Original article

Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions

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Summary The aroma produced in glucose–amino acids (individual and in combination) Maillard reaction, under acidic conditions at 100 °C were determined and compared by trained panellist. Proline produced pleasant, flowery and fragrant aroma. Phenylalanine and tyrosine produced dried roses aroma. Alanine produced fruity and flowery odour, while aspartic acid and serine both produced pleasant, fruity aroma. Arginine, produced a pleasant, fruity and sour aroma at pH 5.2, but not at its natural pH. Glycine, lysine, threonine and valine produced a pleasant caramel-like odour. Isoleucine and leucine gave off a burnt caramel aroma. Methionine developed a fried potato odour. Cysteine and methionine produced savoury, meaty and soy sauce-like flavours. A combination of these amino acids produced different types of aroma, with the stronger note dominating the odour of the mixture. This study will help the prediction of flavour characteristics of hydrolysates from different protein sources.

Keywords: Amino acids, aroma, Maillard reaction, glucose.

Introduction

The parameters that influence the overall flavours and aroma in a Maillard reaction, are the type of amino acid and sugar, pH, temperature, time, moisture content (Lane & Nursten, 1983; Schieberle & Hofmann, 1997) water activity, oxygen, the reaction medium, sulphur dioxide and phosphates (Hurrell & Carpenter, 1977; Namiki, 1988; Shenoy, 1993). Aroma compounds generated from Maillard reaction were mostly studied using simple model systems with amino acids (Baltes *et al.*, 1989; Tressl *et al.*, 1989). Many studies have been done to clarify the mechanisms in organic solvent, rather than in aqueous solution, and using amines not related to food ingredients instead of amino acids (Hofmann, 1998a).

The taste of traditional Japanese foods like miso and soy sauce is due to the release of amino acids from naturally occurring proteins during fermentation. The proper type and level of free amino acids can significantly improve the taste of food products in naturally occurring or intentionally added flavour potentiators. Although people have been heating sugars and amino acids at different pHs and temperatures, there is still no comprehensive study that clearly distinguishes the specific flavour formation and browning development, especially under very acidic conditions in a Maillard reaction. A reaction between one amino acid and one sugar will yield hundreds of volatile compounds (Farmer *et al.*, 1989), which include a range of heterocyclic compounds having a ring structure containing an atom of N, O or S, depending on the heteroatoms present in the amino acid, producing more than one odour in the sensory evaluation.

This study attempts to determine and compare the various aroma resulting from the Maillard reaction of individual and combinations of amino acids to relate to the production of flavours formed in acid-hydrolysed protein. The sensory results of these amino acids on flavour notes may assist in the prediction of possible flavour properties of different hydrolysates by referring to the types of amino acids present through amino acids analysis.

Materials and methods

Materials

L-alanine, L-arginine hydrochloride, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine monohydrochloride monohydrate, L-leucine, L-isoleucine, L-lysine monohydrochloride, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine were purchased from Sigma Chemical Co. (Kuala Lumpur, Malaysia). Glucose monohydrate was obtained from Hamburg Chemical Co. (Hamburg, Germany). Concentrated HCl (37%) and sodium

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hydroxide pellets of analytical grade were obtained from Merck (Kuala Lumpur, Malaysia). Purified water was used except otherwise stated.

Maillard reaction of amino acids and D-glucose

D-glucose monohydrate (10.0 mmol in 20 mL distilled water) were reacted with amino acids (3.3 mmol), individually and in combinations in sealed tubes (Hofmann & Schieberle, 1995) in triplicates, under five different conditions, i.e.

Sample A: At the original pH of the amino acids and heated at 100 ± 1 °C in conventional temperature-controlled thermostatic oven for 24 h;

Sample B: At the original pH of the amino acids and heated at 100 ± 1 °C for 14 h;

Sample C: At pH 5.2 ± 0.1 and heated at 100 ± 1 °C for 24 h;

Sample D: In 6 M HCl as the medium and heated at 100 ± 1 °C for 24 h;

Sample E: In 6 M HCl as the medium and heated at 100 ± 1 °C for 1 h.

After the reaction, the tubes were removed and immediately cooled in ice. Samples D and E were then neutralized to pH 5.2 ± 0.1. The amounts and types of amino acids used for the amino acid combination reaction mixtures are shown in Table 1, with and without sulphur amino acids (cysteine and methionine). These combinations were based on the amino acid profile of a seed protein acid hydrolysate.

