International Food Research Journal 25(3): 1146-1152 (June 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Flavor improvement of mud clam *(Polymesoda erosa)* hydrolysate by using Maillard reaction

*Normah, I. and Noorasma, M.

Department of Food Technology, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, 40450 Selangor Darul Ehsan

Article history

Received: 3 March 2017
Received in revised form: 17 April 2017
Accepted: 18 April 2017

Keywords

Sensory
Maillard reaction products
Hydrolysate
Alcalase
Mud clam

Abstract

Mud clam *(Polymesoda erosa)* hydrolysate (AH) was produced by hydrolysis for 2 hrs using alcalase 2.4L at pH 8.5, 60°C and 3% enzyme-substrate ratio. D-xylose and L-cysteine or D-xylose were added into the hydrolysate and heated at 120°C for 2 hrs to produce the Maillard reaction products (MRPs) labelled as AH-mx or AH-x, respectively. Amino acids, volatile compounds and sensory characteristics of AH and MRPs were evaluated. Amino acids analysis showed that monosodium glutamate (MSG)-like amino acids amount was higher while 2-piperidinone volatile compound that contributes to bitterness was lower in MRPs than AH. Furthermore, sensory results showed that MRPs had more intense umami and less fishy taste compared to AH. Therefore, it was discovered that Maillard reaction improved the flavor of mud clam hydrolysate.

Introduction

Important food properties such as color, flavor and stability can be modified using Maillard reaction. Maillard reaction is a non-enzymatic reaction involving the reaction between carbonyl groups of reducing sugars and amino groups of amino acids, peptides or proteins (Yilmaz and Toledo, 2005). Maillard reaction enhanced flavor effect including continuity and mouthful in consommé soup (Ogasawara *et al*., 2006). Different studies revealed that fish protein can be hydrolysed efficiently through the use of different enzymes. The hydrolysing effect of ten different commercial proteases including alcalase, flavourzyme, protamex, trypsin, bromelain and papain on Alaska Pollock frame has been documented (Hou *et al*., 2011).

Hydrolysis of protein especially protein derives from seafood sometimes give rise to undesirable fishy and bitter off-flavor (Normah and Nur Fazlika Nashrah, 2013; Kouakou *et al.*, 2014). Several efforts have been made to reduce or mask this undesirable flavor such as encapsulation with cyclodextrin, spray drying of hydrolysate, gelatine and soy protein isolate (SPI) mixtures, encapsulation with maltodextrin alone or mixture of maltodextrin and cyclodextrin followed by spray drying as well as addition of sugar followed by Maillard reaction (Szejtli, and Szente, 2005; Favaro-Trindade *et al.*, 2010; Yang *et al.*, 2012; Liu *et al.*, 2012). However, Maillard reaction study on mud clam hydrolysate and its potential to mask or reduce off-flavor has never been explored. In this study, D-xylose or L-cysteine were added into mud clam hydrolysate and heated to produce the MRPs. The objective of this study was to evaluate the taste improvement effects of MRPs produced with the addition of D-xylose and L-cysteine.

Materials and Methods

Materials and chemicals

Mud clam *(Poymesoda erosa)* was bought from a supplier in Kuala Selangor. The clam was placed in ice and then brought to the laboratory. Upon arrival, the flesh was separated manually and then washed. Alcalase 2.4 L with a declared activity of 2.4 Anson Units (AU) g^{-1} was obtained from Novozyme (Bagsvaerd, Denmark). Analytical grade reagents, D-xylose and L-cysteine were purchased from Merck Sdn Bhd, Malaysia.

Preparation of mud clam (Polymesoda erosa) *hydrolysate*

Mud clam hydrolysate was prepared according to the method by Normah and Noorasma (2015). An amount of 453 g flesh and 619 g distilled water was mixed and homogenised in a blender. The mixture was incubated in a circulated water bath. The pH of the mixture was adjusted to pH 8.5 and maintained during hydrolysis using 4N sodium

*Corresponding author.
Email: *norismel@salam.uitm.edu.my*

hydroxide (NaOH). Once the pH and temperature (60°C) stabilized, alcalase at enzyme-substrate ratio of 3% was added and the hydrolysis proceeded for 2 hrs. The mixture was continuously stirred using a stirring propeller throughout the hydrolysis period while the pH was kept constant by the addition of 4N NaOH. At the end of hydrolysis, the enzymatic reaction was terminated by immersing the samples in a water bath at 90°C for 15 min. This was followed by centrifugation at 10 000 rpm, 4°C for 20 min. Supernatant obtained was freeze-dried using the Sanyo-Biomedical freeze dryer (Alpha 1-4, Martin Christ). The powdered hydrolysate named as alcalase hydrolysate (AH) was stored in -20°C before further analysis.

