Food Chemistry **46** (1993) 383–387

Effect of pH on pyrazine formation in glucose–glycine model systems

Gwen L. Bemis-Young, Jeane Huang & Richard A. Bernhard*

Department of Food Science and Technology, University of California, Davis, California 95616, USA

(Received 17 April 1992; accepted 1 June 1992)

Pyrazines produced from a D-glucose/L-glycine model browning system at eight pH values ranging from one to 12 were isolated by extraction and identified by combined gas chromatography/mass spectrometry. Thirty-two compounds were identified in this study including: 19 pyrazines, nine other nitrogen-containing (non-pyrazine) products, and four oxygenated products. As the pH increased, the number of pyrazines produced also increased. The greatest varieties of pyrazines were produced at pH 9·00 and 9·64 in which all 19 pyrazines detected in this study were formed at trace or greater quantities. The major pyrazines generated were 2,3,5-trimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2,3,5,6-tetramethylpyrazine and 2,3-diethylpyrazine.

INTRODUCTION

Non-enzymatic browning reactions (Maillard reactions) have been shown to occur in many heated food systems. During these reactions pyrazines are formed, along with browning products (Hodge, 1953). Pyrazines are a family of nitrogen-containing heterocyclic compounds. These compounds have important flavor and odor characteristics which have a dramatic effect on the sensory aspects of food (Seifert *et al.*, 1972; Nursten & Sheen, 1974; Murray & Whitfield, 1975).

Maillard reactions are initiated when carbonyl and amine groups react. These reactive groups can come from a variety of compounds found in food. The system employed by the authors consisted of glucose and glycine. The reactants were selected since they are quite representative of common food constituents, glucose being one of the most common carbon compounds found in food, as is glycine, the nitrogen source, the simplest amino acid.

The pH of the system plays a crucial role in Maillard reactions, since both carbonyl and amine groups have the potential to be charged or uncharged depending on the hydrogen ion concentration of the system. The relative protonation of these groups is critical, because the initial step of non-enzymatic browning, the condensation of the carbonyl and the amine, is pH-dependent (Koehler & Odell, 1970; Tsuchida *et al.*, 1976; Shibamoto & Bernhard, 1977; Milic & Piletic, 1984; Shu & Ho, 1988). Leahy and Reineccius (1989) used lysine/glucose solutions buffered at pH 5·0, 7·0 and 9·0. They reported rates of pyrazine formation as well as the presence of alkylpyrazines. These were found to increase with both temperature and pH. Using a model system consisting of L-cysteine and D-glucose, Yeo and Shibamoto (1991) investigated microwave-induced volatiles at four pH values. They detected no pyrazines at pH values 2, 5, and 7, but they found eight at pH 9. They attributed this lack of pyrazine formation to the fact that the amino group (pK_a = 10·7) is protonated and therefore it cannot undergo nucleophilic attack.

The specific goal of these studies was to determine the distribution and relative amount of pyrazines formed at the broad range of pH values examined. The authors' rationale was to examine pyrazine formation at precisely defined degrees of protonation. If different pyrazines are produced at the various hydrogen ion concentrations examined, then diverse formation pathways may exist. Assuming this is the case, it may be possible to gain some insight into these mechanisms. Since pyrazines are such powerful flavor and odor compounds, knowledge of the formation mechanisms could be ultimately used in actual food systems to optimize desirable sensory attributes.

MATERIALS AND METHODS

Materials

Anhydrous D-glucose, dichloromethane, glycine, hydrochloric acid and sodium sulfate were purchased from Fisher (Fair Lawn, NJ); sodium hydroxide was obtained from Mallinckrodt (Paris, KY). All authentic reference compounds were obtained commercially at

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/93/$06.00

the highest purity available and were examined for purity by gas chromatography.

Sample preparation

All reaction samples were prepared by dissolving 0·05 mol of D-glucose and 0.15 mol glycine in 90 ml of deionized water, and the final volume was brought to 100 ml. For each sample, the pH was adjusted to the appropriate value by addition of either 2N NaOH or 10% HCl. Eight different pH values were analyzed including both pK_a values and the isoelectric point of glycine. For each pH value, triplicate samples were prepared and triplicate analyses were performed.

