Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 99 (2006) 600–604
Food Chemistry
www.elsevier.com/locate/foodchem

Taste properties of Maillard-reaction products prepared from 1000 to 5000 Da peptide

Masashi Ogasawara *, Tadayoshi Katsumata, Makoto Egi

Food Creation Center, Kyowa Hakko Food Specialties Co. Ltd., 4041-Ami, Ami-machi, Inashiki-gun, Ibaraki 300-0398, Japan

Received 25 April 2005; received in revised form 15 August 2005; accepted 15 August 2005

Abstract

This study investigated the flavour-enhancing properties of the Maillard reaction products from 1000 to 5000 Da peptides. Protein hydrolyzates obtained from enzymatic hydrolysis of soybean protein were fractionated with UF membranes to obtain the 1000–5000 Da fraction. Xylose was added to this fraction and allowed to react for 3.5 h at 95 °C. This reaction mixture was fractionated with 1000 and 5000 Da cut off membranes to obtain the 1000–5000 Da fraction (the "Maillard peptide"). To evaluate the flavour characteristics of the Maillard peptide, an additional test on a umami solution was carried out. It was found that the Maillard peptide produced an enhanced effect on flavour, including umami, continuity and mouthfulness in the umami solution and in consommé soup.

Keywords: Maillard reaction; Peptide; Sugar; Taste enhancer; Simmered taste

1. Introduction

Peptides are thought to be a very important component in the flavour development of fermented and aged foods, such as miso and cheese. There have been some reports on the taste properties of peptides. Glutamic acid-rich oligopeptides have a umami taste (Noguchi, Arai, Yamashita, Kato, & Fujimaki, 1975). A peptide in beef, hydrolyzed by papain, has been isolated (Tamura et al., 1989; Yamasaki & Maekawa, 1978). The 500–1000 Da fraction of hydrolyzed wheat gluten added to a umami solution enhanced its strength (Ishi, Nishimura, Ono, Hatae, & Shimada, 1994). Peptides from cooked pork loins had an effect of sourness-suppression (Tomoyuki, Ryoji, & Toshihide, 2004). From these findings, it can be postulated that peptides play an important role in the taste of foods. The Maillard reaction is a chemical reaction between reducing carbohydrates and amino acids, and it is chiefly responsible for the development of unique aromas and tastes during thermal processing of foods. Such processing includes roasting of meat (Wasserman, 1979), bread baking (Kimpe & Keppens, 1996), roasting of coffee (Baltes & Bochmann, 1987), and fermentation–ripening of foods, as in soy sauce (Nomura, Sasaki, Asao, & Yokotsuka, 1976a, 1976b) and soybean paste (Mori, Kikuchi, & Tabei, 1983). It has also been reported that the reaction gives emulsification and antibacterial properties, which are improved as a result of the coalescence of polysaccharides with egg albumen or lysozymes (Kato, 2002), and that lactosyl β-lactoglobulin is generated when milk and milk products are sterilized (Leonil et al., 1997).

However, taste properties of the peptide Maillard-reaction products have not been reported. This study was conducted to investigate the flavour-enhancing properties of the Maillard-reaction products from 1000 to 5000 Da peptide (the "Maillard peptide").

2. Materials and methods

2.1. Reagents

All chemicals were of reagent grade, or HPLC grade, and purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan), Nacalai Tesque Inc. (Tokyo, Japan) and

* Corresponding author. Tel.: +81 298 888 8041; fax: +81 298 887 2138.
E-mail address: masashi.ogasawara@kvowa.co.jp (M. Ogasawara).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.08.040

Kishida Chemical Co. Ltd. (Tokyo, Japan). Sumizyme FP was purchased from Shin Nihon Chemical Co. Ltd. (Aichi, Japan). Soybean protein, containing 90% protein, was purchased form Fuji Oil Co. Ltd. (Tokyo, Japan).