Preparation of Maillard reaction product

Maillard reaction products (MRPs) were prepared according to the method by Eric *et al.* (2013). An amount of 1g AH, 0.5 g D-xylose and 0.375 g L-cysteine was dissolved with distilled water in a beaker and then made up to a final concentration of 10% (w/v). The mixture was labelled as AH-mx. Another mixture was prepared containing only 1g AH and 0.5 g D-xylose and labelled as AH-x. The mixtures were then transferred to a jam bottle and adjusted to pH 7.4 with 2N NaOH or 2N HCl. The bottles were tightly sealed and heated in thermostatic oil bath (Thermoline, USA) at 120°C for 2 hrs. After 2 hrs, the bottles were immediately cooled under running water and placed in ice-bath. The MRPs obtained was freeze-dried using the Sanyo-Biomedical freeze dryer (Alpha 1-4, Martin Christ) and was stored in -20°C for further analysis.

Determination of amino acid composition

An amount of 0.1 g sample was hydrolysed using 6N HCl at 110°C for 24 hrs and derivatised in AccQ-Fluor reagent prior to AccQ Tag HPLC analysis. The total amino acid was analysed by the AccQ Tag method using an AccQ Tag column (3.9 x 150 mm) at a flow rate of 1 ml/ min equipped with fluorescence detector. The mobile phase used was AccQ Tag Eluent A consists of 100 ml Eluent A and 1000ml deionized water while AccQ Tag Eluent B consists of 60% acetonitrile and 40% deionized water. The total running time per injection was 50 min.

Determination of volatile compound composition

An amount of 1 g sample was placed into a 15 ml headspace vial and pre-equilibrated for 15 minutes at 50°C in thermostatic water bath with vial capped using a silicon septum. Afterward, stainless steel needle; polydimethylsiloxane (PDMS) fiber was pushed through the vial septum. The fiber was pushed out of the housing and exposed to the headspace at 55°C for 30 min. After extraction, the fiber was pulled into the housing and the SPME device was removed from the vial and inserted into the injection port of Gas Chromatography for thermal desorption of the analysis. GC-MS analysis was performed using Agilent GC-MS (Santa Clara, CA, USA). The sample was desorbed in the injection port at 250°C for 2 minutes in splitless mode. HP-5MS analytical capillary column (29 m x 250 µm x 0.25 µm, Agilent) and helium gas at a constant flow rate of 0.8 ml/min was used. The oven temperature was programmed at an initial temperature of 40°C for 2 minutes followed by an increase of 5°C / min to 150°C (held for 5 minutes) and finally at 10°C/min to 220°C (held for 10 min). MS operating conditions (electron impact ionization mode) were an ion source temperature of 200°C, ionization voltage of 70 eV and mass–to–charge ratio of m/z 33-450 at 2.76 scans/s. The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliographic data of known compounds from the Wiley 6 library (Hewlett-Packed Co., Palo Alto,CA) and NIST 98 library (Hewlett-Packed Co., Palo Alto, CA) mass spectral database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). Results are presented as the percent (%) peak area.

Qualitative Descriptive Analysis (QDA)

The QDA was conducted according to method of Ogasawara *et al.* (2006) and Eric *et al.* (2013). Ten panellists were trained three times a week until they were able to detect and memorise the lowest possible taste intensities. Training session was divided into two. In the first session, panellists were given different concentrations of reference solutions comprising of caffeine, monosodium glutamate, fish sauce and burnt sugar to represent bitter, umami, fishy and caramel tastes, respectively. In the sensory evaluation session, panellists were provided with the reference solutions identified during the training session to compare the intensity of each taste with the hydrolysate samples (AH, AH-mx and AH-x). Each panellist was served with 5 ml hydrolysate solution (2.5%, w/v) which was individually dissolved in distilled water at room temperature. Reference solution was coded as 'R' while the samples were coded with three digital codes in random. During this session, panellists have to mark the intensity for each taste on the 15 cm QDA line scale anchored with 'low intensity' to 'high intensity'. Panellists were instructed to rinse their mouth with plain water in between tasting.