Reaction mixtures were placed in pressure bottles (Wheaton, Millville, NJ) sealed with silicone rubber gaskets and porcelain stoppers, and heated at 120°C (oven temperature) for 5 h in a laboratory oven. After heating, the reaction mixture was cooled to room temperature, the pH measured, and then adjusted to pH 10·0 with 2N NaOH or 10% HCl (v/v) to enhance the extraction efficiency of nitrogen-containing heterocyclic compounds. The basic reaction mixture was then extracted for 16 h with 300 ml dichloromethane using a Wehrli continuous liquid–liquid extractor. The extract was dried three times over fresh portions of anhydrous sodium sulfate and then filtered. The solvent was removed using a rotary flash evaporator (40°C and 3·3 kPa). The residue was weighed and then dissolved in a few drops of dichloromethane and transferred to a small vial, which was stored at −10°C until analyzed by high resolution fused silica capillary gas chromatography.

Identification of products formed in the glucose/glycine model system

Qualitative identification of heterocyclic compounds was made by comparing Kovats indices of reaction products to those of authentic compounds. Confirmation of qualitative identification was accomplished by coupling gas chromatography with mass spectrometry (GC/MS). Compounds from reaction extracts were identified by comparing their mass spectra to those of authentic compounds. Further confirmation of particular pyrazines and related compounds was achieved by the method of peak enhancement, in which authentic samples were added to reaction products and cochromatographed.

Instruments

A Varian Model 6000 gas chromatograph equipped with a flame ionization detector and a 30 m × 0·25 mm i.d. (film thickness = 0·25 μm) DB-WAX bonded fused silica capillary column was used for analysis. The GC oven was temperature programmed as follows: 70°C for 8 min, 70–160°C at 2°C/min, and then isothermal hold at 160°C for 87 min. The helium carrier gas flow rate was 30 cm/s and the injector split ratio was 1 : 100. The temperature of the injector and the detector was 200°C. A Shimadzu C-R3A digital integrator was used to determine retention times and peak areas.

A Hewlett Packard 5890 gas chromatograph coupled with a VG Trio-2 mass spectrometer with a VG 11/250 data system was used for mass spectral identification of components. MS operating conditions: ionizing voltage, 70 eV; source temperature, 200°C; accelerating voltage, 4 V; and filament trap current, 100 μA. The gas chromatograph was operated with the same DB-wax column with the operating parameters described above.

Calculations of response factors and relative weight percentages were made by the procedures described by McNair and Bonelli (1969).

RESULTS AND DISCUSSION

In order to determine the effects of pH on pyrazine formation in glucose–glycine model systems, eight different pH levels were examined. The pH values studied represented points below, at, between, and above the pK_a values for glycine (2·34, 9·64).

Because of the wide range of pHs used in this study, the authors chose not to use buffered reaction solutions. Use of buffers would have necessitated employing several different buffers to cover the pH range and thus would introduce additional variables to the system.

Reaction mixtures were extracted with dichloromethane to isolate and recover the pyrazines from the numerous other reaction products. Yields of dichloromethane-soluble compounds varied from 1·56 to 5·01%, with respect to the initial amount of glucose used. Yields were the highest at the extreme basic condition examined (pH 12·00) and the lowest at the isoelectric point (pH 5·97).

All reactions began colorless and upon heating resulted in various shades of yellow to orange to brown, with the exception of the pH 5·97 (isoelectric point) reactions which remained colorless. With the exception noted above, reaction color increased with increasing pH. Since pyrazines are colorless compounds, the colored organic phases must have originated from other Maillard reaction products.

The complexity of the Maillard reactions can be demonstrated by analysis of reaction chromatograms. The dichloromethane-soluble fraction of reaction products produced more than 300 peaks. Thirty-two compounds were identified in this study including: 19 pyrazines, nine other nitrogen-containing (non-pyrazine) products, and four oxygenated products. Means of identification and confirmation of the identities of these reaction products are summarized in Table 1. Compound identity not confirmed by mass spectral analysis should be considered tentative. Mean relative weight percentages for pyrazines formed at the pH values studied are presented in Table 2.