Sensory evaluation was carried independently by eleven trained assessors from the faculty (ASTM, 1968, 1981). The descriptors for sensory analysis were initially discussed, and a set of reference solutions was made. The panel was introduced to these reference solutions in two training sessions.

A set of reference solutions was prepared based on the odour descriptor set, which consisted of bouillon, meaty, soy sauce, smoky, malty, caramel, beany, chicken, fruity, flowery, pandan, vanilla, prawn, seafood, chocolate, coffee, buttery and medicinal. The reference solutions used in order to obtain the most characteristic flavour were:

1 Bouillon: one bouillon cube (beef flavour, from Knorr, CPC/AJI, Kuala Lumpur, Malaysia) dissolved in boiling water.
2 Meaty: commercial acid hydrolysate (Ajieki, Ajinomoto (Malaysia) Bhd., Kuala Lumpur, Malaysia) and concentrated yeast extract (Vegemite, Kraft Foods Limited, Victoria, Australia).
3 Soy sauce: soy sauce (Po-Po, Hung Chun Sdn. Bhd., Ipoh, Malaysia).
4 Smoky: hickory smoke barbecue sauce (Knorr, CPC/AJI).
5 Malty: soymilk with malt flavour (Soyfresh, Lam Soon Singapore Pte Ltd).

Table 1 The types and amounts of amino acids involved in the Maillard reaction of fifteen and seventeen types of amino acids combined

		Without sulphur amino acids		With cysteine and methionine	
Amino acid	Molecular weight	Concentration of amino acid used without sulphur amino acids (mmol)	Amount of amino acids used without sulphur amino acids (g)	Concentration of amino acid used with sulphur amino acids (mmol)	Amount of amino acids used with sulphur amino acids (g)
Asp	133.11	0.4599	0.0122	0.3986	0.0106
Glu	147.13	0.6596	0.0194	0.5717	0.0168
Ser	105.09	0.1385	0.0029	0.1200	0.0025
Gly	75.07	0.1970	0.0030	0.1708	0.0026
His	155.18	0.0000	0.0000	0.0000	0.0000
Arg	174.20	0.3116	0.0109	0.2700	0.0094
Thr	119.12	0.1041	0.0025	0.0902	0.0021
Ala	89.09	0.1371	0.0024	0.1188	0.0021
Pro	115.13	0.1011	0.0023	0.0876	0.0020
Tyr	181.19	0.0560	0.0020	0.0486	0.0018
Val	117.15	0.1656	0.0039	0.1435	0.0034
Met	149.20	0	0	0.2200	0.0066
Cys	240.30	0	0	0.2200	0.0106
Ile	131.18	0.1337	0.0035	0.1159	0.0030
Leu	131.18	0.3895	0.0102	0.3376	0.0089
Phe	165.19	0.3002	0.0099	0.2602	0.0086
Lys	146.19	0.1460	0.0043	0.1265	0.0037
Total		3.3000		3.3000	

6 Caramel: burned sugar.
7 Beany: soymilk (Drinho, Lam Soon Singapore Pte Ltd).
8 Chicken: concentrated chicken stock (Maggi, Nestle Products Sdn. Bhd., Kuala Lumpur, Malaysia).
9 Fruity: raisin (Big Sister, Madina Setia Sdn. Bhd., Kuala Lumpur, Malaysia).
10 Flowery: chrysanthemum tea (Yeo's, Yeo Hiap Seng (Malaysia) Bhd), Kuala Lumpur, Malaysia.
11 Pandan: pandan flavour essence (Star, Bush Boake Allen Sdn. Bhd., Kuala Lumpur, Malaysia).
12 Vanilla: vanilla flavour essence (Star, Bush Boake Allen Sdn. Bhd.).
13 Prawn: prawn flavour 4219 (Matrix Flavours & Fragrances Sdn. Bhd., Kuala Lumpur, Malaysia).
14 Seafood: seafood flavour powder 1879 (Matrix Flavours & Fragrances Sdn. Bhd.).
15 Chocolate: chocolate milk (Dutch Lady, Dutch Lady Milk Ind. Bhd., Kuala Lumpur, Malaysia).
16 Coffee: instant coffee powder (Néscafe Classic, Néstle Products Sdn. Bhd., Kuala Lumpur, Malaysia) was diluted in hot water.
17 Buttery: butter (Fern, New Zealand Dairy Board, Wellington, New Zealand).

