JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



Article

# Influence of Free Amino Acids, Oligopeptides and Polypeptides on the formation of Pyrazines in Maillard Model Systems

Gustavo Luis Leonardo Scalone, Tatiana Cucu, Norbert De Kimpe, and Bruno De Meulenaer

*J. Agric. Food Chem.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.5b01129 • Publication Date (Web): 14 May 2015



## Just Accepted





Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

# Influence of Free Amino Acids, Oligopeptides and Polypeptides on the formation of Pyrazines in Maillard Model Systems

Gustavo Luis Leonardo Scalone [1,2] Tatiana Cucu [1], Norbert De Kimpe [2] and Bruno De Meulenaer [1 *]

[1] Department of Food Safety and Food Quality, nutriFOODchem group, member of Food2Know.

Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

[2] Department of Sustainable Organic Chemistry and Technology.

Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

* To whom correspondence should be addressed. Fax 32 9 2646215.

E-mail: Bruno.DeMeulenaer@UGent.be

## ABSTRACT

Pyrazines are specific Maillard reaction compounds known to contribute to the unique aroma of many products. Most studies concerning the generation of pyrazines in the Maillard reaction have focused on amino acids, while little information is available on the impact of peptides and proteins. The present study investigated the generation of pyrazines in model systems containing whey protein, hydrolyzed whey protein, amino acids and glucose. The impact of thermal conditions, the ratio of the reagents and the aw was measured on the pyrazine formation by HS-SPME-GC-MS. The presence of oligopeptides from hydrolyzed whey protein contributed significantly to an increased amount of pyrazines, while in contrast free amino acids generated during protein hydrolysis contributed to a lesser extent. The generation of pyrazines was enhanced at low aw (0.33) and high temperatures (>120 $^{0}$C). This study showed that the role of peptides in the generation of pyrazines in Maillard reaction systems has been dramatically underestimated.


**KEYWORDS**: Peptides; Maillard; fractions; pyrazines; flavor; model reactions; HS-SPME-GC-MS.

## INTRODUCTION

The Maillard reaction comprises a set of complex chemical reactions, which are initiated when a free amino group of an amino acid, a peptide, a protein or an amine reacts with the carbonyl group of a reducing sugar. This non-enzymatic browning reaction gives rise to modifications in color, aroma, taste and nutritional value of thermally treated foods and is influenced by many factors, such as temperature, time, pH, type of buffer, water activity and reactant concentration.[1] The reaction between free amino acids and carbonyl compounds has been extensively studied[1], whereas the Maillard reaction between peptides and proteins with carbonyl compounds has been less investigated.[2-4] This in fact is quite surprising as the amount of free amino acids in food is generally very low as compared to the amounts of peptides and especially proteins.[5]

A limited number of studies have investigated the generation of volatiles in protein – carbohydrate models. Nevertheless, these studies were performed under unrealistically severe thermal conditions[6-8].

Oligopeptides have been recognized as important flavor enhancers and precursors of the Maillard reaction.[2, 9-11] Moreover, peptides can be a flavor enhancer as such (umami taste). In addition, the formation of flavor compounds due to the reaction between peptides and carbohydrates has mainly been studied in model systems containing glutathione[12-15] and glycine-derived peptides such as diglycine, triglycine, and tetraglycine.[4] In these studies, pyrazines were indeed the most abundant volatiles.

Substituted and unsubstituted pyrazines are specific Maillard reaction products which are known to contribute significantly to the unique roasted, nutty, meaty, earthy

and popcorn-like aroma of many heated food products.[16-17] Oh et al.[4] found that the amount of pyrazines formed from diglycine or tetraglycine was considerably lower compared to that formed from either glycine or triglycine. Van Lancker et al.[18-19] studied the formation of pyrazines from di– and tripeptides with different amino acid sequences. The results of these studies showed significant formation of pyrazines from dipeptides compared to free amino acids and less pyrazine formation in tripeptides over dipeptides and free amino acids.

The general mechanism of pyrazines formation via the Maillard reaction implies in the first step the reaction of a dicarbonyl compound **1** produced by sugar degradation and an amino acid **2**, which leads to the formation of Strecker aldehydes **6** and α-aminoketones **7**. A crucial step in this degradation reaction is the decarboxylation of the intermediate **4** via a cyclic transition state (Figure 1 part I). The second step in the pyrazine formation involves two possible pathways which start with the condensation reaction of two α-aminocarbonyl compounds **7** and **7'** with the formation of a dihydropyrazine **9**. This dihydropyrazine can react in two distinct ways. The dihydropyrazine can oxidize spontaneously with air to the corresponding pyrazine **11** (pathway A). Alternatively, after deprotonation the dihydropyrazine anion **10** can react with a carbonyl compound **6** in an Aldol-type reaction (pathway B). Whenever this reacting carbonyl compound is a Strecker aldehyde **6**, amino acid specific pyrazines **15** are formed (Figure 1 part II).

Peptides cannot follow the typical Strecker degradation due to the absence of a free carboxyl group at the α-carbon with respect to the free amino group, making the decarboxylation impossible. Van Lancker et al.[18] has suggested a mechanism of

pyrazine formation in model systems containing dipeptides and dicarbonyl compounds as shown in Figure 2. In accordance to the reaction with free amino acids, the reaction of the α-dicarbonyl compound **1** with the dipeptide starts with the formation of an α-ketoimine **17**. Afterwards, deprotonation occurs at the α-position of the amide moiety resulting in a 1,5-H-shift leading to enolization of the carbonyl group of intermediate **17**. Hydrolysis of the imino moiety of 4-hydroxy-2-azadiene **18** produces the α-aminoketone **7** and instead of forming the Strecker aldehyde, a complex α-ketoamide **19** is formed.[2]

Although it was shown before that peptides may generate pyrazines in the Maillard reaction, their potential contribution to the overall pyrazine formation in food has not been compared to the contribution of amino acids. Therefore, this study aimed to evaluate the impact of trypsinogenic whey hydrolysis products on the formation of pyrazines in a Maillard reaction model, thereby trying to discriminate the role of free amino acids and peptides generated respectively. In addition the impact of other factors such as heating conditions, the ratio of reagents and the water activity on the formation of pyrazines were investigated.

## MATERIALS AND METHODS

### Chemicals.

DL–norvaline (99%), L–cysteine (99.5%), L–4-hydroxyproline (99%), DL–valine (99%), DL–alanine (99%), L–tryptophan (99.5%), L–citruline (99%), sarcosine (99%), DL–histidine (99%), L–isoleucine (99.5%), DL–leucine (99%) and glutamine (99,5%) were purchased from Fluka (Sigma–Aldrich, Bornem, Belgium). DL–lysine monohydrochloride (98%), DL–methionine (99%), glycine (99.5%), L–arginine hydrochloride (99%), L–phenylalanine (99%), L–glutamic acid (99%), D-(+)-glucose

(99.5%), trypsin from porcine pancreas, trypsin-chymotrypsin inhibitor from glycine max (soybean), insulin from bovine pancreas, insulin chain B oxidized from bovine pancreas, cytochrome C from equine heart, vitamine $B_{12}$, Val-Tyr, pyrazine (99%), 2-methylpyrazine (99%) and sodium bromide (99%) were purchased from Sigma–Aldrich (Bornem). L–lysine (97%), arginine (98%), proline (99%), asparagine monohydrate (99%), 2,5-dimethylpyrazine (99%), 2-ethylpyrazine (98%), 2-ethyl-3,5(6)-dimethylpyrazine (99.5%) and trichloroacetic acid (99%) were purchased from Acros organics, Thermo – Fisher Scientific (Erembodegem, Belgium). Aspartic acid (99%), DL–threonine (99%), L–tyrosine (99%) were purchased from Merck (Darmstadt, Germany). DL–serine, 2,3-dimethylpyrazine (99%) and 2,6-dimethylpyrazine (96%) were purchased from Janssen Chimica (Geel, Belgium). Magnesium chloride hexahydrate (99%), calcium carbonate (99%), potassium iodide (99.5%) and sodium chloride (98%) were purchased from Chemlab Analytical (Zedelgem, Belgium). Whey protein isolate LACPRODAN DI-9224 was donated from Arla foods (Aarhus, Denmark).

