Thesis for the Degree of Master of Fisheries Science

Reaction Mechanism of Maillard Reaction between Glucosamine and Sulfur-containing Amino Acids

by

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글루코사민과 황함유아미노산의 메일라드반응의 반응기작

Advisor: Prof. Yang Bong Lee

by

S.J.Thewarapperuma Arachchi

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Abstract

Maillard reaction is an important reaction in food industry. It is responsible for the formation of color and aroma, as well as toxic compounds as the recent discovered acrylamide. The knowledge of reaction condition to maximize Maillard reaction to form optimum browning and aroma is very important in food processing industry. Maillard reaction is mainly influenced by many factors such as temperature, pH, water activity, heating time, buffer concentration, reactant concentrations and reactant source and sugar involved to the reaction. Even though lots of experiments have been done on this complex network of chemical reactions, very few papers is available on Maillard reaction between glucosamine and sulfur-containing amino acids.

Optimization of Maillard reaction conditions and identification of Maillard reaction products are presented in each of the chapters. The reaction condition of initial pH,

reaction time, reaction temperature and reactant concentration ratio was subjected to optimize by using response surface methodology in aqueous model systems.

Maillard reaction between glucosamine and cysteine (chapter 1) was optimized and found that initial pH and the concentration ratio of the reactants have the significant effects on Maillard reaction. The central composite design matrix showed that the optimum absorbance can be achieved with the independent variable set at 1.30 concentration ratio, initial pH 8.01, temperature 111^{0}C and 2.47 hr reaction time. Its optimum color change can be achieved by the independent variable set at concentration ratio 1.30, initial pH 8.01, temperature 111^{0}C and reaction time 2.47 hours. Sulfur dioxide, methanethiol, carbon disulfide, methanesulfonic anhydride, and ethylene sulfide were identified in Maillard reaction products of glucosamine and cysteine.

The optimum conditions for Maillard reaction between glucosamine and methionine are described in chapter 2. Maillard reaction between glucosamine and methionine was optimized and found that the linear coefficient of pH, reaction time, and reaction temperature and concentration ratio were significant. The optimal reaction conditions for absorbance were 113.9^{0}C reaction temperature, 1.76 hr reaction time, initial pH 8.4 and 1.26 concentration ratio of reactants. The optimal reaction conditions for color development were 93.2^{0}C reaction temperature, 1.9 hr reaction time, initial pH 6.18 and 0.9 concentration ratio of reactants.

The effect of initial pH on Maillard reaction between glucosamine and sulfur-containing amino acids were studied in Chapter 3. Cysteine, methionine and taurine were reacted at

fixed reaction temperature, concentration ratio and reaction time, while pH was varied at pH 5, pH 7 and pH 9. Flavor volatiles were isolated and identified by using GC-MSD. Maillard reaction between glucosamine and taurine showed the highest amount of different kinds of sulfur-containing volatile compounds.

Keywords: Glucosamine, cysteine, sulfur-containing amino acids, Maillard reaction, response surface methodology

Introduction

Non-enzymatic browning reactions between amino acids and reducing sugars are the basis of the Maillard reaction, which takes place in thermally processed foods. The Maillard reaction, or non-enzymatic browning, reported almost a century ago by Maillard (1912), has still not been unravelled or understood completely. Hodge (1953) presented a comprehensive reaction scheme and it was a major achievement in showing the complexity of the chemistry of the Maillard reaction and after that scientists have developed and elaborated this original scheme, steadily advancing knowledge about the Maillard reaction. In general Maillard reaction plays an important role in improving the appearance and taste of foods. It has a direct relationship with aroma, taste and colour. It results the formation of complex mixtures of coloured and colourless reaction products which range from flavour volatiles to melanoidins, which is a series of brown pigments with high molecular weights. This formation of a complex series of compounds called, Maillard reaction products (Mastrocola & Munari, 2000).

Quality controlling of food means controlling chemical, physical and microbiological changes in the food during food processing and storage.

(Horiuchi, Taniguchi,Hayase, Kurata, & Osawa, 2002; Labuza, Reineccius, Monnier, O Brien, & Baynes, 1994).