Colour references were prepared for the samples of Maillard reaction and assessed visually by the panellists as follows:

1 Dark brown: soy sauce (Po-Po, Hung Chun Sdn. Bhd.).
2 Brown: 20% soy sauce in water.
3 Light brown: 5% soy sauce in water.
4 Very light brown: 2% soy sauce in water.
5 Light yellow: 0.5% soy sauce in water.

The references for taste were 1% of the following ingredients in distilled water:

1 Sweet: sucrose (Hamburg).
2 Sour: citric acid (Merck).
3 Salty: sodium chloride (Merck).
4 Bitter: caffeine (Merck).
5 Umami: Monosodium glutamate [Ajinomoto (Malaysia) Bhd.].

The 20-mL samples of the Maillard reaction were served at room temperature and at 60 °C. All the samples were coded with 3-digit numbers and served in a randomised order. Six samples were served per session and the experiments were replicated. The detection and evaluation of the flavour notes of amino acids used only simple descriptive test (ISO, 1985). The results were collated to produce a list of descriptive terms applicable to the samples, based on the frequency of usage of each descriptive word. There were no restrictions as to how many and what terms to use. The odour descriptions included in the results were only those used by four or more panellists ($\chi^2 > 5.18$; significant at $P < 0.05$).

Results and discussion

Flavour notes of individual amino acids

Temperature, substrates ratio, pH and time may affect the flavour notes formed during Maillard reaction. Temperature and substrates ratio were therefore fixed, while pH and time were varied accordingly. Some samples, such as aspartic acid and glutamic acid had low natural pH values (pH 3.0 and 3.2 respectively), while the others were within the range of pH 5.3–5.8. A variety of aromas were detected from arginine, aspartic acid and cysteine, implying the suitability of pH 5.2 towards the flavour formation. Serine, proline and phenylalanine gave significant fruity and flowery aroma at their original pH. Alanine produced a flowery odour, while aspartic acid produced a pleasant odour (Table 2). The aroma detected from isoleucine, leucine, lysine and threonine were caramel-like and became more distinct on extended heating. No odour was detected from arginine, glutamic acid and histidine (Table 2), even after a colour change. This may be due to the very minute amounts of volatile odorous compounds released that cannot be detected by the human olfactory organ. The detection of odour is dependent on the concentration and odour threshold of the key odour impact compounds, and also on the sensitivity of the human nose to that particular compound. Although no odour was detected from arginine when Maillard was performed at its natural pH values, a pleasant, fruity and sour odour were produced at pH 5.2. Leucine, threonine, tyrosine and valine also released slightly stronger aroma at pH 5.2. No aroma were detected from glutamic acid and histidine (Tables 2 and 3), probably because pH 5.2 were not suitable for the production of odorous substances from these two amino acids. Under controlled pH 5.2 conditions, alanine, serine and aspartic acid produced a distinctly pleasant, fruity odour. Cysteine formed a distinct sulphury odour. Glycine, lysine, threonine and valine gave off a pleasant caramel-like odour; while isoleucine and leucine produced a distinct burnt caramel-like aroma. Methionine developed a distinct fried potato odour, while proline produced a distinct flowery, pleasant and pandan-like aroma. Phenylalanine and tyrosine gave off a distinct flowery odour almost similar to dried roses. Under uncontrolled pH, with the initial pH 5.2, most aroma formed were almost the same as under pH 5.2 ± 0.1 controlled conditions except alanine had additional persimmon-like odour, arginine had no odour, glycine and lysine had additional pleasant caramel-like odour, serine had pleasant, slightly ciku, fresh dates-like odour, proline had additional slightly persimmon-like odour, phenylalanine had additional dried roses and almond-like odour, and tyrosine had a slight dried roses odour.

Table 2 Colour and odour of Maillard products of amino acids and glucose formed after heating for 14 and/or 24 h at original pH

