl, or other alkyl group, from the 2-position, resulting in *m/z* 114 from 4-ethyl-2-methyl-3-thiazoline. Fission of the bonds adjacent to the double bond results in fragment *m/z* 82 or 68, depending whether the 4-position on the ring contains ethyl or methyl. The mass spectrum of the compound tentatively identified as 4-ethyl-2-methyl-3-thiazoline agreed with this fragmentation pattern (**Figure 1**).

Table 2. Volatile Compounds Identified in Extruded Wheat Flour Processed under Different Temperatures and Sodium Hydroxide Additions

no. ^a	compound	approximate quantity (ng in headspace from 10 g extrudate) ^b									identification ^c	LRI ^d
		120/0 ^e pH 6.8	120/3 pH 8.0	120/6 pH 8.8	135/0 pH 6.8	135/3 pH 8.1	135/6 pH 8.8	150/0 pH 6.8	150/3 pH 8.1	150/6 pH 8.8		
hydrocarbons												
1	hexane	-	7	-	-	-	-	4	-	-	MS + LRI	600
2	toluene	1	1	1	1	2	2	1	1	1	MS + LRI	773
3	octane	-	-	-	1	-	4	2	2	1	MS + LRI	800
4	ethylbenzene	1	2	1	6	3	3	1	1	1	MS + LRI	872
5	a xylene	1	1	1	-	-	2	5	-	4	ms	881
6	styrene	-	2	1	-	3	4	2	5	5	MS (26)	907
7	an alkylbenzene (mw 120)	3	3	2	2	2	5	-	-	-	ms	1008
8	limonene	9	6	9	6	8	5	5	8	7	MS + LRI	1039
9	undecane	1	-	-	-	-	-	-	-	-	MS + LRI	1100
10	naphthalene	2	-	1	3	5	4	7	4	4	MS + LRI	1218
11	tridecane	-	-	-	3	-	-	-	-	-	MS + LRI	1300
12	tetradecane	1	2	tr	2	-	-	-	-	-	MS + LRI	1400
13	pentadecane	1	1	-	1	1	1	1	tr	-	MS + LRI	1500
14	hexadecane	1	1	1	1	1	-	1	-	1	MS + LRI	1600
15	heptadecane	1	1	1	1	1	-	1	-	-	MS + LRI	1700
alcohols												
16	1-pentanol	4	5	9	4	6	8	6	8	5	MS (26)	783
17	1-hexanol	52	67	65	89	63	74	50	61	51	MS + LRI	885
18	1-octen-3-ol	13	16	13	17	13	13	12	13	12	MS + LRI	993
19	2-methoxy-4-vinylphenol	1	4	2	12	9	9	12	19	25	MS + LRI	1345
aldehydes												
20	3-methylbutanal	5	3	8	1	1	2	1	1	-	MS + LRI	663
21	2-methylbutanal	2	2	7	3	4	5	-	3	-	MS + LRI	668
22	pentanal	1	-	8	-	2	2	1	-	-	MS + LRI	698
23	hexanal	340	370	570	450	280	320	200	290	160	MS + LRI	815
24	heptanal	31	40	32	26	23	21	11	17	16	MS + LRI	915
25	(E)-2-heptenal	2	4	6	-	-	-	-	-	-	MS + LRI	975
26	benzaldehyde	47	66	45	71	40	37	32	32	32	MS + LRI	988
27	octanal	8	17	13	9	5	11	-	8	-	MS + LRI	1016
28	2-ethyl-2-hexenal (E or Z)	5	8	7	6	9	8	4	12	20	MS (26)	1021
29	2-phenylethanal	14	20	15	-	-	-	-	-	-	MS + LRI	1071
30	(E)-2-octenal	6	7	6	2	3	2	3	2	2	MS + LRI	1076
31	nonanal	13	22	14	6	6	-	-	-	-	MS + LRI	1118
32	(E)-2-nonenal	1	2	1	-	-	-	-	-	-	MS + LRI	1177
33	decanal	9	16	11	-	5	-	2	8	-	MS + LRI	1219
34	undecanal	-	1	-	-	-	-	-	-	-	MS + LRI	1320
35	(E,E)-2,4-decadienal	2	-	-	-	-	-	-	-	-	MS + LRI	1342
36	2-butyl-2-octenal (E or Z)	11	11	10	16	10	9	9	8	10	MS (26)	1385
37	dodecanal	1	1	-	-	-	-	-	-	-	MS + LRI	1422
ketones												
38	2,3-pentanedione	-	2	-	-	-	-	-	2	-	MS + LRI	691
39	2-hexanone	1	2	1	3	5	6	3	6	3	MS + LRI	802
40	2-heptanone	69	110	83	150	130	130	82	97	96	MS + LRI	902
41	1-octen-3-one	5	8	4	3	8	13	-	7	2	MS + LRI	986
42	2-octanone	2	5	3	5	6	7	5	7	6	MS + LRI	1002
43	6-methyl-5-hepten-2-one	1	2	1	-	-	-	-	-	-	MS + LRI	1003
44	2-nonanone	1	2	1	1	3	2	1	2	2	MS + LRI	1103
45	geranyl acetone	1	1	1	1	1	-	tr	1	1	MS (26)	1464
furans												
46	2-ethylfuran	-	-	1	-	-	-	-	-	-	MS + LRI	691
47	2-propylfuran	2	3	3	-	2	-	-	1	-	MS + LRI	798
48	3-furfural	1	-	-	-	-	-	-	-	-	MS (27)	837
49	2-furfural	5	8	8	2	4	4	1	5	3	MS + LRI	855
50	2-furanmethanol	1	1	1	-	4	2	-	9	5	MS + LRI	880
51	2-methyl-5-propylfuran	2	2	3	3	3	6	2	3	2	MS (27)	891
52	2-butylfuran	16	25	19	13	10	9	6	8	6	MS + LRI	89
53	2-pentylfuran	130	170	120	90	79	58	31	31	31	MS + LRI	998
54	2-(1-pentenyl)furan (E or Z)	-	2	-	-	-	-	-	-	-	MS (28)	1009
nitrogen-containing compounds												
55	1H-pyrrole	-	-	-	-	1	-	1	2	-	MS + LRI	774
56	1-ethyl-1H-pyrrole	-	-	-	-	-	-	1	2	1	MS (26)	827
57	3-methyl-1H-pyrrole	-	-	-	-	3	1	4	7	7	MS (26)	860
58	2-methyl-1H-pyrrole	-	-	-	-	1	2	1	2	1	MS (26)	866
59	2,3-dihydro-1H-indole	-	-	-	-	6	4	5	8	7	MS (26)	1140
60	2-pentyl-3,5-dibutylpyridine	-	-	-	-	-	-	-	-	1	MS (29)	1919
61	pyrazine	-	1	3	4	8	7	2	3	2	MS + LRI	740
62	methylpyrazine	8	15	9	22	27	26	9	16	14	MS + LRI	843
63	2,5-dimethylpyrazine	16	27	28	51	40	32	16	25	29	MS + LRI	932
64	ethylpyrazine	-	5	2	-	-	4	18	21	34	MS + LRI	934