2.2. Preparation of the 1000–5000 Da peptide

Fifteen grams of soybean protein isolates were dissolved in distilled water to obtain a 15% (w/v) protein slurry and hydrolyzed at pH 5.5, 50 °C, for 48 h with a 0.14 g dosage of Sumizyme FP, which is an *Aspergillus oryzae* protease. This hydrolyzate was incubated at 95 °C for 10 min to inactivate the protease and any precipitate that formed was then removed by centrifugation at 5000*g* for 20 min at 4 °C. The supernatant was ultra-filtered through 1000 and 5000 Da cut-off membranes (Remolino™ UF&RO system, Milipore Ltd., Milford, Mass., USA). The 1000–5000 Da fraction (the "MW 1000–5000 peptide") was freeze-dried and stored at −18 °C until used.

2.3. Preparation of the Maillard peptide

Four grams of xylose and 25 g of the "MW 1000–5000 peptide" were dissolved in distilled water to obtain a 24% (w/v) mixture. This mixture was heated to 95 °C for 3.5 h. This reaction mixture was fractionated with 1000 and MW 5000 Da membranes to obtain the 1000–5000 Da fractions. The 1000–5000 Da fraction (the "Maillard peptide") was freeze-dried and stored at −18 °C until used.

2.4. Chemical analysis

The amino acid composition of the peptide was determined by hydrolyzing with 6 N HCI at 115 °C for 24 h and then analyzed with an amino acid analyzer (JEOL JLC-500/V, Nihon Denshi Datem Co. Ltd. Japan). Fluorescamine assay (Yeboah, Alii, & Yaylayan, 1999) was used to measure the amino groups. A 25 mg sample was dissolved in 10 ml of distilled water and passed though a 22 μm filter. The filtrate was diluted 10 times with distilled water and used as the sample solution. A 200 μl sample solution was added to 4 ml of a 0.2 M potassium borate buffer, pH 8.5, solution. To this solution, 1 ml of 0.015% (w/v) fluorescamine reagent/acetone was added and it was measured for fluorescence at excitation and emission wavelengths of 390 and 475 nm, respectively (the blank used was 4.2 ml of borate buffer, containing 1 ml of the fluorescamine reagent/acetone solution). The degree of modification of amino groups by the Maillard reaction was calculated to express the fluorescence after the Maillard reaction compared to the fluorescence before the Maillard reaction.

The peptides were analyzed by HPLC (Shimadzu LC10, Shimadzu Co. Ltd., Kyoto, Japan) on a gel filtration column (TSKgelG2000SW, Tosoh Corp., Tokyo, Japan).

The filtrate was applied to the column and eluted with an aqueous solution of 45% acetonitrile containing 0.1% trifluoroacetic acid. The flow rate was 0.8 ml/min at a temperature of 40 °C. The UV detector was set at 215 nm and the fluorometer was set to measure total Maillard fluorescence at λ_{ex} 350 nm and λ_{em} 440 nm (Yeboah et al., 1999; Yeboah & Yaylayan, 2001). Aprotinin (MW 6500), oxidized glutathione (MW 612), glycylglycylglycine (MW 189) and glycine (MW 75) were used to create a calibration curve and to calculate molecular weight.

2.5. Sensory evaluation

Threshold values in water were measured by the triangle test. All the test solutions were adjusted to pH 7.0. To

Table 1
Amino acid composition of the "MW 1000–5000 peptide"

	Total amino acid		Free amino acid		Free/total
	mg/g	%	mg/g	%	%
Aspartic acid	96.5	11	0.5	5.1	0.6
Threonine	37.3	4.3	0.3	3.2	0.9
Serine	51.4	5.9	0	0	0
Glutamic acid	131	14.9	1.9	17.8	1.4
Glycine	42.4	4.8	0.1	1.3	0.3
Alanine	49.6	5.7	0.3	3.2	0.7
Valine	49.4	5.6	0.9	8.3	1.8
Cystine	0	0	0	0	0
Methionine	6.2	0.7	0.3	3.2	5.3
Isoleucine	46.3	5.3	1	9.6	2.2
Leucine	64.6	7.4	1.8	17.2	2.8
Tyrosine	10.6	1.2	0.1	0.6	0.6
Phenylalanine	49.8	5.7	0.8	7.6	1.6
Histidine	23.3	2.7	0.4	3.8	1.7
Lysine	83.4	9.5	1.5	14	1.8
Arginine	73.6	8.4	0	0	0
Hyproline	0	0	0	0	0
Proline	60.2	6.9	0.5	5.1	0.9
Total	877	100	10.5	100	1.2

ascertain the flavour characteristics of the Maillard peptide, additional tests with umami solution and consommé soup were carried out.

