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Effects of enzymatic modification of wheat protein on the formation of pyrazines and other volatile components in the Maillard reaction

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ABSTRACT

Enzymatically hydrolysed wheat gluten hydrolysate (WGH) was deamidated using glutaminase to produce deamidated wheat gluten hydrolysate (DWGH). Volatile components were analysed in WGH and DWGH thermally reacted with glucose or fructose. In the reaction system containing glucose, 19 pyrazines, 2 furans, and 5 sulphur-containing components were detected in WGH, while 34 pyrazines, 4 furans, and 7 sulphur-containing components were found in DWGH. In the system containing fructose, 24 pyrazines, 3 furans, and 6 sulphur-containing components were identified in the thermal reaction of WGH, whereas 36 pyrazines, 4 furans, and 8 sulphur-containing components were found in DWGH. The volatile components increased in DWGH, both qualitatively and quantitatively, mainly due to free ammonia released by deamidation. More volatiles were also developed in WGH and DWGH with fructose than with glucose. It was found that ammonia released from wheat protein via deamidation participated in the generation of diverse volatile components including pyrazines in the Maillard reaction.

1. Introduction

Glutamine, which is the most abundant amino acid in wheat protein, is present at high levels in wheat gluten. However, glutamine can be easily hydrolysed by enzymes, as well as heat, acid/base and metal ions, due to deamidation of its susceptible amide residue during the processing and storage of foods (Larre, Chiarello, Blanloeil, Chenu, & Gueguen, 1993). Deamidation is a chemical reaction in which the amide functional group is hydrolysed from an organic compound, releasing free ammonia, while deamination is the loss of the α-amino group from a molecule. The deamidation reaction converts glutamine into glutamic acid and releases free ammonia from its amide residues in proteins, peptides, and free amino acids (Sohn & Ho, 1995).

The Maillard reaction, one of the non-enzymatic browning reactions between free amino groups and carbonyl groups, has been studied for a long time, since it can affect the colour, functional properties, nutritional value and flavour of foods (Ames, Guy, & Kipping, 2001a). Maillard reaction products are influenced by various factors such as the types of amino acids and sugars, temperature, time, pH and water activity. In particular, heterocyclic compounds including pyrazines, pyrroles, thiazoles, oxazoles and furans, which are generated mainly via a series of Maillard reactions, are major contributors to food flavour, since they have relatively low threshold values and characteristic odour notes (Vernin & Parkanyi, 1982).

Pyrazines are considered to provide a representative roasted flavour in foods and commonly exhibit the sensory properties of toasted and nutty odour notes (Maga, 1992). Pyrazines have been investigated mainly due to their characteristic flavour notes in various foods. For example, the relationship between the roasted or toasted aroma note and the level of pyrazines has been analysed in heat-treated foods such as french-fried or potato chips (Zviely, Abushqara, & Hodrien, 2005). In addition, the formation of pyrazines has been studied in different model systems (Maga, 1992) and several mechanisms for their formation have been proposed (Adams, Polizzi, van Boekel, & De Kimpe, 2008; Van Lancker, Adams, & De Kimpe, 2010). It is generally accepted that the formation of pyrazines involves α-aminoketones produced as a result of the condensation of dicarbonyls with amino compounds in the Maillard reaction (Mottram, 1994). However, Hwang, Hartman, Rosen, and Ho (1993) suggested that ammonia released via the deamidation and deamination of amino acids could contribute to the generation of pyrazines since ammonia can react directly with dicarbonyl to produce pyrazines, while the α-amino groups can react with dicarbonyl to generate pyrazine via Strecker degradation (Hwang et al., 1993). In addition, Shu (1998) demonstrated that pyrazines can be generated by the reaction of ammonia released as a result of the decarbonylation of α-amino acid with acyloins, which are the sugar degradation products of deamination (Shu, 1998).

Many studies have focused on the effects of sugar, amino acids, temperature, pH and water activity on the Maillard reaction (Shu &

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Ho, 1988). However, there is only limited information regarding the use of enzymatic hydrolysis (and in particular deamidation) for generating volatile components in thermal reactions. Moreover, there is little data on the effect of the type of sugars on the production of volatile components in the Maillard reaction after deamidation.

The objectives of the present study were to elucidate the effect of deamidation of wheat gluten on the generation of volatile components in the Maillard reaction and to compare the volatile components of deamidated wheat gluten hydrolysates (DWGH) reacted with different reducing sugars.

2. Materials and methods

2.1. Materials and chemicals

Wheat gluten was provided from Sempio foods company (Icheon, South Korea). D-Glucose, D-fructose, 2-vinylpyridine, sodium sulphate and *n*-alkane standards (C_7–C_{22}) were obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade hexane and water were purchased from J.T. Baker (Phillipsburg, NJ, USA). A commercial protease/peptidase (Flavourzyme 500 MG, 500 LAPU/g, endoprotease and exoprotease from *Aspergillus oryzae*) and glutaminase (SD-C100S, 100 GTU/g, from *Bacillus subtilis*) were purchased from Novozymes (Bagsvaerd, Demnark) and Daiwa Kasei (Shiga, Japan), respectively.