Table 2 (Continued)

no. ^a	compound	approximate quantity (ng in headspace from 10 g extrudate) ^b									identification ^c	LRI ^d
		120/0 ^e pH 6.8	120/3 pH 8.0	120/6 pH 8.8	135/0 pH 6.8	135/3 pH 8.1	135/6 pH 8.8	150/0 pH 6.8	150/3 pH 8.1	150/6 pH 8.8		
65	2,3-dimethylpyrazine	-	2	2	1	4	2	-	-	2	MS + LRI	939
66	vinylpyrazine	-	-	-	-	-	1	-	-	1	MS (26)	954
67	2-ethyl-6-methylpyrazine	-	-	-	9	8	-	6	-	-	MS + LRI	1016
68	2-ethyl-5-methylpyrazine	6	8	7	14	9	8	4	12	5	MS + LRI	1021
69	2-methyl-6-vinylpyrazine	-	1	1	-	-	-	-	-	-	MS (26)	1043
70	2-ethyl-3,5-dimethylpyrazine	16	25	20	33	25	21	12	17	15	MS + LRI	1093
71	2-ethyl-3,6-dimethylpyrazine	-	-	-	-	-	-	2	2	2	MS + LRI	1099
72	2,3-diethyl-5-methylpyrazine	-	3	3	-	-	1	-	-	-	MS + LRI	1170
73	pentylpyrazine	1	2	2	3	6	5	-	-	1	MS + LRI	1234
sulfur-containing compounds												
74	3-mercapto-2-butanone	-	1	1	-	-	-	-	-	-	MS + LRI	837
75	1-methylthio-2-propanone	-	-	-	-	-	1	-	1	-	MS (30)	863
76	dimethyl trisulfide	2	-	1	-	-	-	-	-	-	MS (26)	992
77	2-furanmethanethiol	-	-	2	-	-	-	-	-	-	MS + LRI	928
78	2-methyl-3-(methylthio)furan	-	-	-	-	1	1	-	1	1	MS (37)	963
79	2,3-dihydro-6-methylthieno[2,3-c]furan	-	-	1	-	5	4	-	1	4	MS + LRI	1218
80	2-methylthiophene	-	-	3	-	1	1	-	-	-	MS + LRI	780
81	4,5-dihydro-2-methylthiophene	-	-	-	-	1	1	1	-	-	MS (28)	857
82	2-ethylthiophene	-	-	-	-	6	-	-	-	-	MS + LRI	880
83	3,4-dimethylthiophene	-	-	-	-	3	-	-	3	2	MS (26)	890
84	2-vinylthiophene	-	-	-	-	-	-	-	3	3	MS (28)	922
85	2-propylthiophene	-	-	-	-	1	-	-	-	-	MS + LRI	969
86	3-formylthiophene	-	2	3	8	10	11	14	19	21	MS + LRI	1025
87	2-formylthiophene	2	4	3	8	11	12	10	12	13	MS + LRI	1033
88	2-butylthiophene	-	-	-	-	3	-	-	-	-	MS + LRI	1072
89	2-formyl-5-methylthiophene	-	-	-	2	10	17	16	27	26	MS + LRI	1116
90	2-acetylthiophene	-	1	1	5	8	8	7	9	10	MS + LRI	1121
91	2-formyl-3-methylthiophene	-	-	3	3	11	4	8	13	12	MS + LRI	1126
92	a formylmethylthiophene	-	-	-	-	2	2	1	2	2	ms	1152
93	a formylmethylthiophene	1	-	-	-	-	2	1	2	3	ms	1155
94	2-pentylthiophene	2	4	2	2	-	-	-	-	-	MS + LRI	1176
95	an ethylformylthiophene	-	-	-	-	-	-	-	2	2	MS + LRI	1195