Amino acid	Original measured pH	14 and/or 24 hours Colour	Odour
Alanine	5.6	Brown	Fruity** (Persimmon), pleasant/sweet**, flowery**,
Arginine	5.3	Light yellow	None, bitter taste*
Aspartic acid	3.0	Very light brown	Fruity** (*ciku*, fresh dates), pleasant/sweet**
Cysteine	4.5	Light yellow	Sulphury**
Glutamic acid	3.2	No change	None, sour taste**
Glycine	5.7	Brown	Caramel-like**, pleasant/sweet*
Histidine	4.0	Light yellow	None, slight sweet-sour taste
Isoleucine	5.8	Brown	Burnt*, caramel-like*
Leucine	5.7	Brown	Burnt**, caramel-like*
Lysine	5.4	Brown	Pleasant/sweet*, caramel-like**, bitter taste
Methionine	5.6	Brown	Fried potatoes, prawn cracker*
Threonine	5.6	Light brown	Pleasant/sweet**, astringent sweet taste
Serine	5.5	Brown	Fruity* (slightly *ciku*, fresh dates), pleasant/sweet*
Proline	5.7	Light brown	Flowery**, pleasant/sweet**, *pandan***, slightly persimmon, bitter taste
Phenylalanine	5.4	Brown	Flowery** (dried roses), Almond, bitter taste
Tyrosine	5.6	Brown	Flowery** (slightly dried roses), sweet taste
Valine	5.6	Brown	Caramel-like**, bitter taste
None (glucose), control	5.4	No change	None

Ciku = *Manilkara achras* mill.; *pandan* = *Pandanus odorus*, Ridl.
*Results from the chi-square distribution showed the flavour, significantly differed from the blank ($P < 0.05$, $\chi^2 < 4.8$), i.e. identified by at least four or five of the eleven panellists.
**Results from the chi-square distribution showed the flavour very significantly differed from the blank ($P < 0.01$, $\chi^2 < 8.25$), i.e. identified by at least six or more of the eleven panellists.

Table 3 Colour and odour of Maillard products of amino acids and glucose formed after heating for 24 h at starting pH 5.2 and controlled at pH 5.2 ± 0.1 conditions

Amino acid	Colour	Odour
Alanine	Brown	Fruity** (*ciku*, fresh dates), pleasant/sweet**, flowery**
Arginine	Brown	Pleasant/sweet**, fruity*, sour*
Aspartic acid	Brown	Fruity (*ciku*, fresh dates), pleasant/sweet**, caramel-like**
Cysteine	Very light brown	Sulphury**, slightly meaty, boiled chickpeas
Glutamic acid	Brown	None, sour umami taste,
Glycine	Brown	Pleasant/sweet**, flowery*
Histidine	Light yellow	None, sour taste
Isoleucine	Brown	Burnt**, caramel-like**
Leucine	Brown	Burnt**, caramel-like*, biscuit-like
Lysine	Brown	Pleasant/sweet**, *pandan**,bitter taste
Methionine	Brown	Fried potatoes, prawn crackers*
Threonine	Light brown	Pleasant/sweet**, fruity**
Serine	Brown	Pleasant/sweet**
Proline	Brown	Flowery*, pleasant/sweet*, *pandan***
Phenylalanine	Brown (cloudy)	Flowery** (roses), almond, *Mimusops elengi* flower**
Tyrosine	Brown	Flowery*, fruity*, pleasant/sweet, tea-like
Valine	Brown	Caramel-like**, biscuit-like, malty, chocolate, bitter taste
None (glucose)	No change	None

Ciku = *Manilkara achras* mill.; *pandan* = *Pandanus odorus*, Ridl.
*Results from the chi-square distribution showed the flavour, significantly differed from the blank ($P < 0.05$, $\chi^2 < 4.8$), i.e. identified by at least four or five of the eleven panellists.
**Results from the chi-square distribution showed the flavour very significantly differed from the blank ($P < 0.01$, $\chi^2 < 8.25$), i.e. identified by at least six or more of the eleven panellists.

Table 4 Colour and odour of Maillard products of amino acids and glucose formed after heating for 1 h in the presence of 6 M HCl (pH < 0.1)

Amino acid	Colour	Odour
Alanine	Brown	Pleasant/sweet**, fruity* (*ciku*, fresh dates), caramel-like**, slightly burnt**
Arginine	Brown	Caramel-like*, burnt**, slight sweet/pleasant*
Aspartic Acid	Brown	Pleasant/sweet**, fruity (*ciku*, fresh dates), caramel-like**
Cysteine	Dark brown	Sulphury**, meaty*
Glutamic Acid	Brown	Pleasant/sweet**, caramel-like**, burnt**
Glycine	Brown	Pleasant/sweet**, caramel-like**, burnt**
Histidine	Brown	Caramel-like**, burnt*
Isoleucine	Brown	Burnt**, coffee-like*, prune
Leucine	Brown	Burnt*, coffee-like*, caramel-like*, malty*
Lysine	Brown	Pleasant/sweet**, cardboard, Herbal tea*
Methionine	Brown	Meaty**, sulphury**, fried potatoes*
Threonine	Brown	Pleasant/sweet**, fruity** (*ciku*, fresh dates), flowery*, caramel-like**
Serine	Brown	Slightly burnt**, pleasant/sweet*
Proline	Brown	Flowery**, pleasant/sweet**, *pandan**, slightly alkaline
Phenylalanine	Brown	Flowery** (roses)
Tyrosine	Brown	Pleasant/sweet*, caramel-like*, burnt**
Valine	Brown	Pleasant/sweet**, caramel-like**, burnt*, malty