**Hydrolysis of whey protein**.

Whey protein isolate (84% protein) was dissolved in potassium phosphate buffer 0.1M pH 7.8 at a concentration of 5 mg/ml, heated at 95 $^0$C for 5 min and then cooled down at room temperature. Trypsin was added at a ratio of 1:20 (w:w), and then incubated at 37 $^0$C in a water bath during 20 h. The obtained solution was heated at 95 $^0$C for 5 min to inactivate the enzyme and then frozen for posterior use. The free amino acid content of the hydrolyzed protein was analyzed and reported (Table 1). The non-hydrolyzed whey protein isolate did not contain free amino acids in detectable amounts.

The protein hydrolyzate was furthermore characterized with size exclusion chromatography as shown in Figure 1 and outlined below.

**Size exclusion chromatography.**

Samples of whey protein and hydrolyzed whey protein were analyzed with an ÄKTA explorer LC coupled to a UV detector (GE Healthcare, Zaventem, Belgium) equipped with a Superdex Peptide 10/300 GL column (GE Healthcare, Zaventem). Working conditions were: mobile phase - ammonium hydrogen carbonate buffer 0.15M pH 7.8; flow - 0.5 ml/min; UV detection - 214 nm.

**Free amino acid analysis**.

Samples of whey protein and hydrolyzed whey protein were treated with a solution of 15% of trichloroacetic acid, further centrifugated, filtered and analyzed using HPLC – analysis with fluorescence detection.[20]

**Selection of the reaction conditions**.

**Thermal conditions:**

**Sterilization conditions:** Samples were mixed in a volumetric flask and the volume was adjusted to 5 ml with 0.1M potassium phosphate buffer pH 7.8, then transferred to a SPME vial (Gerstel, Mülheim, Germany) and closed with a pressure cap (silicon/PTFE 55º shore A 1.5mm magnetic. Gerstel, Mülheim). The vial was heated in a stirring oil bath at 130 $^{0}$C to obtain an inside temperature of 120 $^{0}$C, for 2 h, after which the vials were immediately cooled down in an ice bath.

**Roasting conditions:** Samples were mixed in a volumetric flask and the volume was adjusted to 5 ml with 0.1M potassium phosphate buffer pH 7.8, then transferred to a SPME vial containing 1.5 g of sand. Subsequently the samples were frozen and

freeze dried in a VaCo5 freeze dryer (Zirbus Technology, Bad Grund, Germany) to ensure sample homogeneity. Finally, the samples were closed with pressure caps and heated in an oven (Memmert, Fisher scientific, Erembodegem) at 180 $^{0}$C for 90 min. Preliminary experiments showed that a considerable improvement of the reproducibility could be obtained dissolving, freezing and freezedrying instead of dry mixing the various ingredients.

**Reactant type.**

Two groups of samples were analyzed to evaluate the yields of pyrazines formation under roasting and sterilization conditions.

Samples containing hydrolyzed whey protein and glucose were prepared at 8 different ratios (protein:glucose) (w:w) ranging from 1:1.33 to 1:0.08 as indicated in Table 2. Samples containing lysine and glucose were prepared at 8 different ratios (lysine: glucose) (w:w) ranging from 1:2.53 to 1:0.15 as indicated in Table 3. The samples were analyzed using HS SPME – GC MS.

**Elucidation of the role of protein hydrolysis products on the formation of pyrazines:**

Four groups of model systems were prepared as follows:

(1) 15 mg of hydrolyzed whey protein and 15 mg of glucose, (2) 15 mg native whey protein and 15 mg glucose, (3) 15 mg native whey protein, 15 mg glucose and the addition of a mixture of free amino acids corresponding to the free amino acid content of the tryptic digestion of whey protein as reported in Table 1, (4) a mixture of free amino acids corresponding to the free amino acid content of the tryptic digestion of whey protein as reported in Table 1 and 15 mg glucose.

The reaction mixtures were dissolved in 5ml 0.1M potassium phosphate buffer pH 7.8 and then transferred to SPME vials containing 1.5 g of sand, freeze dried, closed with pressure caps and thermally treated under previously described roasting conditions. The samples were analyzed using HS SPME – GC MS.

**Impact of water activity on pyrazine generation from hydrolyzed whey protein.**

Samples containing 15 mg of hydrolyzed whey protein and 7.5 mg of glucose were dissolved in 5ml 0.1M potassium phosphate buffer pH 7.8 and transferred to SPME vials containing 1.5 g of sand. Subsequently the vials were freezedried and immediately capped. Afterwards, the aw value of the samples was determined using a 4TEV dew point water activity meter (Aqualab, Decagon Devices, Pullman, USA) obtaining an initial value of 0.16.

Subsequently freezedried samples were transferred to different hermetic plastic recipients containing saturated solutions of: magnesium chloride (32% RH), potassium carbonate (43% RH), sodium bromide (56% RH), potassium chloride (67% RH), sodium chloride (75% RH). All the samples were incubated in triplicates during 7 days at 30 $^{0}$C inside an incubator (Memmert, Fisher scientific, Erembodegem) in order to reach the desired water activity in each sample. After reaching equilibrium, all samples were thermally treated under previously described roasting conditions and analyzed by means of HS SPME – GC MS.

**HS SPME – GCMS analysis.**

The volatiles produced during the different experimental conditions were extracted by means of headspace solid – phase microextraction (HS – SPME) for 30 minutes at 35 $^{0}$C with a DVD/Car/PDMS fiber (Supelco, Bornem) with a multipurpose

sampler (MPS – 2) (Gerstel). GC – MS analyses of the SPME extract were performed with an Agilent 6890 GC Plus apparatus coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium) and equipped with an DB-5 capillary column (30 m length x 0.25 m i.d; 0.25 µm film thickness) (Agilent Technologies, Diegem). Working conditions were: transfer line to MSD 250 $^{0}$C, carrier gas (He) 1.0 ml/min; ionization: EI 70eV; acquisition parameters: scanned m/z: 40-200 (2-10 min), 40-300 (10-20 min), 40-400 (>20 min); oven temperature started at 35 $^{0}$C, held 5 min, programmed from 35 – 80 $^{0}$C at 2 $^{0}$C/min, held 2 min. Pyrazines were identified by comparison of the mass spectrum with mass spectral libraries (Nist 98, Wiley 6$^{th}$ and HPCH2205) and by comparison of the calculated linear retention indices with literature values.

The generation of pyrazines was followed in a semiquantitative way by considering the absolute peak area of each individual pyrazine[18, 21-22]. Although this approach does not allow absolute quantitation of each individual pyrazine, it is generally accepted as suitable to evaluate the pyrazine formation in a reliable way.

**Statistical analysis.**

All analyses were performed with SPSS Statistics version 22 at a significance level of 95% ($p = 0.050$). Data were normally distributed (Kolmogorov-Smirnov test: $p < 0.050$ for all standardized residuals). Therefore, one way ANOVA was selected for statistical analysis. The Games – Howell correction was applied to control the family-wise error rate at 5% for all multiple pairwise comparisons.

## RESULTS AND DISCUSSION

**Selection of the reaction conditions and ratio of the model systems.**

In a first series of experiments the impact of the reactant ratio (glucose and hydrolyed whey protein) on the formation of pyrazines was evaluated for both of thermal treatments applied.

The results shown in Table 2 indicate that during the roasting conditions (180 °C/90 min) significant quantities or the following pyrazines were produced in model systems containing hydrolyzed whey protein and glucose: 2-methylpyrazine, 2,5(6)-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 5-ethyl-2,3-dimethypyrazine.