96	3-ethyl-2-formylthiophene	-	-	-	-	1	-	3	6	7	MS + LRI	1224
97	2-acetyl-4-methylthiophene	-	-	-	-	2	-	-	1	2	MS + LRI	1240
98	a thienothiophene	-	-	-	-	-	-	-	2	3	ms	1287
99	a dihydrothienothiophene	-	-	-	-	2	3	1	3	3	ms	1350
100	2-pentyl-3-formylthiophene	-	-	-	-	-	-	-	2	1	MS (32)	1489
101	4,5-dihydro-3(2H)-thiophenone	-	-	-	1	3	2	1	2	2	MS + LRI	983
102	4,5-dihydro-2-methyl-3(2H)-thiophenone	1	4	3	15	34	35	36	64	77	MS + LRI	1012
103	4,5-dihydro-5-methyl-3(2H)-thiophenone	-	-	-	-	-	-	3	-	-	MS (26)	1015
104	4,5-dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	-	-	-	-	-	-	-	-	1	MS (33)	1045
105	2-ethyl-(2H)-thiapyran	-	3	1	2	5	3	4	5	4	MS + LRI (76)	1028
106	2-butyl-(2H)-thiapyran	-	-	-	-	2	1	-	-	1	MS + LRI (76)	1231
107	2-pentyl-(2H)-thiapyran	-	1	1	2	3	3	3	3	2	MS + LRI (76)	1337
108	3-methyl-1,2-dithiolan-4-one	3	7	5	6	7	7	-	1	-	MS + LRI	1106
109	3-methyl-1,2,4-trithiolane	-	-	-	2	7	5	2	4	3	MS (37)	1158
110	3,5-dimethyl-1,2,4-trithiolane (E or Z)	-	-	-	3	11	9	5	7	5	MS + LRI	1169
111	3,5-dimethyl-1,2,4-trithiolane (E or Z)	-	-	-	-	11	9	-	-	4	MS + LRI	1177
112	1,2-dithian-4-one	1	2	2	-	2	-	-	-	-	MS (33)	1215
113	6-methyl-1,2-dithian-4-one	tr	1	1	5	8	6	3	4	3	ms	1251
114	3-methyl-1,2,4-trithiane	-	-	-	-	4	2	1	3	3	MS + LRI	1290
115	4-methylthiazole	-	1	1	-	2	2	-	1	tr	MS + LRI	836
116	2,5-dimethylthiazole	-	-	-	-	2	-	-	18	-	MS + LRI	934
117	4,5-dimethylthiazole	-	-	-	-	2	-	-	-	-	MS + LRI	951
118	2-acetylthiazole	2	4	4	9	11	8	4	5	4	MS + LRI	1046
119	2,5-dimethyl-4-ethylthiazole	-	-	-	-	-	-	2	3	2	MS + LRI	1077
120	an alkylthiazole (mw141)	-	-	-	-	-	-	-	5	3	ms	1134
121	2-acetyl-4-methylthiazole	-	3	2	3	8	6	-	2	1	MS (34)	1136
122	2-methyl-2-thiazoline	-	-	-	-	2	1	-	1	1	MS (26)	963
123	2,4-dimethyl-3-thiazoline	-	-	-	23	52	47	27	43	47	MS (35)	975
124	2,4,5-trimethyl-3-thiazoline	-	-	-	-	6	2	7	11	10	MS (35)	1005
125	4-ethyl-2-methyl-3-thiazoline	-	-	-	2	9	8	7	11	11	ms	1061
74, 129 (M ⁺ , 82), 114 (78), 82 (23), 68 (20), 83 (17), 56 (15), 96 (12), 59 (11), 41 (10), 86 (10), 45 (9), 46 (8), 53 (7), 55 (7), 85 (7), 87 (7), 42 (6), 54 (6), 47 (5), 71 (5), 99 (5), 116 (5), 130 (5)												