The largest pyrazine distribution patterns were found in neutral to very basic solutions, pH 7·00–12·00. At pH 9·00 and 9·64 the greatest variety of pyrazines were

Table 1. Methods for identification of unknown compounds

Compounds	Kovats	GC–MS	Peak enhancement
Pyrazines			
Pyrazine	+	+	+
2-Methylpyrazine	+	+	+
2,5-Dimethylpyrazine	+	+	+
2,6-Dimethylpyrazine	+	+	+
2-Ethylpyrazine	+	+	+
2,3-Dimethylpyrazine	+	+	+
2-Ethyl-6-methylpyrazine	+	+	+
2-Ethyl-5-methylpyrazine	+	+	+
2,3,5-Trimethylpyrazine	+	+	+
2-Vinylpyrazine	+	–	+
2-Ethyl-3,6-dimethylpyrazine	+	+	+
2,3-Diethylpyrazine	+	+	+
2-Ethyl-3,5-dimethylpyrazine	+	+	+
2,3,5,6-Tetramethylpyrazine	+	+	+
2,3-Diethyl-5-methylpyrazine	+	+	+
5-Methyl-6,7-dihydro-5H-cyclopentapyrazine	+	+	+
5,6,7,8-Tetrahydroquinoxaline	+	–	+
Quinoxaline	+	–	+
2-Methylquinoxaline	+	+	+
Non-pyrazines			
Acetoin	+	+	+
Acetol	+	+	+
Pyrrole	+	–	+
2-Acetylpyridine	+	+	+
1,2-Propanediol	+	–	+
Furfuryl alcohol	+	+	+
Acetamide	+	+	+
Propionamide	+	+	+
1-Furfurylpyrrole	+	–	+
2-Acetylpyrrole	+	+	+
2-Methylimidazole	+	–	+
Imidazole	+	–	+
4-Methylimidazole	+	–	+

+, Detected by this method.
–, Not detected by this method.

produced (Table 2). All 19 pyrazines detected in this study were formed at trace or greater quantities at these pH values. In general, pyrazine production decreased, as compared to pH 9·64, at pH 12·00. Quite probably this is due to increased melanoidin production at the expense of pyrazine formation. This is evidenced by the intense brown color of these reactions in contrast to the colors at pH 9·0 and 9.64.

A minimum number of pyrazines were formed in acidic solutions. At pH 1·00 and 2·34, only seven and eight pyrazines were formed, respectively.

Pyrazines formed in this study can be classified in four different groups, based on the relative weight percentages produced at all pH levels studied. The pyrazine classification groups include: the dominant, less-dominant, minor, and near trace pyrazines.

At least half of all relative weight percentage data for the dominant pyrazines showed them to be present in amounts greater than 15%. For example, relative weight percentages for 2,5-dimethylpyrazine were less than 15% at only two pH levels, pH 1·00 and pH 2·34 (Table 2). The dominant pyrazines include 2,3,5-trimethylpyrazine and 2,5-dimethylpyrazine.

At least half of all relative weight percentage data for the less-dominant pyrazines showed them to be present in amounts between 5 and 15%. This class includes the following: tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethylpyrazine.

At least half of all relative weight percentage data for the minor pyrazines showed them to be present in amounts between 1 and 5%. This group includes: 2-methylquinoxaline, 2,6-dimethylpyrazine, and 2-ethyl-5-methylpyrazine.

Greater than half of all relative weight percentage data for the near trace pyrazines showed them to be present in amounts less than 1%. These pyrazines include the following: pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-vinylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 5-methyl-6,7-dihydro-5H-cyclopentapyrazine, 5,6,7,8-tetrahydroquinoxaline, and quinoxaline. All of this group of reaction products were detectable at a minimum of two different pH levels, but not in quantities great enough to draw any significant quantitative conclusions.