Umami solution consisted of 1.5% monosodium glutamate (MSG) and 0.5% sodium chloride (NaCI). Consommé soup consisted of 1.5% consommé soup premix, comprising 50% salt, 7% sugar, 3% onion extract powder, 3% beef extract powder, 1.5% sodium glutamate, 1.2% caramel, 0.2% white pepper, 0.2% celery powder and 33.9% lactose.

Dextrin DE8-10 (Nissi Co. Ltd., Tokyo, Japan) was added to the umami solution and consommé soup as the control for sensory evaluation.

The ''MW 1000–5000 peptide'' and ''Maillard peptide'' (0.025%) were dissolved in umami solution and consommé soup, and then heated to 60 °C in a water bath. A 60 ml sample, of both the umami and consommé, and 60 ml of control solution were served in opaque disposable plastic cups at the same time. Sensory evaluation was performed in separated sensory booths.

Sensory evaluation was scored on a scale of 1–7, where 3 points was the score given to the dextrin control sample. The test samples were awarded points on the basis of umami, mouthfulness and continuity. A time intensity evaluation was performed using the Compusense Five method (Compusense Co. Ltd. USA). Each of the three test solutions were individually tested by putting 10 ml of solution into the mouth for 15 s, followed by swallowing and recording the taste experienced in 25 s, and the results recorded. The sensory evaluation panel consisted of 6 males and 4 females (age 25–45). All the panel members had extensive experience in tasting and had made a consensus on scoring beforehand for umami, mouthfulness and continuity.

2.6. Statistical analysis

Statistical analysis was performed using Microsoft Excel 2000. The *t*-test was used to determine significant differences between samples. Trends were considered significant when the means of compared sets differed at $P < 0.05$.

3. Results and discussion

3.1. The amino acid composition of the MW 1000–5000 peptide

The amino acid composition of the ''MW 1000–5000 peptide'' was measured and is shown in Table 1. The ''MW 1000–5000 peptide'' contained 90% peptides while the content of free amino acids was approximately 1%. This suggests that most of the free amino acids were removed.

3.2. Characteristics of the ''Maillard peptide''

Table 2 shows the absorbance at 450 nm for a 1% solution of the Maillard peptide. The degree of modification of amino groups was determined by TNBS. It was confirmed that the ''MW 1000–5000 peptide'' fraction had amino groups modified by 30% after browning by the maillard reaction.

Table 2
Absorbance of the ''Maillard peptide'' and the percentage of amino groups

Absorbance at 450 nm (1% solution)	Total amino groups (%)
0.821	70

The gel filtration chromatogram result for the Maillard peptide is shown in Fig. 1. It was found that the Maillard reaction occurred in the peptide fraction within the MW range 350–5000 Da.

3.3. Sensory properties of the Maillard peptide

The threshold values of the ''MW 1000–5000 peptide'' and the ''Maillard peptide'' were found to be 0.05% and 0.03%, respectively.

To evaluate the flavour characters of the ''Maillard peptide'', sensory evaluation with umami solution and consommé soup was carried out using a scoring test. Umami solution was added to the ''MW1000–5000 peptide'' and the Maillard peptide at 0.025% and also to consommé soup with Maillard peptide at 0.025%.