Brown pigment formation is desired during some types of food processing like baking, cocoa and coffee roasting, cooking of meat while it is absolutely undesirable in other food processing like milk drying, thermal treatments for the stabilization of milk, fruit juices and tomatoes. Maillard reaction products have negative consequences not only on the sensory characteristics of foods like colour changes and volatile compound formation but also on the nutritional value like amino acid and protein unavailability (Lerici, Barbanti, Manzano and Cherubin, 1990, Horiuchi, Taniguchi,Hayase, Kurata, & Osawa, 2002).

Recently Maillard reaction has gained more attention due to formation of mutagens and carcinogenic substances during the processing (Finot, 2005). Acrylamide, probably carcinogenic to humans and was found in heat-treated foods, such as French fries, potato crisps, coffee and bread (IARC, 1994; Tareke et al., 2002). Asparagine and reducing sugars are the main precursors to produce acrylamide in Maillard reaction process in foods (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002).

However, Maillard reaction technology is used in flavour and food industry for the production of process reaction flavours and colours or in-process flavours and colours generation.

Maillard reaction occurs in three major stages called initial, intermediate and late stage (Hodge 1953). The reaction depend on factors such as temperature, pH, water activity, heating time, concentration of buffer, concentration of reactant and reactant source and sugar involved to the reaction Changing of any of these products will alter reaction rate, reaction pathways and action end product (Ames, 1990; Wijewickrama, Krejpco & Kitts, 1999).

At the initial stage of the reaction involve the nuclifilic attack by the nitrogen atom of an amino compound on the electropillic carbonil group of reducing sugar form N–substitute glucosamine under heating condition. These N–substituted glucosamine undergoes rearrangement to form Amadori rearrangement products. The degradation of the Amadori product is dependent on the pH of the system (Hodge 1953). At pH 7 or below, it undergoes mainly 1,2-enolisation with the formation of furfural (when pentose are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH >7 the degradation of the Amadori

compound is thought to involve mainly 2,3-enolisation, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMFone), and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed (Fig. 1). All these compounds are highly reactive and take part in further reactions. At the intermediate stage, carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. Dicarbonyl compounds will react with amino acids with the formation of aldehydes and α-aminoketones. This reaction is known as the Strecker degradation. At the late stage, a range of reactions takes place, including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations and further condensations and ultimately, lead to the formation of brown nitrogenous polymers and co- polymers called as melanoidins (Hodge 1953).

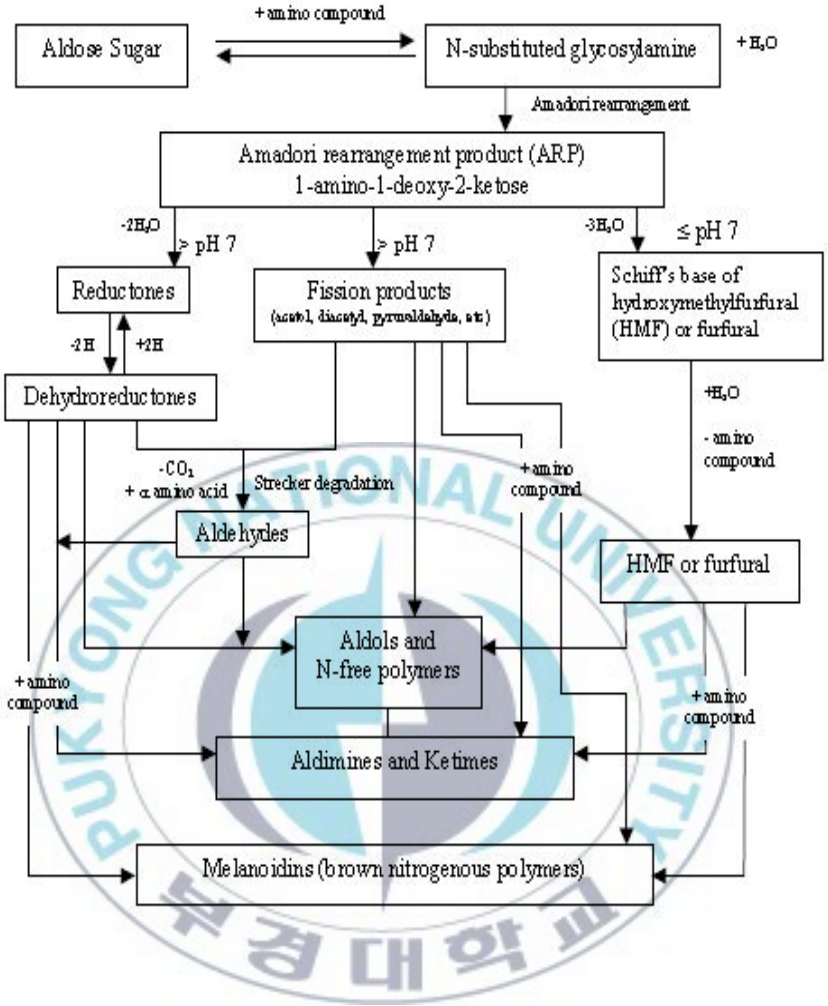

Fig. 1. Maillard reaction scheme adapted from Hodge (1953).