The same reactants under sterilization conditions (120 °C/120 min) yielded mainly 2-methylpyrazine and 2,5(6)-dimethylpyrazine. However, the peak areas of the produced pyrazines were considerably lower compared to those observed during roasting conditions. These observations can be due to the fact that indeed a lower amount of pyrazines was produced, which can be explained by the different reaction conditions: temperature and presence of water in the systems. It should be noted as well, however, that by addition of 2 µg of 2-ethyl-3-methylpyrazine to similar amounts of non-thermally treated samples, a much higher peak area was observed for the dry ($107.89 \times 10^6$) vs the wet sample ($6.68 \times 10^6$). This indicated that the partitioning of the pyrazines between the samples and its headspace is totally different for a dry or aqueous sample.

The ratio between glucose and hydrolyzed whey protein played a crucial role in the formation of pyrazines. The generation of pyrazines under dry roasting conditions was low in model systems where glucose was present in low amounts (ratio 1:0.08 and 1:0.16). This effect can likely be explained since the amount of dicarbonyl compounds

generated from glucose degradation was low, which had an impact on the formation of pyrazines and resulted in lower peak areas. Higher peak areas were detected for ratios up to 1:0.5 and 1:0.67. At higher protein/glucose ratios, a decrease in the formation of pyrazines was again observed. This may be due to the excessive formation of different carboxylic acids such as acetic acid from glucose[23] which might decrease the pH of the model systems. It is generally known that the pH has an impact on the generation of pyrazines[1] hindering their formation at low pH due to the protonation of the amino groups of the amino acids and peptides. Also under sterilization conditions the peak area of the formed pyrazines was affected by the protein/glucose ratio although to a lesser extent.

It is broadly known that in Maillard reaction models free amino acids have a high reactivity. In order to compare the reactivity of the glucose/hydrolyzed whey protein with a glucose/amino acid mixture, lysine was selected. Pyrazine formation at different lysine/glucose ratios was monitored for both heating conditions see (Table 3). Considerably higher amounts of pyrazines were detected in the lysine containing reaction systems under both heating conditions when compared to hydrolyzed whey. In addition, the detected pyrazines were more diverse, particulary for the roasted samples. Under roasting conditions, the detected amounts of 2-methylpyrazine and 2,5(6)-dimethylpyrazine were considerably less influenced by the lysine/glucose ratio. However the lysine/glucose ratio did have a considerable effect on the amount of pyrazines detected. At the lower lysine/glucose ratios, formation of pyrazines in roasting conditions decreased considerably, while this tendency was not observed under sterilization conditions. These experiments showed that depending upon the amino

compounds participating in the reactions such as free amino acids or peptides involved in the pyrazine formation, the impact of the amino compounds/glucose ratio on the pyrazine formation will be different.

As it was noticed that considerable more pyrazines were detected under roasting conditions (180°C/90 min) either for glucose/hydrolyzed whey protein and glucose/lysine models, this heat treatment was further on used.

**Elucidation of the role of protein hydrolysis products on the formation of pyrazines:**

From the previous experiments it was obvious that the hydrolyzed whey proteins were able to generate pyrazines in Maillard reaction systems. However, it was not clear if the compounds responsible for the pyrazine generation are the free amino acids produced during hydrolysis (**Table 1**), or the peptidic fraction, or both. Therefore the formation of pyrazines by the hydrolyzed whey protein was compared with the pyrazine generation in a mixture of non-hydrolyzed proteins to which the free amino acids formed upon trypsinogenic hydrolysis were added according to the amounts reported in (**Table 1**). Non-hydrolyzed whey protein was included as a control. In addition, glucose together with the free amino acids generated upon trypsinogenic hydrolysis of whey was considered as well. Experiments were conducted under dry heating conditions considering two protein/glucose ratios.

In model systems containing free amino acids as described in Table 1 and glucose, no pyrazines were detected (data not shown). Most likely the amount of reagents was not sufficient to generate sufficient Maillard products. Model systems containing hydrolyzed whey protein and glucose led to the formation of the highest

amounts of pyrazines (Table 4). In model systems containing native whey protein and glucose, the generation of pyrazines was significantly lower than in samples of hydrolyzed whey. The native whey protein isolate did not contain any free amino acids, suggesting that the limited generation of pyrazines was due to thermal degradation of the protein in the selected reaction conditions, releasing amino compounds which can react with glucose and form pyrazines. Model systems, containing native whey with the addition of the mixture of free amino acids (**Table 1**) and glucose, produced comparable and statistically similar amounts of pyrazines as the model systems containing the native whey protein isolate and glucose.

These results allowed to conclude that the amount of free amino acids present in the whey hydrolyzate was not sufficient to produce pyrazines in measurable amounts using the heating conditions earlier described. Compared to the results from the experiments with the hydrolyzed whey, it is obvious that the peptides generated upon hydrolysis played an important role in the generation of pyrazines.

Regarding the pathways that involve the peptidic fraction into the generation of α-aminoketones and their posterior condensation into pyrazines, Oh et al.[4] studied model systems with glycine containing di-, tri- and tetrapeptides. The authors suggested that tetraglycine could be degraded to diglycine and further degraded into glycine to produce pyrazines. Simultaneously, triglycine was suggested to be degraded to diglycine and glycine. However, these results are limited to glycine-containing peptides. Yan et al.[24] studied the peptide bond cleavages during the Maillard reaction and their results show that some peptide bonds can be more resistant or labile depending on the amino acid sequence in the peptide chain. Therefore, the use of di-, tri- and tetraglycine can only

represent some peptides. The mechanism previously depicted in Figure 2 implies the formation of pyrazines in model systems containing dipeptides and dicarbonyl compounds. The alternative mechanism was suggested since dipeptides cannot follow the typical Strecker degradation due the absence of a free carboxyl group to form α-aminoketones.

It is obvious that a similar mechanism can be responsible for the formation of pyrazine from oligopeptides as well.

**Impact of water activity on pyrazine generation from hydrolyzed whey protein.**

Lu et al.[25] studied the effects of the water content on volatile generation and peptide degradation in the Maillard reaction using glycerol to limit the water content of the samples. However, Smarrito-Menozzi.[26] reported that glycerol is an underestimated flavor precursor in the Maillard reaction. Therefore, the water content of the samples was controlled by equilibrating them in recipients filled with saturated salt solutions, obtaining atmospheres with fixed relative humidities as described by Greenspan et al.[27]. Thus, after equilibration, samples with a particular water activity were obtained.

The results presented in Table 5 show that the model systems incubated with a water activity of 0.33 are the ones which produced the highest amounts and variety of pyrazines. This may be explained by the potential effect of water activity on the peptide degradation. Oliyai et al.[28] studied the chemical stability of a hexapeptide under several factors such as pH, temperature and moisture content finding that the moisture content degraded significantly the hexapeptide, leading to an increased reactivity. In addition water activity has been pointed out to have an impact on reaction rates[29] since water in food often acts as a plasticizer, leading to enhanced mobility and chemical reactivity as

well.[30] However, increasing the water activity to values higher than 0.33 lead progressively to a lower generation of pyrazines, which can be explained by the fact that water is a product of several condensation steps in the Maillard reaction.[31].

This is the first paper systematically evaluating the impact of peptides generated by trypsinogenic hydrolysis on the formation of pyrazines via the Maillard reaction. The results suggest that the contribution of peptides to the generation of pyrazines is considerably high, while the role of free amino acids is only minor and more than likely less important.

The role of peptides in food on the generation of pyrazines has probably been underestimated and requires more attention.

**Aknowledgements**

The authors thank the Erasmus Mundus program for the financial support of this investigation. Siele Ceuppens, Monica Ovando Chaves, Laurens De Coen and Luke Sebastian Scalone are acknowledged for their valuable advice.