Table 1. Total amino acids composition (g $100g^{-1}$) based on taste characteristic

Amino acid	AH	AH-x	AH-mx
MSG-like*			
Asp	0.96±0.02	2.69±0.42	3.16±0.10
Glu	4.10±0.38	4.12±0.31	3.63±0.24
Total	5.06	6.81	6.79
Sweet*			
Ala	1.86±0.53	8.56±0.16	1.89±0.14
Gly	1.71±0.51	0.66±0.23	0.61±0.10
Ser	3.57±0.14	1.45±0.11	1.71±0.25
Thr	2.79±0.47	1.03±0.17	1.01±0.34
Total	9.93	12.03	5.22
Bitter*			
Arg	5.29±0.83	1.44±0.51	1.40±0.16
His	5.69±0.91	7.55±0.17	2.51±0.21
Ile	2.45±0.56	1.32±0.12	1.21±0.37
Leu	4.89±0.42	2.03±0.10	3.48±0.22
Met	3.71±0.32	0.56±0.27	0.58±0.13
Phe	21.56±0.41	10.81±0.31	11.15±0.10
Val	3.17±0.59	1.44±0.11	1.49±0.12
Total	46.76	25.15	21.82
Tasteless*			
Cys	6.54±0.70	3.79±0.31	0.24±0.17
Lys	2.97±0.86	1.25±0.14	3.81±0.26
Pro	2.72±0.23	1.66±0.10	1.81±0.41
Total	12.23	6.70	5.86

Values are expressed as means ± standard deviation from triplicate determinations

*Amino acids grouping based on taste (Tseng *et al*., 2005).

AH: alcalase hydrolysate

AH-x: AH + D-xylose

AH-mx: AH + D-xylose + L-cysteine

Statistical analysis

The data obtained was analysed using the Analysis of Variance (ANOVA) to determine significance at 5% level. Duncan Multiple Range Test (DMRT) was used to identify differences between means. Statistical analysis was performed using the Statistical Package for Social Science (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, 2011).

Results and Discussion

Amino acids composition

The amino acids grouping based on their taste characteristic as described by Tseng *et al.* (2005) are shown in Table 1. Total amino acid decreased after Maillard reaction especially for amino acids that contribute to bitterness. Decrease in total amino acids indicated crosslinking in between the low molecular weight peptide fraction with xylose (Liu *et al.*, 2012). Bitter amino acids were predominant in all hydrolysates as indicated by the higher amount of these amino acids compared to others. However, AH treated with D-xylose and L-cysteine (AH-mx) had the lowest amount of bitter taste amino acids (21.82%). This result was in line with sensory data where AH-mx was least bitter compared to AH and AH-x (Table 3).

The amount of MSG-like amino acids (aspartic acid and glutamic acid) were 6.81% in AH-x followed by AH-mx (6.79%) and AH (5.06%). The higher amount of these amino acids in MRPs indicated that the crosslinking process was dominant in thermal reaction of peptide fractions with xylose which cause increment of umaminess in MRPs (Liu *et al.*, 2012). During the thermal treatment of reducing sugar and peptide, peptide will undergo two chemical changes; on one side, peptide degrades to smaller fraction and amino acids, on the other hand, peptide can crosslink directly with sugar (Lu *et al*., 2005; Lan *et al*., 2010). These amino acids are found abundantly in various seaweed species where aspartic acid exhibits interesting properties in flavor development while glutamic acid is the major component in the taste sensation of umami (Kato *et al.,* 1989; Dawczynski *et al*., 2007). Therefore, MRPs contributed to the increased umami taste as supported by the sensory evaluation results in Table 3.

Volatile compounds

The formation of volatile compound in Maillard reaction depends on the amino acid composition, molecular weight distribution and peptide formation of hydrolyzed protein (Su *et al.*, 2011). Degradation of amino acid by Maillard reaction formed more volatile compounds (Liu *et al.*, 2012). The volatile compounds detected and identified were seven, fourteen and eleven in AH, AH-x and AH-mx, respectively (Table 2).