Maillard reaction product have ability to retard lipid oxidation, as well as to inhibit certain oxidoreductases, such as PPO Maillard reaction products have antioxidant ability through scavenging oxygen radicals or chelating metals according to the findings of Bersuder et al. (2001), Lingnert et al. (1983), Monti et al. (1990), and Tan & Harris, (1995).

Generally, by the reaction of the Amadori product or dicarbonyls colour pigments for brown colour were formed.

Colour formation

The colour of a food is the result of coloured natural products associated with raw material and/or coloured compounds generated as a result of processing (Rizzi, 1997). Colour formation was due to the formation of high molecular weight (>12, 000 Daltons) of polymeric compounds called melanoidins. Most of the studies investigating the mechanism and rate of the Maillard reaction are corresponding with the formation of brown colour. The fluorescence and browning developments can be used as indicator in Maillard reaction rate and Maillard reaction products formation (Yeboah et al., 1999). Hodge's (1953) studies focused mainly on Maillard browning Fig. 1 and Josek, et al., (2010) explained a

framework that covered important reaction routes for the formation of aroma compounds Fig. 2.

Maillard intermediates are formed in the initial phase of the Maillard reaction by Amadori rearrangement of the corresponding N-glucosamine. N-glucosamine condenses with amino acids and aldoses such as glucose as shown in the pathway of Fig. 2. Then, it causes to produce flavour compounds (Josek, et al., 2010). Strecker pathway not only to produces colour but also produces off-flavours in Maillard reaction (Namiki, 1988). Maillard reaction leads to produce many important classes of flavour compounds including furans, pyrazines, oxazoles, thiophenes, thiavoles and other heterocyclic compound. The lager number of different reactive intermediates that can be generated in this complex reaction pathway (Mottram, 1994)

Measurement of volatiles released from food matrix is a main tool that uses in food quality control. This tool has been developing rapidly by introducing modern techniques for the collection of volatiles (headspace, purge and trap etc.) and their instrumental analysis (capillary GC, GC/MS).

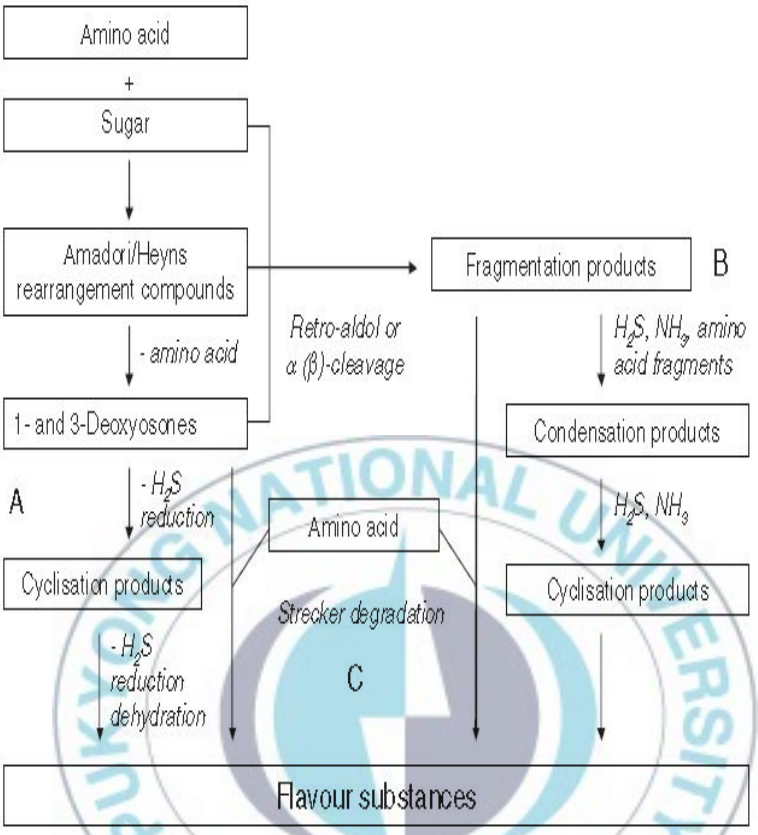

Fig. 2. Major Pathways for the formation of flavor substance during Maillard reaction (Josek, et al., 2010).