Table 2 (Continued)

no. ^a	compound	approximate quantity (ng in headspace from 10 g extrudate) ^b								identification ^c	LRI ^d	
		120/0 ^e pH 6.8	120/3 pH 8.0	120/6 pH 8.8	135/0 pH 6.8	135/3 pH 8.1	135/6 pH 8.8	150/0 pH 6.8	150/3 pH 8.1			150/6 pH 8.8
		miscellaneous										
126	acetic acid	-	3	-	-	3	1	-	-	4	MS + LRI	682
127	1-formyl-5-ethylcyclopentene	14	18	13	9	7	6	7	7	7	MS (31)	1053

^a Compounds with numbers shown in bold letter type were used in the principal component analysis (PCA). ^b Estimated quantity (ng) in headspace of 10 g of extrudate expressed as the peak area relative to that of 130 ng 1,2-dichlorobenzene internal standard; values are the mean of duplicate analyses; tr, <0.5 ng; -, not detected above detection limit of approximately 0.1 ng. ^c MS + LRI, mass spectrum and LRI agree with the authentic compound analyzed under similar GC-MS conditions; MS, tentative identification by comparison with mass spectrum reported in the literature; ms, tentative identification by interpretation of mass spectrum. ^d Linear retention index on BPX-5 column. ^e Extrusion variables of temperature (°C) and added sodium hydroxide (g/kg).

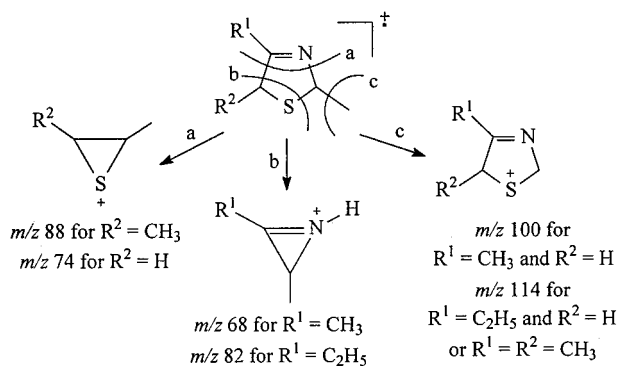


Figure 1. Suggested fragmentation of 2,4-dimethyl-, 2,4,5-trimethyl-, and 4-ethyl-2-methyl-3-thiazoline explaining the major mass spectral ions.

Thiazolines have not been reported previously in extruded cereals, but were found in relatively high quantities in the 135 and 150 °C extruded samples, with 2,4-dimethyl-3-thiazoline present in the highest concentration. No thiazolines could be detected in the extrudates from the lowest extrusion temperature. Several 3-thiazolines have been reported previously in yeast extracts, including the unstable 5-hydroxy-substituted species (17). Recently, several alkyl-3-thiazolines, with long-chain alkyl substituents in the 2-position, have been identified in cooked beef and lamb (18, 19). In a dry, heated (180 °C) cysteine model system thiazolidines and 2-alkylthiazolines were identified (20). It has been shown that 3-thiazolines can be formed by reacting α -dicarbonyls, such as butanedione, or hydroxyketones with hydrogen sulfide and ammonia in the presence of an aliphatic aldehyde (21, 22). This is the most likely route to the thiazolines in the extruded cereal, where the degradation of cysteine provided acetaldehyde, hydrogen sulfide, and ammonia.

The extrudates contained relatively high quantities of lipid degradation products, including aldehydes, ketones, furans, and alcohols. In other work in our laboratory, we have noted that the quantities of volatiles from lipid oxidation varied with the age of the flour (5). Although no rancid off-odor was noticeable in any of the wheat flours prior to extrusion, extrudates from flours which had been stored for longer periods showed higher quantities of lipid degradation products. Higher levels of free fatty acids from residual lipase activity may explain the higher content of lipid oxidation products in such extrudates. In a recent study of oat flour extrusion, some samples had residual lipase activity resulting in higher levels of free fatty acids. Extrudates from such oat flour contained significantly higher quantities of lipid-derived volatiles (23).

Other volatile compounds present in relatively high amounts were thiophenones, pyrazines, and thiophenes. 2-Pentyl-3,5-dibutylpyridine, pentylpyrazine, 2-pentylthiophene, some tri-thiolanes, and several thiophenes have not been previously

identified in extruded wheat. The heterocycles exhibiting long-chain aliphatic substituents were likely to be products from the interaction between Maillard-derived products and aldehydes from lipids (24). The dithiolanones and trithiolanes were probably formed by the reaction of cysteine with reducing sugars, and mechanisms for their formation have been published recently (13, 14).

Temperature and pH Effects. The effects of extrusion temperature and sodium hydroxide addition on the generation of volatiles were summarized by PCA. Thirty-six compounds did not explain systematic variation and were removed in the analysis (Table 2). A PCA model with 3 significant components was obtained which explained 84% (58% after validation) of the total variance. The third PC explained 4% of the validated variance and was not used further in the interpretation of the results.