Fig. 2 shows the results of the sensory evaluation in the umami solution and Fig. 3 shows the results of the sensory evaluation in the consommé soup. The ''MW1000–5000 peptide'' and the ''Maillard peptide'' had little aroma or basic taste in distilled water at a concentration of 0.025%. From Fig. 2, the addition of Maillard peptide significantly increased the intensities of mouthfulness and continuity in the umami solution compared to the control and the addition of the ''MW 1000–5000 peptide''. From Fig. 3, the addition of ''Maillard peptide''significantly increased the intensities of mouthfulness and continuity in consommé soup compared to the control.

To evaluate the flavour characteristics of the Maillard peptide, a further test on the umami solution was carried out using a time intensity method. Fig. 4 shows the results of the time–intensity method with the umami solution. From Fig. 4, the addition of ''Maillard peptide'' increased the taste intensity and gave longer taste continuity than did the control or the addition of the ''MW 1000–5000 peptide''. These results suggest that the Maillard peptide is behaving as a flavour enhancer. There have been various instances reported of taste-enhancing peptides. For instance, some glutamic acid-containing peptides have a umami taste like MSG (Noguchi et al., 1975). The ''Maillard peptide'' did not have any strong taste, and might have some characteristics different from the glutamic acid-containing peptides.

More than 40 years ago, the first flavour synergism was reported between the umami-like monosodium L-glutamate

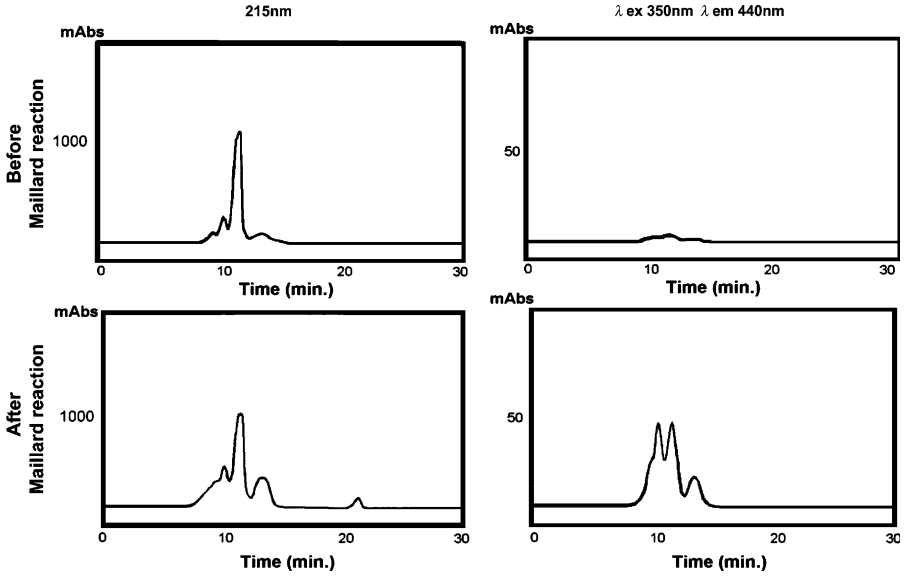

Fig. 1. Gel filtration chromatogram pattern of the "MW 1000–5000 Maillard peptide".

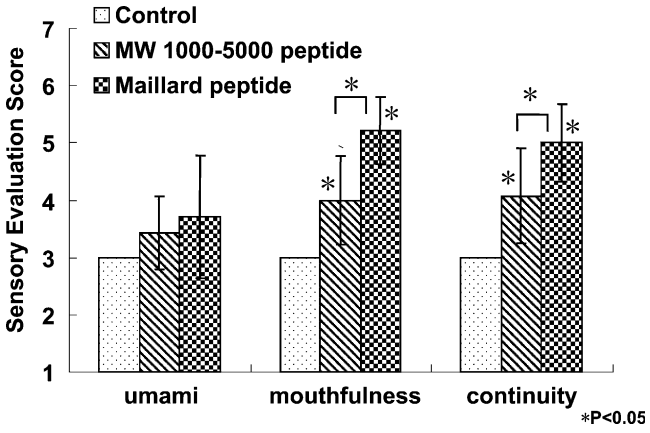

Fig. 2. Taste performance of the "Maillard peptide" in umami solution by sensory evaluation.