Ciku = *Manilkara achras* mill.; *pandan* = *Pandanus odorus*, Ridl. All products contained black sediment. After 24 h most of the samples had burnt, smoky aroma, except phenylalanine and proline samples retained their pleasant, flowery aroma.

*Results from the chi-square distribution showed the flavour, significantly differed from the blank ($P < 0.05$, $\chi^2 < 4.8$), i.e. identified by at least four or five of the eleven panellists.

**Results from the chi-square distribution showed the flavour, very significantly differed from the blank ($P < 0.01$, $\chi^2 < 8.25$), i.e. identified by at least six or more of the eleven panellists.

During the Strecker degradation, sulphur-containing amino acids will lose one carbon as CO_2 to form highly odorous thioaldehydes. For example, methionine will form methional, which produces a significant potato flavour (Self, 1967) as similarly found for methionine in this study (Tables 2–4). Compounds of particular importance for the development of 'meaty' flavours, reportedly have a furan or thiophene ring with a thiol group in the 3-position, while similar compounds with a thiol in the 2-position tend to be 'burnt' and 'sulphurous' (Farmer, 1994). In this study, cysteine was found to emit a sulphury odour at its natural pH, and both sulphury and meaty aroma in acidic pH.

Maillard-type reaction between the amino acid proline and reducing carbohydrates is well known for generating popcorn-like, roasty aroma upon thermal treatment (Hunter *et al.*, 1969). Nine key odourants are generated in thermally treated proline/glucose reaction mixtures, of which one gave buttery odour, another gave caramel-like odour, six gave popcorn-like odour and the last unknown odourant gave a roasty odour (Hofmann & Schieberle, 1998a). The distillate obtained by boiling and simultaneously extracting an aqueous mixture of proline and D-glucose, elicited an intense roasty, popcorn-like odour (Hofmann & Schieberle, 1998a). The difference in the flavour developed from proline-glucose interaction between this study and those reported previously is probably due to the acid pH of the reaction medium, and that the panellists were more familiar with pandan than popcorn flavour.

The factors influencing the generation of aroma from cysteine/carbohydrate reaction mixtures are still not fully understood, particularly because the contribution of a single odorant to the key flavour notes has not been established. A sensory evaluation of cysteine/glucose mixture revealed meat-like and pungent odour notes as the predominant odour qualities, while a cysteine/rhamnose mixture revealed roasty, meat-like and sulphury odour notes with the highest intensities and cysteine/ribose mixture produced an additional caramel-like and a seasoning-like odour note (Salter *et al.*, 1988; Hofmann & Schieberle, 1998b). Similar results were found in this study.

Effect of extreme low pH

At 6 M HCl (which is the concentration of HCl used in producing acid protein hydrolysates), most samples turned brown after 1 h at 100 °C (Table 4). Cysteine was dark brown in colour after the 1-h heating, and gradually turned black after 24 h. Tyrosine also exhibited colour change from light to a darker colour with prolonged heating. Results clearly show that 1-h heating at an extremely low pH is sufficient, and some samples produced a burnt odour. Other samples such as aspartic acid, cysteine, methionine, threonine, proline and

phenylalanine emitted a pleasant odour. When the heating period was prolonged to 24 h, more samples with the exception of methionine, proline and phenylalanine emitted burnt odour.

Serving temperature

Table 5 shows that while serving temperature had no effect on the colour, apparent differences in odour were detected. Glutamic acid and histidine had no detectable odour when served at room temperature, but when served at 60 °C, odour was easily detected. Glycine, lysine and serine also emitted additional aroma. This might be due to the minute amounts of volatiles probably hydrogen bonded to the aqueous medium which made detection at room temperature difficult.