These methods are common in volatile quality and quantification in Maillard reaction. Glucosamine ($C_6H_{13}NO_5$) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine is part of the structure of the polysaccharides chitosan and chitin. It is the major component of the exoskeletons of crustaceans and other arthropods, as well as the cell walls of fungi. Glucosamine is one of the most abundant monosaccharide and it is produced commercially by the hydrolysis of crustacean exoskeletons. Glucosamine is the first precursor that produced in Maillard reaction between sugar and anima acids.

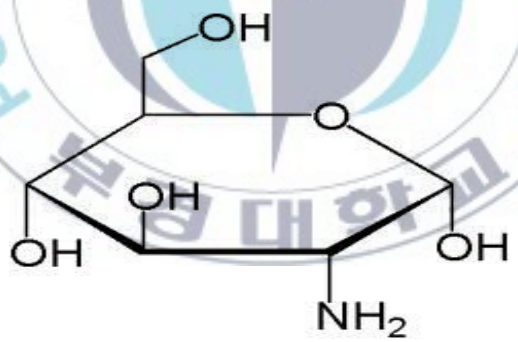

Fig. 3. Chemical structures of the D- glucosamine

Methionine, cysteine and taurine are the common sulfur-containing amino acids, and only Methionine, Cysteine, are incorporated into proteins. Taurine is 2-aminoethanesulfonic acid and it is an organic acid widely distributed in animal tissues.

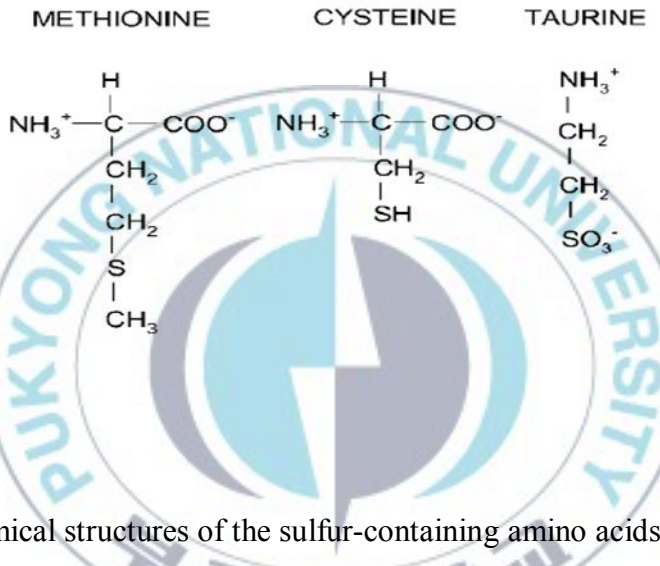

Fig. 4. Chemical structures of the sulfur-containing amino acids.

Maillard reactions with sulfur containing amino acids produce sulfur-containing volatiles. Volatile sulfur compounds are important contributors to the characteristic flavours and off-flavours of many foods. As a class, sulphur-containing flavour volatiles have low sensory detection thresholds; sulphur-containing flavour compounds are typically present in

foods at extremely low levels, often at sub parts-per billion concentrations. Some of these volatile components provide background sensory nuances to the flavor (McGorrin, R. J. 2011). Some of the sulfure volatiles contribute to off-flavors also. Light struck off-flavor in beer due to 3-methyl-2-butene-1-thiol, when beer expose to light, S-Methyl hexanethioate in beer cabbage,rubbery smell , and cat urine smell in beer due to 3-Mercapto-3-methylbutylformate (Qian, M., et al.,2011)

In this thesis, the chemistry of the Maillard reaction was studied between glucosamine and sulfur-containing amino acid to achieve maximum colour change and maximum flavor volatiles. Specifically, in this thesis, different studies have been performed in order to understand the reaction mechanism and the optimum conditions for the Maillard reaction and identify the volatiles. On the other hand this study can use as a pilot research to replaced expensive sugars by cheaper ones without changing the resulting properties. Even though lots of experiments have been done on glucosamine and amino acids, it is very rear to find information on Maillard reaction between glucose amine and sulfure- containing amino acids.