## REFERENCES

(1) Nursten, H. E., *The Maillard reaction chemistry, biochemistry, and implications*. Royal Society of Chemistry: 2005.
(2) Van Lancker, F.; Adams, A.; De Kimpe, N., Chemical modifications of peptides and their impact on food properties. *Chemical Reviews* **2011,** *111*, 7876-7903.
(3) Ho, C. T.; Oh, Y. C.; Zhang, Y. G.; Shu, C. K., Peptides as flavor precursors in model Maillard reactions *Acs Symposium Series* **1992,** *490*, 193-202.
(4) Oh, Y. C.; Shu, C. K.; Ho, C. T., Some volatile compounds formed from thermal interaction of glucose with glycine, diglycine, triglycine, and tetraglycine. *J. Agric. Food Chem.* **1991,** *39*, 1553-1554.
(5) Arnoldi, A., Thermal processing and nutritional quality. *The Nutrition Handbook for Food Processing* **2002**, 265-286.
(6) Jing, H.; Kitts, D. D., Chemical and biochemical properties of casein-sugar Maillard reaction products. *Food Chem. Toxicol.* **2002,** *40*, 1007-1015.
(7) Ferretti, A.; Flanagan, V. P.; Ruth, J. M., Nonenzymatic browning in a lactose-casein model system. *J. Agric. Food Chem.* **1970,** *18*, 13-18.
(8) Ferretti, A.; Flanagan, V. P., Lactose-casein (Maillard) browning system - volatile components. *J. Agric. Food Chem.* **1971,** *19*, 245-249.
(9) Schlichtherle-Cerny, H.; Amado, R., Analysis of taste-active compounds in an enzymatic hydrolysate of deamidated wheat gluten. *J. Agric. Food Chem.* **2002,** *50*, 1515-1522.
(10) Hashiba, H., Glucose-diglycine condensation product participating in oxygen-dependent browning. *J. Agric. Food Chem.* **1975,** *23*, 539-542.
(11) Jiang, Z. M.; Brodkorb, A., Structure and antioxidant activity of Maillard reaction products from alpha-lactalbumin and beta-lactoglobulin with ribose in an aqueous model system. *Food Chem.* **2012,** *133*, 960-968.
(12) Zhang, Y. G.; Chien, M. J.; Ho, C. T., Comparison of the volatile compounds obtained from thermal-degradation of cysteine and glutathione in water. *J. Agric. Food Chem.* **1988,** *36*, 992-996.
(13) Zhang, Y. G.; Ho, C. T., Volatile compounds formed from thermal interaction of 2,4-decadienal with cysteine and glutathione. *J. Agric. Food Chem.* **1989,** *37*, 1016-1020.
(14) Zhang, Y. G.; Ho, C. T., Comparison of the volatile compounds formed from the thermal-reaction of glucose with cysteine and glutathione. *J. Agric. Food Chem.* **1991,** *39*, 760-763.
(15) Zhang, Y. G.; Ho, C. T., Formation of meatlike aroma compounds from thermal-reaction of inosine 5'-monophosphate with cysteine and glutathione. *J. Agric. Food Chem.* **1991,** *39*, 1145-1148.
(16) Maga, J. A., Pyrazine update *Food Reviews International* **1992,** *8*, 479-558.
(17) Adams, A.; De Kimpe, N., Chemistry of 2-acetyl-1-pyrroline, 6-acetyl-1,2,3,4-tetrahydropyridine, 2-acetyl-2-thiazoline, and 5-acetyl-2,3-dihydro-4H-thiazine: Extraordinary Maillard flavor compounds. *Chemical Reviews* **2006,** *106*, 2299-2319.
(18) Van Lancker, F.; Adams, A.; De Kimpe, N., Formation of pyrazines in Maillard model systems of lysine-containing dipeptides. *J. Agric. Food Chem.* **2010,** *58*, 2470-2478.
(19) Van Lancker, F.; Adams, A.; De Kimpe, N., Impact of the N-terminal amino acid on the formation of pyrazines from peptides in maillard model systems. *J. Agric. Food Chem.* **2012,** *60*, 4697-4708.
(20) Mestdagh, F.; Kerkaert, B.; Cucu, T.; De Meulenaer, B., Interaction between whey proteins and lipids during light-induced oxidation. *Food Chem.* **2011,** *126*, 1190-1197.
(21) Adams, A.; Borrelli, R. C.; Fogliano, V.; De Kimpe, N., Thermal degradation studies of food melanoidins. *J. Agric. Food Chem.* **2005,** *53*, 4136-4142.

(22) Yu, A. N.; Zhang, A. D., The effect of pH on the formation of aroma compounds produced by heating a model system containing L-ascorbic acid with L-threonine/L-serine. *Food Chem.* **2010,** *119*, 214-219.
(23) Davidek, T.; Robert, F.; Devaud, S.; Vera, F. A.; Blank, I., Sugar fragmentation in the Maillard reaction cascade: Formation of short-chain carboxylic acids by a new oxidative alpha-dicarbonyl cleavage pathway. *J. Agric. Food Chem.* **2006,** *54*, 6677-6684.
(24) Yang, C.; Wang, R.; Song, H. L., The mechanism of peptide bonds cleavage and volatile compounds generated from pentapeptide to heptapeptide via Maillard reaction. *Food Chem.* **2012,** *133*, 373-382.
(25) Lu, C. Y.; Hao, Z. G.; Payne, R.; Ho, C. T., Effects of water content on volatile generation and peptide degradation in the Maillard reaction of glycine, diglycine, and triglycine. *J. Agric. Food Chem.* **2005,** *53*, 6443-6447.
(26) Smarrito-Menozzi, C.; Matthey-Doret, W.; Devaud-Goumoens, S.; Viton, F., Glycerol, an underestimated flavor precursor in the Maillard reaction. *J. Agric. Food Chem.* **2013,** *61*, 10225-10230.
(27) Greenspan, L., Humidity fixed-points of binary saturated aqueous-solutions. *J Res Nbs a Phys Ch* **1977,** *81*, 89-96.
(28) Oliyai, C.; Borchardt, R. T., Chemical pathways of peptide degradation. VI. Effect of the primary sequence on the pathways of degradation of aspartyl residues in model hexapeptides. *Pharm. Res.* **1994,** *11*, 751-8.
(29) Labuza, T. P., The effect of water activity on reaction-kinetics of food deterioration. *Food Technol-Chicago* **1980,** *34*, 36-40.
(30) Roos, Y. H., Glass transition-related physicochemical changes in foods. *Food Technol-Chicago* **1995,** *49*, 97-102.
(31) Eichner, K.; Karel, M., Influence of water-content and water activity on sugar amino browning reaction in model systems under various conditions. *J. Agric. Food Chem.* **1972,** *20*, 218-223.