Pyrazine formation is closely related to non-enzymatic browning. The condensation of the carbonyl and amine groups is regarded as the initial step of pyrazine formation, and is one of the rate-determining steps for non-enzymatic browning. The maximum rate of many carbonyl reactions of this type occurs between pH 4 and pH 5 (Westheimer, 1934). Only the uncharged form of the amine can condense with the carbonyl group (Katchalsky & Sharon, 1953; Yeo & Shibamoto, 1991). The equilibrium ratio of the amine species depends upon the pH of the system. As the uncharged form is depleted by reaction, the equilibrium is driven towards producing more of this species. Therefore, even under extreme acidic conditions, some uncharged species is available for reaction. Under acidic conditions, the rate of sugar-amine condensation reactions would be expected to be diminished, due to the relatively low concentration of the uncharged form. Conversely, the protonation of the carbonyl group should enhance the nucleophilic attack of the amino group on the carbonyl. However, this effect is offset by the relatively low reactivity of the protonated amine group at the highly acidic pH levels necessary for protonation of the carbonyl group (Namiki, 1988).

After condensation of the carbonyl and amine groups, fragmentation of the carbohydrate occurs through the following reactions: reverse aldol, enolization, and dehydration (Shibamoto & Bernhard, 1977). These three reactions are all base-catalyzed. The optimum pH for these reactions is difficult to measure because acids are produced during the reaction, thus lowering the pH level (Katchalsky & Sharon, 1953; Wong & Bernhard, 1988). Also, sugar fragmentation in neutral and slightly acidic conditions result in α-dicarbonyl compound production (Speck, 1958).

Table 2. Mean relative weight percentages of the pyrazines formed at all pH values studied[a]

Pyrazine	pH 1·00	pH 2·34	pH 4·00	pH 5·97	pH 7·00	pH 9·00	pH 9·64	pH 12·0
Pyrazine	ND	ND	ND	1·31	0·79	0·48	0·06	ND
2-Methylpyrazine	ND	ND	0·69	0·75	1·14	2·22	1·93	0·20
2,5-Dimethylpyrazine	3·73	3·03	22·42	20·65	18·43	18·58	18·55	53·44
2,6-Dimethylpyrazine	1·03	ND	0·21	0·60	0·65	3·84	5·03	2·24
2-Ethylpyrazine	ND	ND	ND	ND	1·04	TR	0·09	0·11
2,3-Dimethylpyrazine	ND	ND	ND	ND	0·63	1·94	2·84	0·99
2-Ethyl-6-methylpyrazine	ND	ND	ND	ND	ND	0·22	0·46	2·41
2-Ethyl-5-methylpyrazine	ND	ND	3·31	2·95	3·83	3·24	2·78	TR
2,3,5-Trimethylpyrazine	18·72	21·52	28·66	38·53	37·06	39·55	47·42	31·18
2-Vinylpyrazine	ND	ND	ND	ND	ND	0·08	0·03	TR
2-Ethyl-3,6-dimethylpyrazine	ND	ND	0·65	ND	1·04	0·48	0·35	1·65
2,3-Diethylpyrazine	1·31	1·51	5·81	7·91	11·25	3·13	0·54	0·06
2-Ethyl-3,5-dimethylpyrazine	71·36	55·43	5·31	2·45	3·89	4·07	4·70	1·76
2,3,5,6-Tetramethylpyrazine	2·13	1·19	7·29	10·41	13·17	12·03	10·23	2·71
2,3-Diethyl-5-methylpyrazine	1·36	16·65	24·13	11·94	0·18	0·22	0·06	0·12
5-Methyl-6,7-dihydro-5H-cyclopentapyrazine	ND	ND	ND	ND	0·83	0·62	TR	0·05
5,6,7,8-Tetrahydroquinoxaline	ND	ND	0·95	ND	0·29	0·46	TR	ND
Quinoxaline	ND	0·29	TR	ND	0·60	TR	TR	0·10
2-Methylquinoxaline	ND	0·75	1·53	3·67	5·75	8·63	4·27	1·23

[a] Data are the mean value of triplicate determinations.
ND, Not detected.
TR, Trace.

Yields of pyrazines produced depend upon the mechanisms of formation of the fragments, the relative abundance of each fragment produced, and the rates of reactions. It is quite probable that pH plays a major role in each of these components. All of the above evidence could explain the increase in pyrazine production found in our systems under basic conditions.