Principal component (PC) 1 described mainly a temperature effect, separating the low temperature (120 °C) from the other extrudates (Figure 2a). In addition, PC1 showed the differences in the extrudates (135 and 150 °C) obtained by addition of sodium hydroxide. The second PC separated the 135 and 150 °C extrudates, but did not exhibit a strong pH effect. Overall, the largest variation in the data set could be attributed to the extrusion temperature.

The correlation loadings for the volatile compounds with the PCs showed a group of lipid-derived furans together with aldehydes from lipids and Strecker degradation that were negatively correlated with PC1. These compounds were present in higher amounts in the 120 °C extrudates than in the higher temperature extrudates. The decrease of aldehydes and aldehyde-derived furans in the higher temperature extrudates could have been due to the further interaction of aldehydes with other compounds formed during the extrusion, such as products of the Maillard reaction. This effect was also observed in previous studies on maize flour and oat flour extrudates (15, 23). Lipid-derived alcohols, ketones, and *n*-alkanes were much less affected by the extrusion temperature.

Compounds which correlated positively with PC1 included several thiophenes, thiophenones, thiazolines, thiapyrans, polythiacycloalkanes, and sulfur-containing furans. These compounds were generated in higher quantities in the 135 and 150 °C extrudates, especially when alkali was added to the extrusions. Two recent papers examined the formation of sulfur-containing volatiles in reaction systems containing 4-hydroxy-5-methyl-3(2H)-furanone (HMF) and cysteine or hydrogen sulfide at pHs 4.5 and 6.5 (13, 14). The pH had a significant effect on the profile of sulfur compounds produced. HMF is an intermediate in Maillard reactions involving pentoses, and a similar effect of pH had been shown earlier with model systems containing cysteine and ribose (10). At pH 4.5 thiols and sulfides

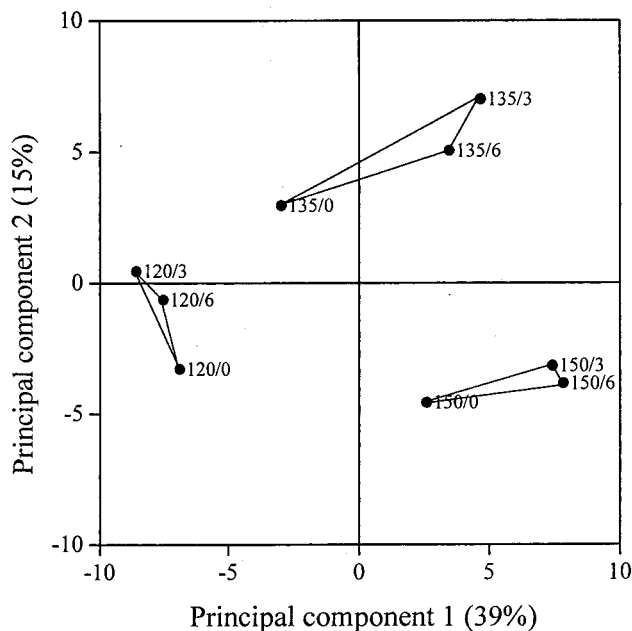


Figure 2. Scores and correlation loadings plots from PCA of selected volatiles in the extrudates. The concentric circle in the loadings plot represents 50% explained variance, and the outer circle represents the maximum explained variance for the variables.

were dominant, thiophenes were formed at both pHs, whereas thiophenones were produced in larger quantities at pH 6.5, and trithiolanes and dithianes were found only at pH 6.5. These results follow the same trend as those for the extrudates and clearly demonstrate an effect of pH on sulfur-containing volatiles formed in the Maillard reaction.

The quantities of thiazoles and thiazolines increased with addition of sodium hydroxide, but there was little difference between the 3 and 6 mg/kg additions. The increased availability of free ammonia or NH_2 groups at higher pHs is the most likely explanation, and in the HMF–cysteine model systems discussed above, thiazoles and pyrazines were formed only at the higher pH. The loadings of most pyrazines, acetylthiazoles, and 4-methylthiazole, as well as thianones and thiolanes, showed a positive correlation with PC2. These compounds were predominantly generated at 135 °C, especially when sodium hydroxide was added. However, 2-ethyl-3,6-dimethylpyrazine, ethylpyrazine, and alkylpyrroles were negatively correlated with PC2. Formation of these compounds required the highest extrusion temperature, which was also found in previous studies (15). The addition of sodium hydroxide in the extrusions of wheat tended to increase the numbers of alkylpyrazines. However, relatively small increases in the concentrations of pyrazines were observed. In model systems containing glycine (9) or cysteine (25) and a reducing sugar, the formation of pyrazines has been shown to increase under alkaline conditions. The optimal pH for alkylpyrazines in these model studies varied with the nature of the alkyl substituents. In general, the greatest numbers and quantities were formed at pH 9 or slightly higher. Such a pH effect on pyrazine formation was not obvious in this study. However, in the heating of a food system a network of reactions occurs involving amino acids, sugars, lipids, thiamin, and other components. Competition between the reactions for available reactants and interactions between the various products of the reactions can affect the volatile profile. Such interactions were observed recently in the extrusion cooking of oat flour, where levels of pyrazines decreased as the lipid oxidation products increased (23).