## TABLES AND ARTWORK

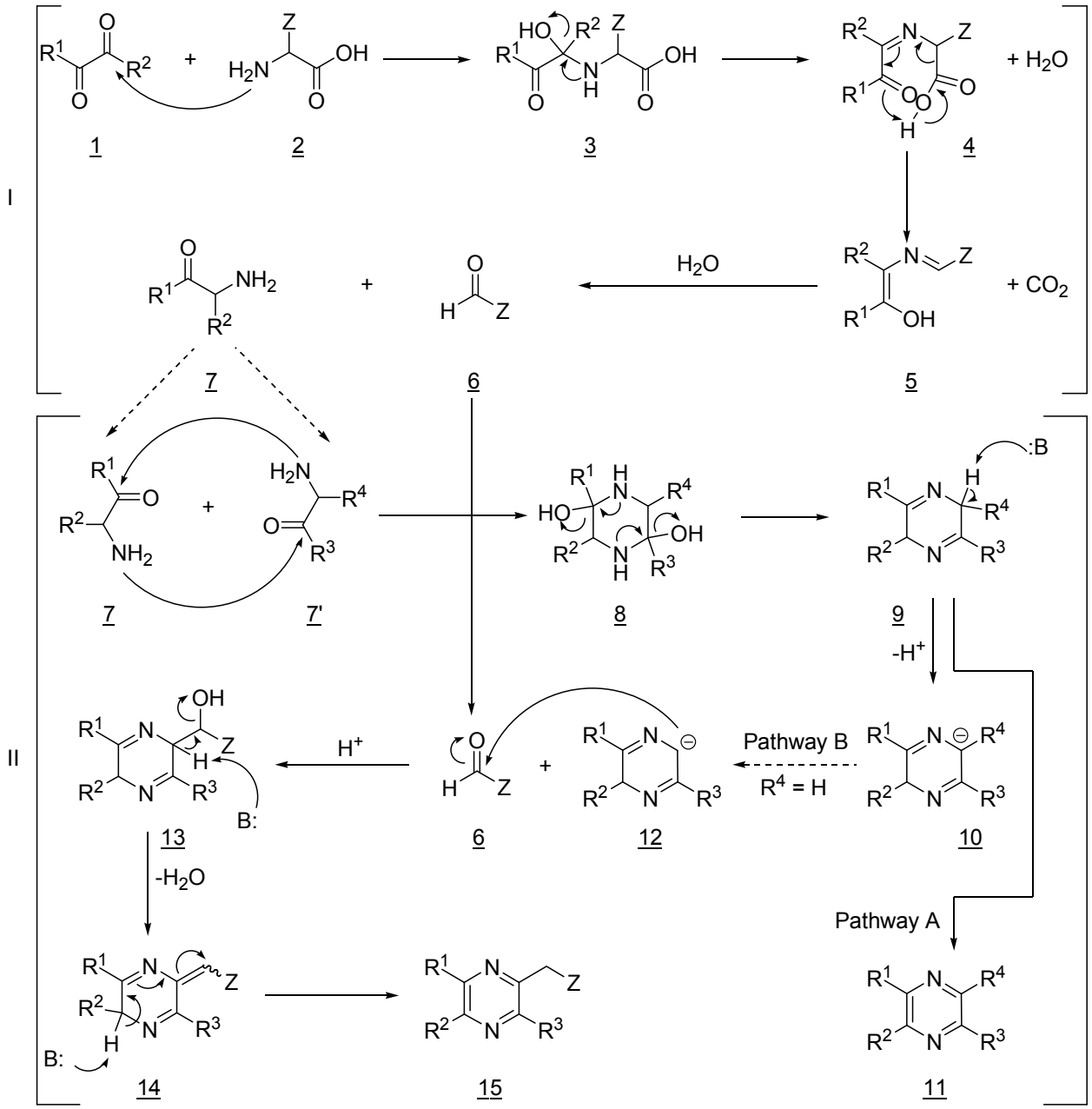


**Figure 1**. Strecker degradation (part I) and pyrazine formation (part II)

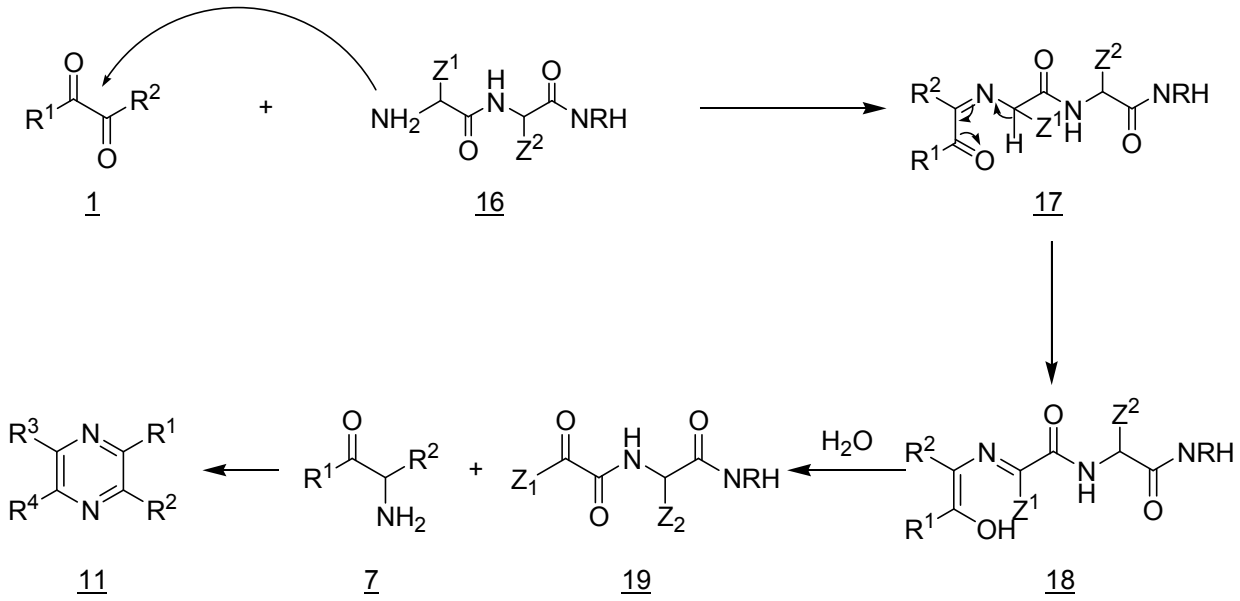


**Figure 2.** Hypothetical formation mechanism of α-aminoketones from the reaction of peptides with an α-dicarbonyl compound. Adapted from Van Lancker et al. [18]

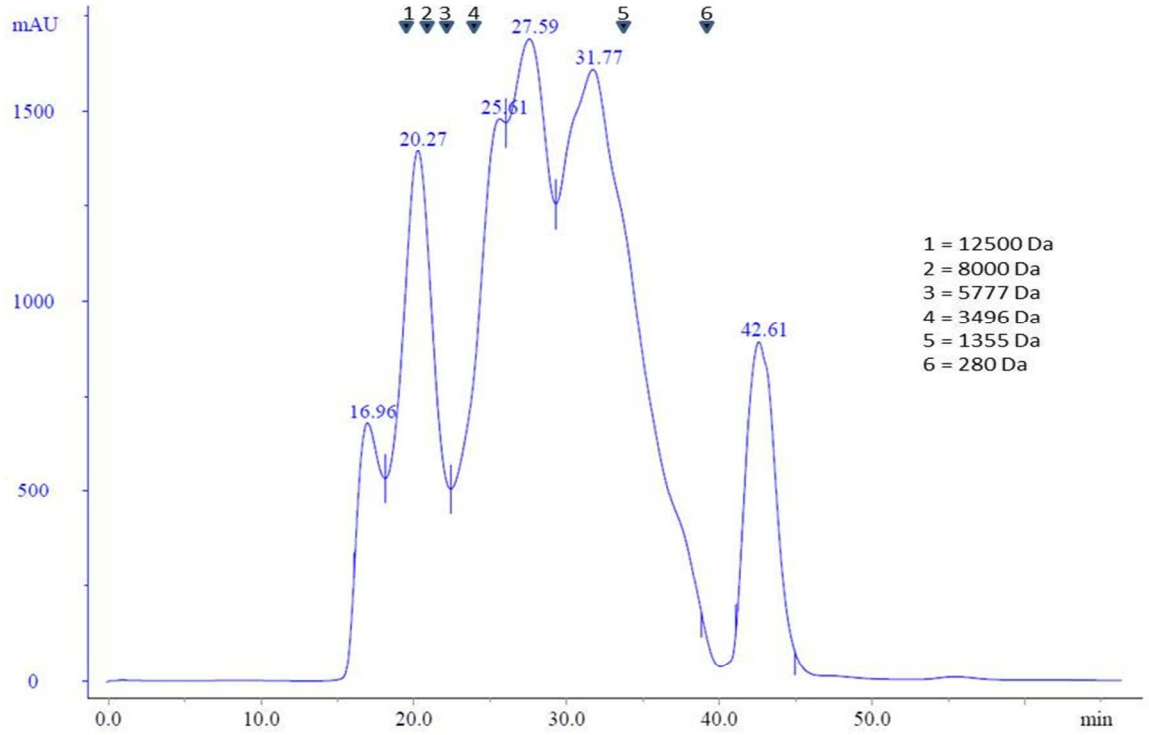


**Figure 3**. Gel permeation chromatogram of hydrolyzed whey protein after tryptic hydrolysis. Non-hydrolyzed whey elutes as one unique peak at 16.96 minutes. Peak position for weight markers are shown on top.