Previously the authors have proposed mechanisms that make use of a series of α-amino carbonyl fragments, and 10 of these fragments were described (Shibamoto & Bernhard, 1977). These fragments are formed from carbonyl (from glucose) and amine (from glycine) reactions that take place in the system. The authors suggest that the pyrazines are formed by combining these α-amino carbonyl fragments. The proposed mechanisms explain the formation of the following pyrazines: pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine. In addition to these mechanisms, the authors propose that 2,3-diethyl-5-methylpyrazine may be formed by combination of 2-aminopropanal (**I**) (Shibamoto & Bernhard, 1977) and 2-hydroxy-3-amino-4-hexanone (**II**) followed by loss of three moles of water (Fig. 1). Fragment **II** may be formed from 2-hydroxybutanal as shown in Fig. 1. The 2-hydroxybutanal could arise from glucose after a series of dehydration, keto-enol tautomerisms and Cannizzaro reactions to produce a 2,4-diketohexanal (Umano & Shibamoto, 1984). All of the above pyrazines were identified in the present study.

As stated earlier, nine nitrogen-containing non-pyrazine compounds and four oxygenated compounds were also identified in this study. Many of these compounds were formed in greater quantities than the pyrazines studied. Undoubtedly, some of these compounds also have important flavor/odor properties.

These compounds are important in this study because they were formed from the same starting materials as the pyrazines and were also dichloromethane-soluble products. Table 3 presents the relative weight percentages of these compounds versus pH. The greatest

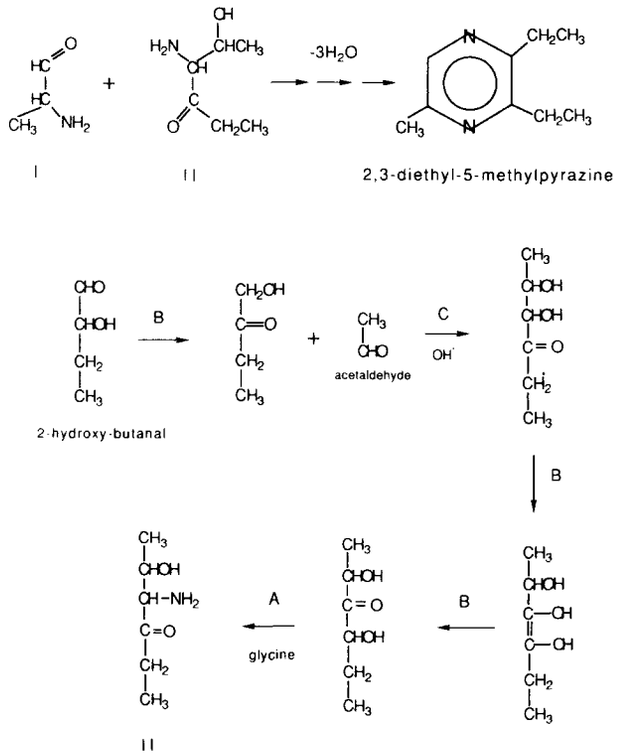

Fig. 1. Proposed mechanisms for the formation of fragment **II** and 2,3-diethyl-5-methylpyrazine. (A, Amadori rearrangement; B, keto-enol tautomerism; C, dehydration reaction).

Table 3. Mean relative weight percentages of the non-pyrazine compounds formed as a function of pH[a]

Compound	pH 1·00	pH 2·34	pH 4·00	pH 5·97	pH 7·00	pH 9·00	pH 9·64	pH 12·00
Acetoin	TR	1·49	4·10	5·26	6·03	3·68	8·97	2·42
Acetol	63·21	44·61	33·51	32·87	29·22	19·17	23·92	0·56
Pyrrole	ND	ND	0·11	ND	0·08	0·17	0·90	4·61
2-Acetylpyridine	ND	ND	ND	ND	TR	0·15	TR	0·24
1,2-Propanediol	ND	TR	0·47	ND	0·17	0·04	TR	ND
Furfuryl alcohol	10·15	6·39	9·79	16·43	12·12	12·97	2·06	0·31
Acetamide	ND	1·82	TR	8·26	0·21	0·95	2·01	0·44
Propionamide	3·88	ND	TR	ND	TR	0·13	0·23	0·08
1-Furfurylpyrrole	10·95	23·36	2·61	3·48	2·83	6·98	5·49	4·90
2-Acetylpyrrole	3·91	23·35	54·27	40·78	48·82	18·40	TR	2·07
2-Methylimidazole	8·81	ND	0·25	2·75	0·26	19·62	31·46	83·93
Imidazole	ND	ND	TR	TR	0·10	7·60	15·41	0·67
4-Methylimidazole	ND	ND	ND	TR	0·13	1·04	2·61	1·51

[a] Data are the mean value of triplicate determinations.
ND, Not detected.
TR, trace.

number of non-pyrazine compounds were produced at pH values equal to or greater than pH 7·00. This formation pattern parallels the trend observed for pyrazine formation.