Odor of Extrudate Components. Odor assessment of the extrudates, mixed with water, was made by six experienced

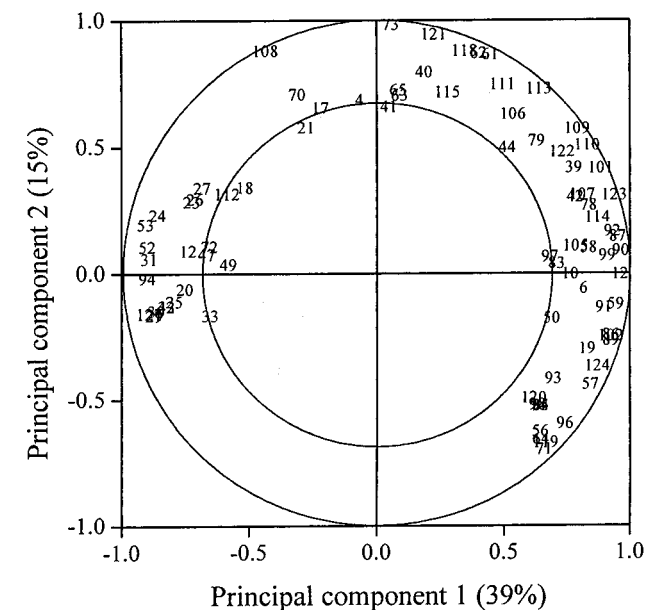


Table 3. Odor Descriptions of Extruded Wheat Processed under Different Temperatures and Levels of Sodium Hydroxide Addition

product temp	NaOH (g/kg)	pH	odor description ^a
120 °C	0	6.8	cooked milk, honey-like, biscuit-like, bread, stale, wet wheat flakes-like, toasted, puffed wheat
135 °C	0	6.8	cooked milk, meaty, porridge-like, popcorn, baby cereal-like, bran-like, toasted, rubbery, pungent
150 °C	0	6.8	bread, wheaty, porridge-like, milky, rancid, popcorn, bran-like, toasted, puffed wheat, rubbery
120 °C	3	8.0	wheaty, bread, oats, porridge-like, popcorn, toasted, puffed wheat, burnt, rubbery
135 °C	3	8.1	wheaty, bread, woody, bran-like, toasted, puffed wheat, rubbery, sulfury
150 °C	3	8.1	wheaty, bread, creamy, roast pork, puffed wheat, burnt, rubbery, sulfury
120 °C	6	8.8	bland, cooked milk, stale popcorn, onion-like, nutty rubbery, pungent
135 °C	6	8.8	onion, meaty, eggy, like burnt matches, toasted, rubbery, sulfury
150 °C	6	8.8	biscuit-like, sweet, woody, vegetable, earthy, crackly pork, sugar puffs, rubbery, sulfury, pungent

^a Descriptive terms generated by six assessors to indicate the odors from the extrudates.

assessors (Table 3). This indicated that strong burnt, sulfury odors characterized the most alkaline samples. The intermediate 3 g/kg addition of sodium hydroxide gave extrudates with more pronounced wheat-like and puffed wheat odor notes. Such terms were generally weaker in the extrudates processed without addition of sodium hydroxide, where cooked milk notes dominated. Increasing the extruder feedstock pH stimulated the burnt, sulfury odors, which were generated at lower temperature than in the absence of sodium hydroxide. These findings corresponded with the higher levels of sulfur compounds in the extrudates from more alkaline extrusion processing.

Table 4. Odor-Active Regions in GCO Analyses that Coincide with Components in the Headspace from Extruded Wheat (temperature, 150 °C; moisture, 18%; and sodium hydroxide, 6 g/kg feedstock)

LRI interval	number of odor responses ^a	odor descriptors	coinciding compound
657–670	5	cheesy, malty	2- and/or 3-methylbutanal
715–725	5	sulfury, garlic-like, cabbage-like	dimethyl disulfide ^b
810–822	6	grassy, green	hexanal
879–888	4	caramel, woody, toasted, sweet	2-furanmethanol
890–896	3	garlic-like, burnt	3,4-dimethylthiophene
922–926	4	stale, burnt, green	2-vinylthiophene
927–931	3	sulfury, burnt	2-furanmethanethiol
932–938	6	mushroom, savory	unknown
939–950	6	biscuit-like, toasted, popcorn	2-acetyl-1-pyrroline ^b
978–984	3	fruity, woody, vegetable	unknown
988–994	6	sulfury, garlic-like	dimethyl trisulfide
994–1000	5	mushroom-like	1-octen-3-ol
1020–1028	3	fruity, medicinal	2-ethyl-2-hexenal
1047–1056	6	biscuit-like, toasted, burnt bread	2-acetylthiazole
1078–1088	5	garlic-like, sulfury, onion-like	2,5-dimethyl-4-ethylthiazole
1105–1119	6	biscuit-like, breadly, sharp	unknown
1120–1142	5	biscuit-like, toasted, sweet, woody	unknown

^a Number of replicates from a total of 6 in which the odors were detected.