**Table 1**. Free amino acid composition of native whey protein isolate under tryptic hydrolysis

| Amino acid | mg/100 mg hydrolyzed whey |
|---|---|
| **Lysine** | 0.9916 |
| **Leucine** | 0.1340 |
| **Valine** | 0.0960 |
| **Isoleucine** | 0.0728 |
| **Arginine** | 0.0546 |
| **Tyrosine** | 0.0421 |
| **Threonine** | 0.0389 |
| **Tryptophan** | 0.0359 |
| **Phenylalanine** | 0.0297 |
| **Methionine** | 0.0218 |
| **Alanine** | 0.0104 |

**Table 2**. Pyrazines (GC-MS peak Area x 10 $^{6}$) detected in model reactions of dry glucose and hydrolyzed whey mixtures under roasting conditions (180 ºC/90 min.), and aqueous glucose and hydrolyzed whey solutions under sterilization conditions (120 ºC/120 min.).

| Compund | Experimental conditions | Hydrolyzed whey 15 mg Glucose 1.25 mg | Hydrolyzed whey 15 mg Glucose 2.5 mg | Hydrolyzed whey 15 mg Glucose 5 mg | Hydrolyzed whey 15 mg Glucose 7.5 mg | Hydrolyzed whey 15 mg Glucose 10 mg | Hydrolyzed whey 15 mg Glucose 15 mg | Hydrolyzed whey 15 mg Glucose 17.5 mg | Hydrolyzed whey 15 mg Glucose 20 mg |
|---|---|---|---|---|---|---|---|---|---|
| **2-Methylpyrazine** $^{\alpha}$ | Roasting | 4.15 ± 0.12 $^{a}$ | 9.46 ± 0.23 $^{b}$ | 16.21 ± 0.47 $^{c}$ | 23.10 ± 0.36 $^{d}$ | 22.54 ± 1.95 $^{cd}$ | 20.97 ± 1.20 $^{cd}$ | 18.86 ± 1.47 $^{cd}$ | 20.87 ± 0.65 $^{d}$ |
| | Sterilization | N/D | N/D | 0.94 ± 0.04 $^{a}$ | 1.75 ± 0.01 $^{b}$ | 3.46 ± 0.24 $^{c}$ | 5.45 ± 0.08 $^{de}$ | 5.35 ± 0.29 $^{de}$ | 6.32 ± 0.39 $^{e}$ |
| **2,5(6)-Dimethylpyrazine** $^{\alpha}$ | Roasting | 24.45 ± 1.35 $^{a}$ | 35.22 ± 1.88 $^{b}$ | 37.81 ± 1.23 $^{bd}$ | 42.39 ± 3.04 $^{d}$ | 35.25 ± 2.01 $^{b}$ | 25.41 ± 1.83 $^{a}$ | 22.75 ± 2.22 $^{a}$ | 20.22 ± 1.26 $^{a}$ |
| | Sterilization | N/D | 5.65 ± 0.52 $^{a}$ | 6.75 ± 0.66 $^{ac}$ | 9.64 ± 0.41 $^{be}$ | 7.49 ± 0.19 $^{c}$ | 11.16 ± 0.04 $^{d}$ | 8.32 ± 0.20 $^{e}$ | 9.66 ± 0.14 $^{b}$ |
| **2,3-Dimethylpyrazine** $^{\alpha}$ | Roasting | N/D | N/D | 1.13 ± 0.03 $^{a}$ | 1.80 ± 0.07 $^{b}$ | 2.10 ± 0.15 $^{c}$ | 2.16 ± 0.01 $^{c}$ | 2.54 ± 0.14 $^{d}$ | 2.11 ± 0.03 $^{c}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethylpyrazine** $^{\alpha}$ | Roasting | N/D | N/D | 3.45 ± 0.04 $^{a}$ | 4.24 ± 0.19 $^{b}$ | 4.29 ± 0.16 $^{b}$ | 4.73 ± 0.06 $^{c}$ | 4.96 ± 0.22 $^{c}$ | 4.92 ± 0.12 $^{c}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethyl-5-methylpyrazine** $^{\alpha}$ | Roasting | 3.79 ± 0.20 $^{a}$ | 8.22 ± 0.44 $^{bd}$ | 10.84 ± 0.21 $^{c}$ | 11.00 ± 0.51 $^{c}$ | 9.38 ± 0.48 $^{d}$ | 8.80 ± 0.05 $^{bd}$ | 9.32 ± 0.50 $^{d}$ | 7.92 ± 0.37 $^{b}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **3-Ethyl-2,5-dimethylpyrazine** $^{\alpha}$ | Roasting | 3.77 ± 0.18 $^{a}$ | 6.70 ± 0.39 $^{b}$ | 6.78 ± 0.33 $^{b}$ | 5.14 ± 0.13 $^{c}$ | 3.59 ± 0.33 $^{a}$ | 3.37 ± 0.18 $^{a}$ | 3.43 ± 0.21 $^{a}$ | 2.42 ± 0.14 $^{da}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethyl-3,5-dimethylpyrazine** $^{\beta}$ | Roasting | N/D | N/D | 2.07 ± 0.04 $^{a}$ | 2.28 ± 0.14 $^{a}$ | 2.04 ± 0.16 $^{a}$ | 1.54 ± 0.08 $^{ba}$ | 1.26 ± 0.56 $^{b}$ | 1.27 ± 0.09 $^{b}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **5-Ethyl-2,3-dimethylpyrazine** $^{\beta}$ | Roasting | N/D | N/D | 1.22 ± 0.05 $^{a}$ | 1.06 ± 0.06 $^{a}$ | 1.03 ± 0.08 $^{a}$ | 1.16 ± 0.08 $^{a}$ | 1.23 ± 0.11 $^{a}$ | 1.12 ± 0.03 $^{a}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |

N/D (not detected). $^{\alpha}$ Identification confirmed by GC retention index and mass spectra of authentic compounds. $^{\beta}$ Tentatively identified by matching mass spectra library.
Data points represent mean values of 3 independent determinations. Values in the same line followed by different superscript letters are significantly different ($p < 0.05$).

**Table 3**. Pyrazines (GC-MS peak Area x $10^{6}$) detected in model reactions of dry glucose and lysine mixtures under roasting conditions (180 ºC/90 min.), and aqueous glucose and lysine solutions under sterilization conditions (120 ºC/120 min.).