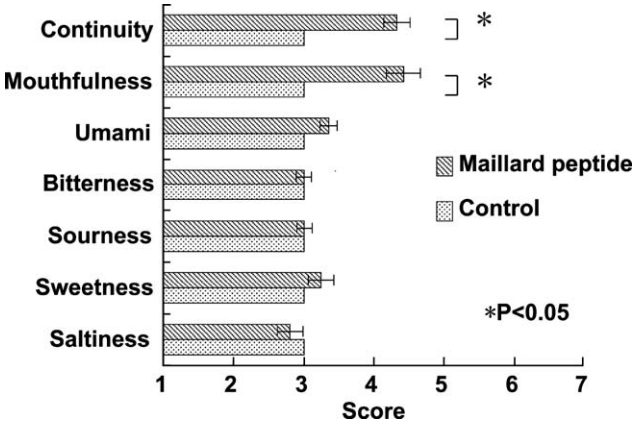

Fig. 3. Taste performance of the "Maillard peptide" in consommé soup by sensory evaluation.

(MSG), purine-5′-ribonucleotides and analogues of both groups (Kuninaka, 1967; Yamaguchi, 1967; Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). Recently, it has been reported that peptides added to beef extract created a more intense meaty or savoury taste (Wang, Maga, & Bechtel, 1996), glutathione had a characteristic flavour including continuity, mouthfulness and thickness in a umami solution (Ueda, Yonemitsu, Tsubuku, & Sakaguchi, 1997), and alaphyridaine was identified as a general taste enhancer (Solod, Blank, & Hofmann, 2003).

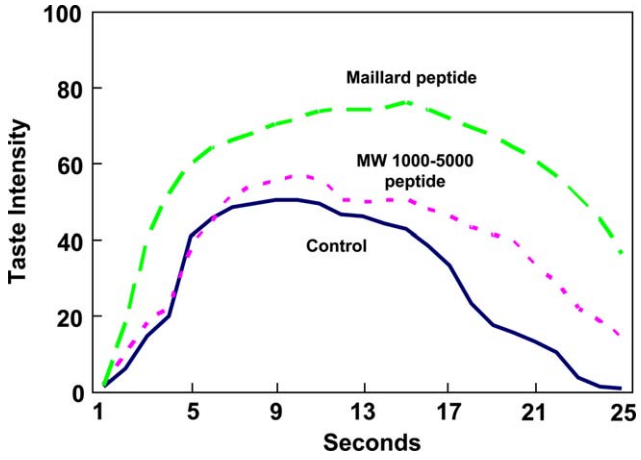

Fig. 4. Performance of different solutions by sensory analysis from time–intensity measurement.

Flavour enhancers are widely used in the food industry and studied by food chemists. We need more analysis in order to clarify the taste properties of "Maillard peptide" in greater detail.

4. Conclusions

The "Maillard peptide" from the 1000 to 5000 Da fraction of the hydrolyzed soybean protein does not have a strong taste but when added to different solutions it

significantly increased the intensity of mouthfulness and continuity in umami solution and consommé soup. From the flavour profile described by sensory evaluation, the flavour-enhancing effect by the ''Maillard peptide'' could be defined as ''simmered taste'', the so called ''koku'' taste perception in Japanese.

Acknowledgements

The authors would like to thank Ms. Hiroko Nakakuki for her technical assistance.

References

Baltes, W., & Bochmann, G. (1987). Model reactions on roast aroma formation. 1. Reaction of Serine and threonine with sucrose under the conditions of coffee roasting and identification of new coffee aroma compounds. *Journal of Agricultural and Food Chemistry, 35*, 340–346.

Ishi, K., Nishimura, T., Ono, T., Hatae, K., & Shimada, J. (1994). Taste of peptides in wheat gluten hydrolyzate by protease. *Journal of Home Economics Japan, 45*, 615–620.

Kato, A. (2002). Industrial applications of Maillard-type protein-polysaccharide conjugates. *Food Science and Technology Research, 8*, 193–199.