^b Tentative assignment based on odor character and LRI of the authentic compound.

In GCO evaluation of the extrudate from the most severe treatment (150 °C, 6 g/kg sodium hydroxide) 17 odor-active regions were identified (Table 4). Volatile compounds that eluted in the vicinity of odors were 2-acetylthiazole, 2,5-dimethyl-4-ethylthiazole, 2-vinylthiophene, 3,4-dimethylthiophene, 2-furanmethanethiol, dimethyl trisulfide, 2-furanmethanol, 2 and 3-methylbutanal, hexanal, 2-ethyl-2-hexenal, and 1-octen-3-ol. In addition, odors which had the retention time and odor characters associated with 2-acetyl-1-pyrroline and dimethyl disulfide were found in the GCO. These compounds have low odor threshold values and have been previously identified in cereal extrudates (15). No odors could be related to the three thiapyrans and other long alkyl-substituted heterocyclic compounds from Maillard–lipid interactions. These compounds, therefore, may not be directly involved in the extrudate aroma. However, they may modify the cereal flavor by removing precursors that otherwise could generate other flavor components.

LITERATURE CITED

- Palkert, P. E.; Fagerston, I. S. Determination of flavor retention in pre-extrusion flavored textured soy protein. *J. Food Sci.* **1980**, *45*, 526–533.
- Bredie, W. L. P.; Mottram, D. S.; Hassell, G. M.; Guy, R. C. E. Sensory characterisation of the aromas generated in extruded maize and wheat flour. *J. Cereal Sci.* **1998**, *28*, 97–106.
- Ho, C.-T.; Bruechert, L. J.; Kuo, M. C.; Izzo, M. T. Formation of volatile compounds from extruded corn-based model systems. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; American Chemical Society: Washington, DC, 1989; pp 504–511.
- Izzo, H. V.; Ho, C.-T. Ammonia affects Maillard chemistry of an extruded autolyzed yeast extract: pyrazine aroma generation and brown color formation. *J. Food Sci.* **1992**, *57*, 657–659.
- Bredie, W. L. P. Flavour Generation in Extruded Cereals in Relation to Feedstocks and Cooking Conditions. Ph.D. Thesis, The University of Reading, U. K., 1996.
- Riha, W. E.; Ho, C.-T. Formation of flavors during extrusion cooking. *Food Rev. Int.* **1996**, *12*, 351–373.
- Shibamoto, T.; Bernhard, R. A. Investigation of pyrazine formation pathways in sugar–ammonia model systems. *J. Agric. Food Chem.* **1977**, *25*, 609–614.
- Shu, C.-K.; Ho, C.-T. Effect of pH on the volatile formation from the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Agric. Food Chem.* **1988**, *36*, 801–803.
- Bemis-Young, G. L.; Huang, J.; Bernhard, R. A. Effect of pH on pyrazine formation in glucose–glycine model systems. *Food Chem.* **1993**, *46*, 383–387.
- Meynier, A.; Mottram, D. S. The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chem.* **1995**, *52*, 361–366.
- Bates, L.; Ames, J. M.; MacDougall, D. B. The development and control of colour in extrusion cooked foods simulated using a model reaction cell. In *Developments in Food Engineering*; Yano, T., Matsumo, R., Nakamura, K., Eds.; Blackie: London, 1994; pp 876–878.
- Ames, J. M.; Defaye, A. B.; Bates, L. The effect of pH on the volatiles formed in an extruded starch–glucose–glycine model system. *Food Chem.* **1997**, *58*, 323–327.
- Whitfield, F. B.; Mottram, D. S. Investigation of the reaction between 4-hydroxy-5-methyl-3(2H)-furanone and cysteine or hydrogen sulfide at pH 4.5. *J. Agric. Food Chem.* **1999**, *47*, 1626–1634.
- Whitfield, F. B.; Mottram, D. S. Heterocyclic volatiles formed by heating cysteine or hydrogen sulfide with 4-hydroxy-5-methyl-3(2H)-furanone at pH 6.5. *J. Agric. Food Chem.* **2001**, *49*, 816–822.
- Bredie, W. L. P.; Mottram, D. S.; Guy, R. C. E. Aroma volatiles generated during the extrusion cooking of maize flour. *J. Agric. Food Chem.* **1998**, *46*, 1479–1487.
- Elmore, J. S.; Mottram, D. S. Formation of 2-alkyl-(2H)-thiapyrans and 2-alkylthiophenes in cooked beef and lamb. *J. Agric. Food Chem.* **2000**, *48*, 2420–2424.
- Werkhoff, P.; Bretschneider, W.; Emberger, R.; Güntert, M.; Hopp, R.; Köpsel, M. Recent developments in the sulfur flavor chemistry of yeast extracts. *Chem. Mikrobiol. Technol. Lebensm.* **1991**, *13*, 30–57.
- Elmore, J. S.; Mottram, D. S.; Enser, M.; Wood, J. D. Novel thiazoles and 3-thiazolines in cooked beef aroma. *J. Agric. Food Chem.* **1997**, *45*, 3603–3607.
- Elmore, J. S.; Mottram, D. S.; Enser, M.; Wood, J. D. The effects of diet and breed on the volatile compounds of cooked lamb. *Meat Sci.* **2000**, *55*, 149–159.
- Umamo, K.; Hagi, Y.; Nakahara, K.; Shyoji, A.; Shibamoto, T. Volatile chemicals formed in the headspace of a heated D-glucose/L-cysteine Maillard model system. *J. Agric. Food Chem.* **1995**, *43*, 2212–2218.
- Takken, H. J.; van der Linde, L. M.; de Valois, P. J.; van Dort, H. M.; Boelens, M. Reaction products of α -dicarbonyl compounds, aldehydes, hydrogen sulfide and ammonia. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; American Chemical Society: Washington, DC, 1976; pp 114–121.
- Elmore, J. S.; Mottram, D. S. Investigation of the reaction between ammonium sulfide, aldehydes and α -hydroxyketones or α -dicarbonyls to form some lipid–Maillard interaction products found in cooked beef. *J. Agric. Food Chem.* **1997**, *45*, 3595–3602.
- Parker, J. K.; Hassell, G. M. E.; Mottram, D. S.; Guy, R. C. E. Sensory and instrumental analysis of volatiles generated during extrusion cooking of oat flours. *J. Agric. Food Chem.* **2000**, *48*, 3497–3506.
- Whitfield, F. B. Volatiles from interactions of Maillard reactions and lipids. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1–58.
- Yeo, H. C. H.; Shibamoto, T. Microwave-induced volatiles of the Maillard model system under different pH conditions. *J. Agric. Food Chem.* **1991**, *39*, 370–373.