SUMMARY

In this study the effect of pH on pyrazine formation in glucose–glycine model systems was investigated. The data obtained during this study clearly show that pH does affect pyrazine formation in glucose–glycine model systems. Under these conditions, specific pyrazines and their respective relative amounts produced are pH-dependent.

A mechanism has been proposed for the formation of 2-ethyl-3,5-dimethylpyrazine.

REFERENCES

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *J. Agric. Food Chem.*, **1**, 928–43.

Katchalsky, A. Sharon, N. (1953). Kinetics of aldose-amino acid interaction. *Biochim. Biophys. Acta.*, **10**, 290–301.

Koehler, P. E. & Odell, G. V. (1970). Factors affecting the formation of pyrazine compounds in sugar-amine reactions. *J. Agric. Food Chem.*, **18**, 895–8.

Leahy, M. M. & Reineccius, G. A. (1989). Kinetics of the formation of alkylpyrazines-effects of pH and water activity. In *Thermal Generation of Aromas*, ed. T. H. Parliment, R. J. McGorrin & C.-T. Ho. ACS Symposium Series 409, American Chemical Society, Washington, DC, pp. 196–208.

McNair, H. M. & Bonelli, E. J. (1969). In *Basic Gas Chromatography* (5th edn). Varian Associates, Walnut Creek, CA, pp. 140–4.

Milic, B. L. & Piletic, M. V. (1984). The mechanism of pyrrole, pyrazine, and pyridine formation in non-enzymatic browning reactions. *J. Food Chem.*, **13**, 165–80.

Murray, K. E. & Whitfield, F. B. (1975). The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food Agric.*, **26**, 973–86.

Namiki, M. (1988). Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Adv. Food Res.*, **32**, 120–3.

Nursten, H. E. & Sheen, M. R. (1974). Volatile flavor compounds of baked potatoes. *J. Sci. Food Agric.*, **25**, 643–63.

Seifert, R. M., Buttery, R. G., Guadagni, D. G., Black, D. R. & Harris, J. G. (1972). Synthesis and odor properties of some additional compounds related to 2-isobutyl-3-methoxypyrazine. *J. Agric. Food Chem.*, **20**, 135–7.

Shibamoto, T. & Bernhard, R. A. (1977). Investigation of pyrazine formation pathways in glucose-ammonia model systems. *Agric. Biol. Chem.*, **41**, 143–53.

Shu, C. K. & Ho, C. T. (1988). Effect of pH on volatile formation from the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Agric. Food Chem.*, **36**, 801–3.

Speck, J. C., Jr (1958). Lobry De Bruyn–Alberta van Ekenstein transformations. *Advan. Carbohyd. Chem.*, **13**, 63.

Tsuchida, H., Komoto, M., Kato, H. & Fujimaki, M. (1976). Differences in the formation of pyrazine derivatives from glucose-ammonia reaction in weakly acidic medium and in strongly alkaline medium. *Nippon Nogei Kagaku Kaishi.*, **50** (4), 187–9.

Umano, K. & Shibamoto, T. (1984). Chemical studies on heated starch/glycine model systems. *Agr. Biol. Chem.*, **34**, 1387–93.

Westheimer, F. H. (1934). Semicarbazone formation in sixty per cent methyl cellosolve. *J. Am. Chem. Soc.*, **56**, 1962–5.

Wong, J. M. & Bernhard, R. A. (1988). Effect of nitrogen source on pyrazine formation. *J. Agric. Food Chem.*, **36**, 123–9.

Yeo, H. C. H. & Shibamoto, T. (1991). Microwave-induced volatiles of the Maillard model system under different pH conditions. *J. Agric. Food Chem.*, **39**, 370–3.