Flavour notes of amino acids combinations

Amino acids combinations heated at their natural pH values at 100 °C for 24 h produced an odour similar to soy sauce, hydrolysed yeast extract (Marmite) and slightly of dried salted fish (Table 6). When they are heated in the presence of 6 M HCl as for the production of acid-hydrolysed proteins, a strong chemical-like disinfectant and chlorine-like aroma were produced. This may be due to the usage of a strong acid in the reaction. Heating at pH 5.2, produced a burnt, smoked and slightly soy sauce-like aroma. Cysteine and methionine may be involved in the production of soy sauce odour, which may have been destroyed when heated in the presence of 6 M HCl. A good odour was produced under a very strong acidic condition, after just 1 h of heating at 100 °C. Heating the amino acids at

Table 5 Comparison of colour and odour of Maillard products of amino acids and glucose heated and maintained at pH 5.2 ± 0.1 served at room temperature and 60 °C

	Served at room temperature		Served at 60 °C	
Amino acid	**Colour**	**Odour**	**Colour**	**Odour**
Arginine	Brown	Pleasant/sweet**, fruity*, sour*	Brown	Pleasant/sweet**, caramel-like**, fruity, sour
Glutamic acid	Brown	None**, fruity sour umami taste	Brown	Pleasant/sweet**, caramel-like*, biscuit-like*
Glycine	Brown	Pleasant/sweet**, flowery*	Brown	Pleasant/sweet**, flowery*, caramel-like**
Histidine	Light yellow	None**, sour taste	Light yellow	Pleasant/sweet**, caramel-like*
Lysine	Brown	Pleasant/sweet**, *pandan**	Brown	Pleasant/sweet**, *pandan**, flowery*
Threonine	Very light brown	Pleasant/sweet**, fruity**	Very light brown	Pleasant/sweet**, fruity*
Serine	Brown	Pleasant/sweet**	Brown	Pleasant/sweet**, caramel-like**
Tyrosine	Brown	Flowery*, fruity*, Pleasant/sweet*, tea-like	Brown	Flowery** (roses), fruity, Pleasant/sweet, tea-like
Valine	Brown	Caramel-like**, biscuit-like, malty, chocolate	Brown	Caramel-like**, biscuit-like, malty, chocolate, burnt**

*Results from the chi-square distribution showed the flavour, significantly differed from the blank ($P < 0.05$, $\chi^2 < 4.8$), i.e. identified by at least four or five of the eleven panellists.

**Results from the chi-square distribution showed the flavour, very significantly differed from the blank ($P < 0.01$, $\chi^2 < 8.25$), i.e. identified by at least six or more of the eleven panellists.

Table 6 Mixture of aroma detected from the Maillard reaction of a combination of amino acids (as in Table 1 in the presence of sulphur amino acids) with glucose under five different reaction conditions

Samples	Description of odour
A: At the original pH of the amino acids and heated at 100 ± 1 °C in conventional temperature-controlled thermostatic oven for 24 h	Soy sauce**, marmite*, salted fish-like*
B: At the original pH of the amino acids and heated at 100 ± 1 °C for 14 h	Chemical*, strong disinfectant*, chlorine-like*
C: At pH 5.2 ± 0.1 and heated at 100 ± 1 °C for 24 h	Burnt*, smoky*, slightly soy sauce-like*
D: In 6 M HCl as the medium and heated at 100 ± 1 °C for 24 h. Neutralised to pH 5.2 ± 0.1 after immediate cooling in ice	Flowery*, caramel-like*
E: In 6 M HCl as the medium and heated at 100 ± 1 °C for 1 h. Neutralised to pH 5.2 ± 0.1 after immediate cooling in ice	Chemical*, strong disinfectant*, chlorine-like*

*Results from the chi-square distribution showed the flavour, significantly differed from the blank ($P < 0.05$, $\chi^2 < 4.8$), i.e. identified by at least four or more of the eleven panellists.

**Results from the chi-square distribution showed the flavour, very significantly differed from the blank ($P < 0.01$, $\chi^2 < 8.25$), i.e. identified by at least six or more of the eleven panellists.

100 ± 1 °C for 14 h, under the original pH, released a flowery and caramel-like aroma. No soy sauce-like flavours were released when cysteine and methionine were absent. The sensation of odour is produced by the volatile chemical substances, which stimulate the receptors in the nasal epithelium.