| Compund | Experimental conditions | Lysine 14.61 mg Glucose 2.5 mg | Lysine 14.61 mg Glucose 4.5 mg | Lysine 14.61 mg Glucose 9 mg | Lysine 14.61 mg Glucose 13.5 mg | Lysine 14.61 mg Glucose 18 mg | Lysine 14.61 mg Glucose 22.5 mg | Lysine 14.61 mg Glucose 27 mg | Lysine 14.61 mg Glucose 31.5 mg | Lysine 14.61 mg Glucose 37 mg |
|---|---|---|---|---|---|---|---|---|---|---|
| **2-Methylpyrazine** $^{\alpha}$ | Roasting | 49.05 ± 0.11 $^{a}$ | 36.80 ± 0.15 $^{b}$ | 35.96 ± 0.83 $^{b}$ | 42.74 ± 0.01 $^{c}$ | 48.49 ± 0.87 $^{a}$ | 45.16 ± 0.01 $^{d}$ | 40.69 ± 1.13 $^{e}$ | 43.36 ± 0.18 $^{cd}$ | 40.57 ± 0.08 $^{e}$ |
| | Sterilization | N/D | 6.75 ± 0.47 $^{a}$ | 13.28 ± 0.19 $^{b}$ | 15.11 ± 0.39 $^{bc}$ | 16.97 ± 0.40 $^{c}$ | 25.45 ± 1.15 $^{d}$ | 30.33 ± 1.46 $^{e}$ | 34.82 ± 2.04 $^{f}$ | 38.87 ± 1.14 $^{g}$ |
| **2,5(6)-Dimethylpyrazine** $^{\alpha}$ | Roasting | 430.58 ± 0.01 $^{a}$ | 426.55 ± 3.67 $^{a}$ | 402.27 ± 1.03 $^{b}$ | 450.48 ± 1.28 $^{c}$ | 448.55 ± 0.51 $^{c}$ | 433.84 ± 3.74 $^{a}$ | 392.45 ± 9.19 $^{b}$ | 293.06 ± 0.63 $^{d}$ | 251.48 ± 1.27 $^{e}$ |
| | Sterilization | N/D | 8.92 ± 0.77 $^{a}$ | 19.07 ± 1.37 $^{b}$ | 25.24 ± 0.59 $^{dc}$ | 20.95 ± 1.74 $^{b}$ | 27.52 ± 0.57 $^{c}$ | 29.69 ± 1.33 $^{c}$ | 25.57 ± 0.45 $^{c}$ | 24.37 ± 1.14 $^{d}$ |
| **2,3-Dimethylpyrazine** $^{\alpha}$ | Roasting | 2.23 ± 0.03 $^{a}$ | 4.12 ± 0.02 $^{b}$ | 10.35 ± 0.22 $^{c}$ | 13.81 ± 0.11 $^{d}$ | 15.11 ± 0.13 $^{e}$ | 14.62 ± 0.07 $^{f}$ | 12.62 ± 0.27 $^{g}$ | 10.62 ± 0.03 $^{c}$ | 8.07 ± 0.05 $^{h}$ |
| | Sterilization | N/D | 1.65 ± 0.16 $^{a}$ | 2.39 ± 0.06 $^{b}$ | 2.86 ± 0.17 $^{bc}$ | 2.42 ± 0.03 $^{b}$ | 3.17 ± 0.12 $^{c}$ | 3.34 ± 0.29 $^{c}$ | 3.41 ± 0.29 $^{c}$ | 3.20 ± 0.23 $^{c}$ |
| **2-Ethylpyrazine** $^{\alpha}$ | Roasting | N/D | N/D | 8.37 ± 0.12 $^{a}$ | 13.44 ± 0.16 $^{b}$ | 15.37 ± 0.08 $^{c}$ | 15.31 ± 0.33 $^{c}$ | 11.81 ± 0.06 $^{d}$ | 10.58 ± 0.06 $^{e}$ | 8.88 ± 0.02 $^{f}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethyl-5-methylpyrazine** $^{\alpha}$ | Roasting | 45.50 ± 0.50 $^{a}$ | 68.30 ± 0.16 $^{b}$ | 211.13 ± 2.29 $^{c}$ | 239.29 ± 0.69 $^{d}$ | 278.17 ± 0.20 $^{e}$ | 263.29 ± 1.66 $^{f}$ | 182.96 ± 0.53 $^{g}$ | 117.95 ± 2.19 $^{h}$ | 75.22 ± 0.04 $^{i}$ |
| | Sterilization | N/D | N/D | N/D | 1.10 ± 0.05 $^{a}$ | 1.56 ± 0.08 $^{b}$ | 2.00 ± 0.05 $^{c}$ | 2.53 ± 0.05 $^{d}$ | 2.75 ± 0.25 $^{d}$ | 2.84 ± 0.08 $^{d}$ |
| **3-Ethyl-2,5-dimethylpyrazine** $^{\alpha}$ | Roasting | 47.89 ± 0.02 $^{a}$ | 67.30 ± 0.57 $^{b}$ | 89.62 ± 0.92 $^{c}$ | 115.96 ± 2.02 $^{d}$ | 121.61 ± 0.80 $^{e}$ | 135.47 ± 1.47 $^{f}$ | 91.24 ± 0.07 $^{c}$ | 48.87 ± 0.56 $^{a}$ | 26.95 ± 0.57 $^{g}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethyl-3,5-dimethylpyrazine** $^{\beta}$ | Roasting | 4.80 ± 0.10 $^{a}$ | 10.58 ± 0.20 $^{b}$ | 32.99 ± 0.17 $^{cd}$ | 31.58 ± 0.51 $^{d}$ | 31.68 ± 0.32 $^{d}$ | 32.41 ± 2.70 $^{cd}$ | 35.29 ± 0.55 $^{c}$ | 17.83 ± 0.43 $^{e}$ | 9.96 ± 0.11 $^{b}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **5-Ethyl-2,3-dimethylpyrazine** $^{\beta}$ | Roasting | 7.86 ± 0.09 $^{a}$ | 22.07 ± 0.20 $^{b}$ | 101.59 ± 3.64 $^{c}$ | 191.70 ± 5.51 $^{d}$ | 222.19 ± 0.69 $^{e}$ | 219.87 ± 6.20 $^{e}$ | 85.99 ± 0.39 $^{f}$ | 32.70 ± 0.01 $^{b}$ | 13.72 ± 0.79 $^{ab}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2,5-Diethylpyrazine** $^{\beta}$ | Roasting | N/D | 9.69 ± 0.09 $^{a}$ | 56.94 ± 0.90 $^{b}$ | 89.30 ± 1.28 $^{c}$ | 94.04 ± 0.60 $^{c}$ | 80.42 ± 4.16 $^{d}$ | 23.77 ± 0.10 $^{e}$ | 8.07 ± 0.58 $^{a}$ | 2.70 ± 0.17 $^{f}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2,3-Diethyl-5-methylpyrazine** $^{\beta}$ | Roasting | N/D | 3.11 ± 0.07 $^{a}$ | 7.44 ± 0.07 $^{b}$ | 11.18 ± 0.54 $^{c}$ | 14.75 ± 0.01 $^{d}$ | 16.65 ± 0.54 $^{e}$ | 11.59 ± 0.29 $^{c}$ | 5.59 ± 0.02 $^{f}$ | 2.75 ± 0.07 $^{a}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **3,5-Diethyl-2-methylpyrazine** $^{\beta}$ | Roasting | N/D | 2.95 ± 0.09 $^{a}$ | 10.30 ± 0.16 $^{b}$ | 14.60 ± 0.32 $^{c}$ | 18.20 ± 0.03 $^{d}$ | 15.09 ± 0.75 $^{c}$ | 8.40 ± 0.15 $^{e}$ | 3.36 ± 0.13 $^{a}$ | 1.37 ± 0.06 $^{f}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Ethyl-3,5,6-trimethylpyrazine** $^{\beta}$ | Roasting | N/D | 3.29 ± 0.06 $^{a}$ | 8.43 ± 0.16 $^{b}$ | 13.05 ± 0.70 $^{c}$ | 19.25 ± 0.11 $^{d}$ | 16.99 ± 0.43 $^{e}$ | 9.88 ± 0.14 $^{f}$ | 4.50 ± 0.02 $^{g}$ | 2.38 ± 0.23 $^{a}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |
| **2-Isopropyl-3,5-dimethylpyrazine** $^{\beta}$ | Roasting | N/D | 11.11 ± 0.10 $^{a}$ | 8.86 ± 0.06 $^{b}$ | 9.38 ± 0.10 $^{c}$ | 10.72 ± 0.19 $^{d}$ | 12.41 ± 0.08 $^{e}$ | 9.38 ± 0.11 $^{c}$ | 6.59 ± 0.09 $^{f}$ | 2.78 ± 0.11 $^{g}$ |
| | Sterilization | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D | N/D |

N/D (not detected). $^{\alpha}$ Identification confirmed by GC retention index and mass spectra of authentic compounds. $^{\beta}$ Tentatively identified by matching mass spectra library. Data points represent mean values of 3 independent determinations. Values in the same line followed by different superscript letters are significantly different ($p < 0.05$).