2.2. Preparation of wheat gluten hydrolyate (WGH) and deamidated wheat gluten hydrolysate (DWGH)

In order to decide the degree of hydrolysis (DH) of wheat gluten, a modified formol titration was employed. Wheat gluten (10 g) and protease/peptidase (1 g) were mixed in 100 ml of distilled water in an Erlenmeyer flask and kept in a shaking incubator (BS-31, JEIOTECH Co. Ltd., Daejeon, Korea) at 55 °C and 180 rpm for 0, 2, 4, 6, 8 and 24 h. Fifty millilitre of the hydrolysate adjusted to pH 7 using 0.1 M NaOH and 25 ml of neutralised formaldehyde solution (37% v/v) were mixed and stirred magnetically for 10 min. The mixture was adjusted to pH 8.9 with 0.1 M NaOH. For acidic hydrolysate, it was considered that the wheat protein treated with 6 M HCl at 110 °C for 24 h was 100% hydrolysed. DH was calculated using equation of $(V_1/V_0) \times 100$, where V_1 is the volume of NaOH solution for sample titration and V_0 is the volume of NaOH solution for acidic hydrolysate titration. The measurement of DH was performed in triplicate. The mixture was placed in a boiling water bath at 100 °C for 5 min in order to inactivate the enzyme and then cooled down to 4 °C. Hydrolysed wheat gluten was then fractionated by using ultrafiltration membranes with cut-offs of MW 3000 (Millipore, Billerica, MA, USA) in a cold room at 4 °C, and the resulting hydrolysate was considered to be wheat gluten hydrolysate (WGH). In order to obtain deamidated wheat gluten hydrolysate (DWGH), 1 g of glutaminase was added into the fractionated WGH. The mixture was then transferred into an Erlenmeyer flask and kept in a shaking incubator at 65 °C and 180 rpm for 2 h. After incubation, the mixture was placed in boiling water bath for 5 min at 100 °C to inactivate the enzyme and then cooled down to 4 °C.

2.3. Analysis of glutamine, glutamic acid and ammonia content

One hundred microlitres of each WGH and DWGH was derivatized using EZ:faast™ amino acid analysis kits (Phenomenex Inc., Torrance, CA, USA) following the manual included. Glutamine and glutamic acid in both WGH and DWGH were analysed using a HP 6890N gas chromatograph-5975 mass selective detector (GC–MSD) (Hewlett–Packard, Palo Alto, CA, USA) equipped with a ZB-AAA capillary column (10 m length × 0.25 mm i.d., Phenomenex Inc.). The carrier gas was helium at a constant flow rate of 1.1 ml/min. One microlitre of the derivatized sample was injected at a split ratio of 1:15. The oven temperature was initially held at 110 °C for 1 min, and then raised to 320 °C at a rate of 30 °C/min. Injector and transfer line temperatures were 250 and 320 °C, respectively. The MS was operated in the electron impact (EI) ion source mode at 70 eV, an ion source temperature of 240 °C and a scanning range of 35–350 amu.

Mass spectrometric data were recorded in selected ion monitoring (SIM) mode. SIM was set to monitor *m/z* 84 for glutamine, *m/z* 230 for glutamic acid and *m/z* 158 for internal standard (Norvaline). The identification of glutamine and glutamic acid was confirmed by comparison of their retention times and mass spectral data with those of authentic standard compounds. The quantification was obtained using external calibration curves. Calibration curves were based on the ratio of the peak areas of each amino acid to that of the internal standard compound [100 μl of norvaline (20 mM)] versus the concentrations of amino acids in the range of 1–1000 ppm. The analysis of amino acids in WGH and DWGH were performed in triplicate.

The free ammonia in WGH and DWGH was determined using a spectrophotometric ammonium assay kit K-AMIAR (Megazyme, Wicklow, Ireland) on the basis of the enzymatic conversion of NADPH to NADP in the presence of 2-oxoglutarate and glutamate dehydrogenase. The kit contains triethanolamine buffer, 2-oxoglutarate, NADPH, and glutamate dehydrogenase (GDH). A NADPH tablet was dissolved with triethanolamine buffer and stirred intermittently over 2–3 min in a cuvette. This was followed by the addition of distilled water (2 ml) and the fractionated WGH (100 μl), respectively. After being mixed and left standing for 2 min, the absorbance of the sample solutions was determined at 340 nm using a UV spectrophotometer (Spectronic genesys 10, Thermo Electron Corp., Waltham, MA, USA). The reaction started immediately by the addition of GDH. After the mixing, the absorbance of the mixture was measured at 340 nm. The content of ammonia was determined by the difference in the absorbances before and after adding GDH into the hydrolysate. The analysis of ammonia in WGH and DWGH was performed in triplicate.

2.4. Thermal reaction

WGH or DWGH (30 ml) was mixed with glucose or fructose (final concentration of 0.05 M), respectively, and adjusted to pH 7 with 1 M NaOH. The mixture (30 ml) was transferred into a stainless steel cylinder, before it was reacted at 180 °C in a drying oven (LDO-250N, Daihan labtech Co. Ltd., Namyangju, Korea) for 90 min. The reaction was immediately stopped by cooling the cylinders under a stream of cold water.

2.5. Analysis of volatile components

The thermal reaction solution, enriched with 0.1 ml of 2-vinylpyridine [1000 ppm (v/v) in hexane] as an internal standard, was extracted with 30 ml of hexane by magnetically stirring at 400 rpm for 30 min. The extracts were poured into a separatory funnel to obtain upper organic phase layer and, then, washed twice with 50 ml of distilled water. The organic extracts were dehydrated over anhydrous sodium sulphate and, then concentrated under gentle stream of nitrogen gas to obtain a final volume of 0.1 ml. This experiment was conducted in triplicate.

The volatile components analysis was carried out using a HP 6890N gas chromatograph-5975 mass selective detector (GC–MSD) (Hewlett–Packard, Palo Alto, CA, USA) equipped with a DB-5MS fused silica capillary column (30 m length × 0.25 mm