- (26) NIST/EPA/MSDC *Mass Spectral Database (Versions for PC and for Mass Spectrometer Data Systems)*. Office of Standard Reference Data of the National Institute of Standards and Technology: Gaithersburg, MD, 1992.
- (27) Heyns, K.; Stute, R.; Scharmann, H. Massenspektrometrische Untersuchungen. XIII. Die Massenspektren von Furanen. *Tetrahedron*, **22**, 2223–2235.
- (28) ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Compounds in Food*; Institute for Nutrition and Food Research TNO: Zeist, The Netherlands, 1979.
- (29) Hwang, S. S.; Carlin, J. T.; Bao, Y.; Hartma, G. J.; Ho, C.-T. Characterization of volatile compounds generated from the reactions of aldehydes with ammonium sulfide. *J. Agric. Food Chem.* **1986**, *34*, 538–542.
- (30) Nakamura, S.; Nishimura, O.; Masuda, H. Identification of volatile flavor components of the oil from roasted sesame seeds. *Agric. Biol. Chem.* **1989**, *53*, 1891–1899.
- (31) Werkhoff, P.; Brüning, J.; Emberger, R.; Güntert, M.; Hopp, R. Flavor chemistry of meat volatiles: new results on flavor components from beef, pork and chicken. In *Recent Developments in Flavor and Fragrance Chemistry*; Hopp, R., Mori, K., Eds.; VCH: Weinheim, 1993; pp 183–213.
- (32) Farmer, L. J.; Whitfield, F. B. Some aroma compounds formed from the interaction of lipid in the Maillard reaction. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured: Carol Stream IL, 1993; pp 387–390.
- (33) Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.* **1989**, *49*, 347–368.
- (34) Stoll, M.; Winter, M.; Gautschi, F.; Flament, I.; Willhalm, B. Recherches sur les arômes. Sur l'arôme de café. I. *Helv. Chim. Acta* **1967**, *50*, 682–694.
- (35) Mussinan, C. J.; Wilson, R. A.; Katz, I.; Hruza, A.; Vock, M. H. Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulphur and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; American Chemical Society: Washington, DC, 1976; pp 133–145.

Received for review August 28, 2001. Revised manuscript received November 28, 2001. Accepted November 28, 2001. This study was financially supported by the Ministry of Agriculture, Fisheries and Food (U.K.) and a consortium of food companies under the Food Processing Sciences LINK Scheme (Project CSA 2172).

JF0111662