**Table 4.** Pyrazines (GC-MS peak Area x 10 $^{6}$) detected in model reactions of dry mixtures of glucose and hydrolyzed whey, whey and whey with the addition of an amount of free amino acids as described in Table 1, under roasting conditions (180 ºC/90 min)

| Compound | Hydrolyzed whey 15 mg Glucose 7.5 mg | Hydrolyzed whey 15 mg Glucose 15 mg | Whey 15 mg Glucose 7.5 mg | Whey 15 mg Glucose 15 mg | Whey 15 mg + AA (*) Glucose 7.5 mg | Whey 15 mg + AA (*) Glucose 15 mg |
|---|---|---|---|---|---|---|
| **2-Methylpyrazine** $^{\alpha}$ | 23.10 ± 0.36 $^{a}$ | 20.97 ± 1.20 $^{b}$ | 8.99 ± 0.62 $^{c}$ | 8.53 ± 0.26 $^{c}$ | 9.66 ± 1.05 $^{d}$ | 8.06 ± 0.66 $^{e}$ |
| **2,5(6)-Dimethylpyrazine** $^{\alpha}$ | 42.39 ± 3.04 $^{a}$ | 25.41 ± 1.83 $^{b}$ | 9.02 ± 0.69 $^{c}$ | 9.29 ± 0.82 $^{c}$ | 10.99 ± 1.17 $^{d}$ | 9.37 ± 0.84 $^{c}$ |
| **2,3-Dimethylpyrazine** $^{\alpha}$ | 1.80 ± 0.07 $^{a}$ | 2.17 ± 0.01 $^{a}$ | N/D | N/D | N/D | N/D |
| **2-Ethylpyrazine** $^{\alpha}$ | 4.23 ± 0.19 $^{a}$ | 4.73 ± 0.06 $^{a}$ | N/D | N/D | N/D | N/D |
| **2-Ethyl-5-methylpyrazine** $^{\alpha}$ | 10.70 ± 0.11 $^{a}$ | 8.81 ± 0.05 $^{b}$ | 1.64 ± 0.05 $^{c}$ | 3.50 ± 0.13 $^{d}$ | 3.53 ± 0.12 $^{d}$ | 3.44 ± 0.42 $^{d}$ |
| **3-Ethyl-2,5-dimethylpyrazine** $^{\alpha}$ | 5.14 ± 0.13 $^{a}$ | 3.38 ± 0.18 $^{b}$ | N/D | N/D | N/D | N/D |
| **2-Ethyl-3,5-dimethylpyrazine** $^{\beta}$ | 2.28 ± 0.14 $^{a}$ | 1.54 ± 0.08 $^{b}$ | N/D | N/D | N/D | N/D |
| **5-Ethyl-2,3-dimethylpyrazine** $^{\beta}$ | 1.03 ± 0.01 $^{a}$ | 1.16 ± 0.08 $^{a}$ | N/D | N/D | N/D | N/D |

N/D (not detected). (*) concentration of amino acids as described in Table 1. α Identification confirmed by GC retention index and mass spectra of authentic compounds. β Tentatively identified by matching mass spectra library.
Data points represent mean values of 3 independent determinations. Values in the same line followed by different superscript letters are significantly different ($p < 0.05$).

**Table 5.** Pyrazines (GC-MS peak Area x 10 $^{6}$) detected in model reactions of dry glucose and hydrolyzed whey mixtures incubated at different relative humidities under roasting conditions (180 ºC/90 min)

| Compound | 0.16 aw | 0.33 aw | 0.38 aw | 0.53 aw | 0.62 aw | 0.75 aw |
|---|---|---|---|---|---|---|
| **2-Methylpyrazine** $^{\alpha}$ | 23.10 ± 0.36 $^{a}$ | 54.45 ± 4.70 $^{b}$ | 37.99 ± 1.23 $^{c}$ | 30.40 ± 1.25 $^{d}$ | 22.39 ± 0.86 $^{a}$ | 19.71 ± 0.89 $^{a}$ |
| **2,5(6)-Dimethylpyrazine** $^{\alpha}$ | 42.39 ± 3.04 $^{a}$ | 419.99 ± 13.07 $^{b}$ | 343.52 ± 16.20 $^{c}$ | 327.52 ± 14.63 $^{c}$ | 287.13 ± 5.82 $^{d}$ | 211.5 ± 12.18 $^{e}$ |
| **2,3-Dimethylpyrazine** $^{\alpha}$ | 1.8 ± 0.07 $^{a}$ | 5.43 ± 0.26 $^{b}$ | 5.73 ± 0.54 $^{b}$ | 5.02 ± 0.48 $^{b}$ | 4.78 ± 0.37 $^{b}$ | 4.59 ± 0.33 $^{b}$ |
| **2-Ethylpyrazine** $^{\alpha}$ | 4.24 ± 0.19 $^{a}$ | 6.69 ± 0.46 $^{b}$ | 5.62 ± 0.40 $^{b}$ | 4.84 ± 0.43 $^{ca}$ | 5.07 ± 0.28 $^{ca}$ | 4.98 ± 0.32 $^{ca}$ |
| **2-Ethyl-5-methylpyrazine** $^{\alpha}$ | 11.00 ± 0.51 $^{a}$ | 122.23 ± 8.24 $^{b}$ | 101.74 ± 6.33 $^{b}$ | 97.00 ± 6.95 $^{cd}$ | 107.68 ± 6.33 $^{bc}$ | 85.2 ± 8.00 $^{d}$ |
| **3-Ethyl-2,5-dimethylpyrazine** $^{\alpha}$ | 5.14 ± 0.13 $^{a}$ | 262.84 ± 12.30 $^{b}$ | 249.39 ± 13.11 $^{b}$ | 247.43 ± 12.75 $^{b}$ | 254.57 ± 4.56 $^{b}$ | 163.00 ± 11.38 $^{c}$ |
| **2-Ethyl-3,5-dimethylpyrazine** $^{\beta}$ | 2.28 ± 0.14 $^{a}$ | 30.34 ± 2.08 $^{b}$ | 24.82 ± 1.93 $^{c}$ | 22.04 ± 2.05 $^{cd}$ | 22.26 ± 1.37 $^{c}$ | 16.61 ± 1.39 $^{d}$ |
| **5-Ethyl-2,3-dimethylpyrazine** $^{\beta}$ | 1.06 ± 0.06 $^{a}$ | 28.34 ± 2.46 $^{b}$ | 19.73 ± 1.92 $^{c}$ | 22.68 ± 1.15 $^{c}$ | 30.82 ± 2.75 $^{b}$ | 22.60 ± 1.67 $^{c}$ |
| **2,5-Diethylpyrazine** $^{\beta}$ | N/D | 6.78 ± 0.33 $^{a}$ | 5.10 ± 0.43 $^{a}$ | 5.09 ± 0.45 $^{a}$ | 9.92 ± 0.77 $^{b}$ | 7.43 ± 0.70 $^{ca}$ |
| **2,3-Diethyl-5-methylpyrazine** $^{\beta}$ | N/D | 18.72 ± 0.49 $^{a}$ | 16.19 ± 1.05 $^{ab}$ | 15.20 ± 0.46 $^{b}$ | 18.59 ± 1.21 $^{a}$ | 13.49 ± 1.27 $^{b}$ |
| **3,5-Diethyl-2-methylpyrazine** $^{\beta}$ | N/D | 20.44 ± 0.37 $^{a}$ | 18.07 ± 1.33 $^{a}$ | 19.14 ± 1.28 $^{a}$ | 26.53 ± 1.77 $^{b}$ | 20.04 ± 1.93 $^{a}$ |
| **2-Ethyl-3,5,6-trimethylpyrazine** $^{\beta}$ | N/D | 13.03 ± 0.93 $^{a}$ | 11.46 ± 0.84 $^{a}$ | 10.60 ± 0.72 $^{ab}$ | 12.83 ± 1.02 $^{a}$ | 8.81 ± 0.65 $^{b}$ |
| **2,5-Dimethyl-3-(3-methyl-butyl)pyrazine** $^{\beta}$ | N/D | 47.25 ± 2.70 $^{a}$ | 46.88 ± 1.58 $^{a}$ | 43.83 ± 1.14 $^{a}$ | 42.22 ± 4.01 $^{a}$ | 22.55 ± 1.39 $^{b}$ |
| **3-Furan-2-ylmethyl-2,5-dimethylpyrazine** $^{\beta}$ | N/D | 11.80 ± 0.95 $^{a}$ | 0.39 ± 0.03 $^{b}$ | 0.10 ± 0.01 $^{b}$ | 0.07 ± 0.01 $^{b}$ | N/D |

N/D (not detected). $^{a}$ Identification confirmed by GC retention index and mass spectra of authentic compounds. $^{\beta}$ Tentatively identified by matching mass spectra library.
Data points represent mean values of 3 independent determinations. Values in the same line followed by different superscript letters are significantly different ($p < 0.05$).

For table of contents only

TOC graphic