Furfural and 5-methyl-2-furfural are described as

Table 2. Major volatile compounds in alcalase hydrolysate (AH) and MRPs (AH-x and AH-mx)

Volatile compounds	Percent area (%)		
	AH	AH-x	AH-mx
Hexadecanoic acid, methyl ester	48.96	0.12	0.56
Octadecanoic metyl ester	16.61	n.d.	1.13
n-decanoic acid	n.d.	0.32	n.d.
Tetradecanoic acid	n.d.	2.92	n.d.
Benzoic acid, 3-methyl-2-trimethyl	n.d.	n.d.	6.22
2,4-Nonadienal	n.d.	n.d.	1.00
2,4-Decadienal	n.d.	n.d.	1.11
Cylopentasiloxane, decamethyl	2.17	0.41	3.65
Heptadecane	0.27	0.29	n.d.
Octadecane	0.13	0.19	n.d.
2,5 dimethylpyrazine	1.01	9.87	n.d.
Pyridine	n.d.	n.d.	2.16
2- piperidinone	6.79	0.95	n.d.
Fulfural	n.d.	18.98	n.d.
2-furanmetanethiol	n.d.	n.d.	2.41
3- furanmethanol	n.d.	12.68	n.d.
2-n-Butyl furan	n.d.	6.60	n.d.
4-pyradinamine,2,6-dimethyl	n.d.	0.31	n.d.
Benzeneacetyldehyde	n.d.	1.72	n.d.
1-H-Pyrole,1-(2-furanmethyl)	n.d.	0.22	n.d.
Benzofuran-2-one-2.3-dihydro-3,3-dimethyl-4-nitro	n.d.	n.d.	1.00
Bis (2-fufuryl) disulphide	n.d.	n.d.	2.97
Oxime- methoxy-phenyl	n.d.	n.d.	6.22

n.d. = not detected
AH: alcalase hydrolysate
AH-x: AH + D-xylose
AH-mx: AH + D-xylose + L-cysteine

having a caramel, sweet and fruity odor (Mottram, 1994). Furfural was only detected in AH-x (18.98%). Thiol group is known to be very important for meaty flavor (Mottram and Nobrega, 2002; Cerny and Davidek, 2003). This volatile compound was found in AH-mx in the form of 2-furanmetanethiol (2.41%) which demonstrated that the AH-mx is more meaty-like flavor. Bis (2-fulfuryl) disulphide was only detected in AH-mx (2.97%). This may be due to the addition of L-cysteine that contains sulphur amino acid. Sulphide volatile compound exhibited either a meaty odor or an onion/cabbage odor with low odor threshold values (Gasser and Grosch, 1988; Mottram, 1994; Manley and Ahmedi, 1995). This compound has been reported in heated aqueous soy extract and has a major influence on the flavor of the hydrolysed vegetable protein and generated in extruded potato snack (Coleman *et al*., 1996; Aaslyng *et al.*, 1998; Majcher and Jelen, 2007). In addition, sulphide volatile compounds are also responsible for the flavor and aroma to stewed beef juice, boiled trout, french fries, bread crust, cooked chicken, roasted chicken, boiled beef, cocoa powder, peanuts, pilsner, roasted beef, popcorn and coffee (Cerny, 2008).

2-piperidinone that contributed to bitterness was not detected in AH-mx. It has been postulated that 2-piperidinone could be reduced from thermal degradation of MRPs through the interaction between carbonyl compound and sulfur containing amino acid (cysteine) to form the sulfur containing flavor compounds such as 2-furanmetanethiol and Bis(2-furfuryl) disulphide (Guentert *et al*., 1990). This result revealed the ability of Maillard reaction to reduce bitterness. Similarly, 2, 5 dimethylpyrazine that gives rise to roasted, nutty and burnt notes of cooked food was not detected in AH-mx. This indicates that the addition of L-cysteine may not have led to other reactions competing with the formation of pyrazines (Eric *et al*., 2013). Two types of aldehyde were found in AH-mx (2, 4-Nonadienal and 2, 4-Decadienal) and Benzenecetyldehyde in AH-x. Trace amount of aldehyde gives special aroma such as fruit fragrance and nut aromas (Zhang *et al*., 2010; Eric *et al*., 2013). Therefore, the presence of favorable aroma compounds in AH-x and AH-mx suggested that Maillard reaction indeed improved the overall flavor of AH.