Kimpe, D. K., & Keppens, M. (1996). Novel synthese of the major flavor components of bread and cooked rice. *Journal of Agricultural and Food Chemistry, 44*, 1515–1519.

Kuninaka, A. (1967). Flavor potentiators. In H. W. Schultz, E. A. Day, & L. M. Libbey (Eds.), *Symposium on foods the chemistry and physiology of flavors* (pp. 515–535). Westport, CT: AVI.

Leonil, J., Molle, D., Fauquant, J., Maubois, J. L., Pearce, R. J., & Bouhallab, S. (1997). Characterization by ionization mass spectrometry of lactosyl β-lactoglobulin conjugates formed during heat treatment of milk and whey and identification of one lactose-binding site. *Journal of Dairy Science, 80*, 2270–2281.

Mori, Y., Kikuchi, K., & Tabei, H. (1983). Flavor components of miso: basic fraction. *Agricultural and Biological Chemistry, 47*, 1487–1492.

Noguchi, M., Arai, S., Yamashita, M., Kato, H., & Fujimaki, M. (1975). Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from a fish protein hydrolysate. *Journal of Agricultural and Food Chemistry, 23*, 49–53.

Nomura, N., Sasaki, M., Asao, Y., & Yokotsuka, T. (1976a). Identification of volatile components in shoyu (soy sauce) by gas chromatography–mass spectrometry. *Agricultural and Biological Chemistry, 40*, 485–490.

Nomura, N., Sasaki, M., Asao, Y., & Yokotsuka, T. (1976b). Isolation and identification of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanon, as a flavor component in shoyu (soy sauce). *Agricultural and Biological Chemistry, 40*, 491–495.

Solod, T., Blank, I., & Hofmann, T. (2003). Alapyridaine a general taste enhancers? *Chemical Senses, 28*, 371–379.

Tamura, M., Nakatsukasa, T., Tada, M., Kawasaki, Y., Kikuchi, E., & Okai, H. (1989). The relationship between taste and primary structure of 'delicious peptide' (Lys-Gly-Asp-Glu-Glu-Ser-Leu-leu) from beef soup. *Agricultural and Biological Chemistry, 53*, 319–325.

Tomoyuki, O., Ryoji, Y., & Toshihide, N. (2004). Sourness-suppressing peptides in cooked pork loins. *Bioscience Biotechnology and Biochemistry, 68*, 1657–1662.

Ueda, Y., Yonemitsu, M., Tsubuku, T., & Sakaguchi, M. (1997). Flavor characteristics of glutathione in raw and cooked foodstuffs. *Bioscience Biotechnology and Biochemistry, 61*, 1977–1980.

Wang, K., Maga, J. A., & Bechtel, P. J. (1996). Taste properties and synergisms of beefy meaty peptide. *Journal of Food Science, 61*, 837–839.

Wasserman, A. E. (1979). Symposium on meat flavor chemical basis for meat flavor a review. *Journal of Food Science, 44*, 6–11.

Yamaguchi, S., Yoshikawa, T., Ikeda, S., & Ninomiya, T. (1971). Measurement of the relative taste intensity of some L-a-amino acids and 5′-nucleotides. *Journal of Food Science, 36*, 1761–1765.

Yamaguchi, S. (1967). The synergistic taste effect of monosodium glutamate and disodium 5′-inosinate. *Journal of Food Science, 32*, 473–478.

Yamasaki, Y., & Maekawa, K. (1978). A peptide with delicious taste. *Agricultural and Biological Chemistry, 42*, 1761–1765.

Yeboah, F. K., & Yaylayan, V. A. (2001). Analysis of glycated proteins by mass spectrometric techniques: qualitative and quantitative aspects. *Nahrung/Food, 45*, 164–171.

Yeboah, F. K., Alii, I., & Yaylayan, V. A. (1999). Reactivities of D-glucose and D-fructose during glycation of bovine serum albumin. *Journal of Agricultural Food Chemistry, 47*, 3164–3172.