A combination of these amino acids produced different types of aroma. The types of odour produced were a combination of aroma of each individual amino acid with the stronger note dominating and becoming the main odour of the samples (Table 6). For example, sample D produced a flowery odour, most probably caused by phenylalanine and tyrosine. When cysteine and methionine were added to the reaction composition, the main odour was different. It produced soy sauce, hydrolysed yeast extract (Marmite) and slightly salted fish-like aroma as in sample A of Table 6. On the other hand, the lack of cysteine and methionine in samples D and E caused the production of a non-soy sauce odour. This clearly shows that these two sulphur-containing amino acids (cysteine and methionine) are the main amino acids that are responsible for the production of a meaty flavour. The determined flavour notes of the amino acids Maillard reactions are closely related to the flavour produced by vegetable protein hydrolysates.

Colour

Glutamic acid did not exhibit any colour changes at its original pH even after heating for 24 h (Table 2). The Maillard reaction may have already taken place long before any colour changes were observed. Under controlled pH, the mixture turned brown in colour at the end of the reaction. Variation of odour was more prominent than the colour changes under both reaction conditions. Most samples had similar colours when reacted at different pH values for 24 h, as this long period of time is believed to allow maximum interaction of the substrates. Arginine, aspartic acid, cysteine, glutamic acid and proline exhibited prominent colour changes especially at pH 5.2.

The complexity of the nonenzymatic browning reactions is known to be at least partly due to the sugar caramelisation processes (Greenshields, 1973). In this study, glucose solution without amino acid was also treated under similar conditions to examine the appearance of the brown colour. Sensory results show no changes in colour when glucose was present alone in the reaction medium at approximately pH 5.4, as previously reported (Ajandouz & Puigserver, 1999), and that the intensity of browning increased with pH values, and the presence of almost all of the essential amino acids. None of these amino acids, with the exception of tryptophan, gave rise to any browning in the absence of glucose.

Maillard reaction is temperature dependent and the reaction rate may increase two to three times for each 10 °C rise in temperature of model systems (Birch, 1977), and even more than this in the natural systems. Up to 60 °C, browning is normally a zero order reaction, but at higher temperatures, changes may follow a first order reaction (Labuza & Saltmarch, 1981). The effect of temperature on Maillard reaction, is also related to other variables such as acidity and water activity. Activation energy decreases with increasing water activity, resulting in increasing browning rate. Reaction at 37 °C occurred over a period of days, while at above 100 °C, reaction time occurred within minutes. In a mixture of albumin and glucose, 76% of the ε-amino lysine were unavailable after 30 days at 37 °C, compared with 85% in 15 min at 121 °C (Armstrong, 1994; Mustapha, 1997). pH value has a major influence on many important pathways of the Maillard reaction and the products (Ames, 1988). The browning rate of glucose/glycine peptides below pH 6 is greater than glucose/glycine at the same molar concentration, but the reverse was observed at a higher pH (Labuza & Schmidl, 1986). Increasing the pH values of the reaction medium will generally enhance the reaction (Labuza *et al.*, 1983; Ashoor & Zent, 1984; Petriella *et al.*, 1985). The type and the amount of final products may differ if the experimental conditions are not exactly identical (Ames *et al.*, 1997), and if the pH is not controlled during the reaction (Ames *et al.*, 1993). The present results agree with the previous researchers' observations, and new aromas are reported here due to the slight differences in reaction conditions.

The likelihood of mutagenic or carcinogenic compounds formed under the conditions of the reaction is beyond the scope of this study. The formation and occurrence of carcinogenic heterocyclic amines in glucose–amino acids model systems have been reported (Johansson *et al.*, 1995) and reviewed (Skog *et al.*, 1998).

Conclusions

In this study, most amino acids produced a pleasant or acceptable flavour with only a few producing unpleasant burnt and sulphury aroma. Consequently, the Maillard reaction can be used as a basis for the production of specific flavouring products by carefully selecting the sugars and amino acids, and controlling the processing conditions within narrow specifications. Both pH and time play an important role in controlling the type of odorous compounds that are produced and the use of extreme pH, such as 6 M HCl would produce a burnt odour even with only an hour of heating. Prolonged heating time under this condition would cause the unpleasant odour to become even more intense. Combination of amino acids produced different results. Slightly acidic amino acid mixtures heated for 24 h (Table 6, sample C) produced a better odour than amino

acid mixtures heated at their natural pH for (sample B) 14 h. The serving temperature also plays an important role in the detection of odour. At 60 °C, higher concentrations of volatile compounds are being released.

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