Qualitative Descriptive Analysis

Sensory profiles of AH, AH-x and AH-mx is shown in Table 3. In determining the sensory of AH and MRPs, reference solutions were used to identify and define descriptive terms (bitter, umami, fishy and caramel) for the samples. AH and MRPs significantly differed ($p<0.05$) in all the evaluated attributes. Sensory evaluation involving ten trained panellists showed that AH-x and AH-mx were less bitter, less fishy but more umami and had more

Table 3. Sensory profiles of AH, AH-x and AH-mx in terms of bitter, umami, fishy and caramel taste

	Taste			
Sample	Bitter	Umami	Fishy	Caramel
AH	6.57 ^{b} ±0.07	0.53 ^{d} ±0.04	10.19 ^{a} ±0.20	0.29 ^{d} ±0.06
AH-x	2.28 ^{c} ±0.12	4.55 ^{c} ±0.10	4.34 ^{b} ±0.24	5.16 ^{a} ±0.21
AH-mx	1.56 ^{d} ±0.11	7.54 ^{b} ±0.08	2.54 ^{c} ±0.08	3.44 ^{c} ±0.14
Reference solution	9.49 ^{a} ±0.16	8.97 ^{a} ±0.01	2.30 ^{c} ±0.23	4.36 ^{b} ±0.20

Values are expressed as means ± standard deviation. Different letters within each column indicate significant different at $p < 0.05$.
AH: alcalase hydrolysate
AH-x: AH + D-xylose
AH-mx: AH + D-xylose + L-cysteine

caramel taste than AH. The bitter characteristics of AH was reflected by the presence of large amount of bitter amino acids (histidine, valine, methionine, and proline). Moreover, bitterness may be correlated with high amount of 2-piperidinone bitter compound in AH. 2-piperidinone was either reduced in AH-x or not detected in AH-mx. As shown in Table 3, umaminess of AH increased after Maillard reaction. Higher degree of umaminess in AH-x and AH-mx might be due the higher content of umami like amino acids such as glutamic and aspartic acid (Table 1). Therefore, the addition of D-xylose and L-cysteine increased the umami taste in the hydrolysate. This might be due to the domination of degradation reactions of high molecular peptide in hydrolysate with added xylose and cysteine.

Maillard reaction reduced the bitterness and increased the caramel and umami taste of AH. Caramel like taste was due to furfural contents (Van Boekel, 2006).The higher caramel taste in AH-x compared to AH-mx and AH might be in part attributed to the larger amount of furfural (Table 2). Furfural could be produced by sugar caramelization and carbohydrate degradation (Shibamoto, 1980). This suggested that the addition of L-cysteine might not favour the formation of furfural compound due to less caramel taste in AH-mx. The acceptance of fish protein hydrolysates in food has been precluded because of the problems with fishy off-flavors (Adler-Nissen, 1986). In the present study, the fishy flavor was reduced suggesting that Maillard reaction was capable of reducing the fishy flavor in AH. The improvement might be due to the formation of Maillard peptide exhibiting flavor-enhancing properties (reaction of protein hydrolysate with xylose) and producing volatile compounds characterized by grilled notes such as pyrazines and sulphur containing compounds (Ogasawara *et al*., 2006). Thus, this study reveals that the Maillard reaction provided an upgradeable process that can be utilized in debittering and fishy flavor removal from mud clam hydrolysate.

Conclusion

The bitter taste amino acids were reduced from 46.76% in AH to 25.15% in AH-x and 21.82% in AH-mx. MSG-like amino acids were higher in MRPs compared to AH. 2-piperidinone volatile compound that contributes to bitter compound was reduced in AH-x and not detected in AH-mx. The sensory data revealed that bitter and fishy taste was also reduced in MRPs. Therefore, these findings suggested that Maillard reaction in addition with the presence of D-xylose and L-cysteine successfully improved the taste of mud clam hydrolysate.

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