Objectives

- Optimization of Maillard reaction between glucosamine and cysteine using response surface methodology.
- Optimization of Maillard reaction between glucosamine and Methionine using response surface methodology.
- pH effect on the formation of Maillard reaction volatiles between glucosamine and sulfur-containing amino acids such as cysteine, methionine and taurine.

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CHAPTER 1

Optimization of Maillard Reaction between Glucosamine and Cysteine using Response Surface Methodology

Abstract

Sulfur-containing amino acids play important roles in good flavor generation in Maillard reaction of nonenzymatic browning, so aqueous model systems of glucosamine and cysteine were studied to investigate the effects of reaction temperature, initial pH, reaction time and concentration ratio of glucosamine and cysteine. Response surface methodology was applied to optimize the independent reaction parameters of cysteine and glucosamine in Maillard reaction. Box–Behnken factorial design was used with 30 runs of 16 factorial levels, 8 axial levels and 6 central levels. The degree of Maillard reaction was determined by reading absorption at 425 nm in a spectrophotometer and Hunter's L, a, and b values. Delta E was consequently set as the fifth response factor. Maillard reaction products were subjected to the combine system of purge and trap, gas chromatography and mass selective detector (GC-MSD) to check volatile compounds as a sixth response factor. According to the results of statistical analysis, initial pH and the concentration ratio of the reactants showed significant effects on Maillard reaction between glucosamine and cysteine, but the effects of temperature and time were not significantly affected. Sulfur-containing compounds identified from a reaction product by using GC-MSD were sulfur dioxide, methanethiol, carbon disulfide, methanesulfonic

anhydride, ethylene sulfide and tetrathiane which are thought to be important in their flavors.

Key words: Glucosamine, cysteine, sulfur-containing amino acids, Maillard reaction, response surface methodology.

Introduction

Maillard reaction is one of most important and complex process in food processing due to participate large number of complex mixtures of product through different pathways. The basis of Maillard reaction is non-enzymatic browning reaction between amino acids and reducing sugars, which take place in thermally processed (Maillard, 1912). Maillard reaction plays a major role in food stability, flavour development, nutrition and health (Waller & Feather, 1983; Fujimarki et al., 1986; Fino et al., 1990; Labuza et al., 1994). It results formation of complex mixtures of coloured and colourless range of flavor volatile to melanoidins, a series of brown pigments with high molecular weight. Generally, alkyl pyrazine have been reported as important product of Maillard reaction for unique sensory properties in food industries (Davidek et al., 1990) and formation of colour directly related with these products (Wong & Shibamoto., 1996). At the early stage of Maillard reaction, the free amino group of amino acid reacts with the carbonyl group of reducing sugar and form reversible Schiff base and rearrange to Amadori rearrangement products or Heyns rearrangement product (Jing & Kitts, 2002; Rizzi, 1994). The presence of open chain of sugar molecules in aqueous solution of aldehyde or ketone

is less than 1% of total sugar in solution. Therefore, ring opening reaction is initiated by nucleophilic attack of nitrogen atom of the amino group of amino acid to electrophilic carbonyl group of reducing sugar (Yaylayan & Huyghues-Despointes, 1994). At the intermediate stage, highly UV absorbing and colourless compounds are formed and in the advance phase of recation, amadori products undergo further transformation to fluorescent, coloured substances and cross linked polymers (Ames, 1990; Van Boekel, 2001). The final result is the formation of reductions, furfurals, pyrazines and other cyclic substances.

Maillard reaction properties give desirable characters to some kind of food processing (baking, cocoa and coffee roasting and cooking of meat) and undesirable characters to other processing (milk, fruit juice). Although the Maillard reaction products have negative effect on sensory characteristics of the food (colour changes and volatile compound formation) and nutrition metabolism in human body (amino acid unavailability for metabolism), (Leric, Barbati, Manzano and Cherubin, 1990) it has beneficial effects, not only in food processing but also in food preservation. Maillard reaction products have antioxidant ability through scavenging oxygen radicals or chelating metals according to the finding of

Bersuder et al. (2001), Lingnert et al. (1983), Wijewickrama et al. (1999) and Monti et al. (1999).

Thus, Maillard reaction is a complex reaction, science it is influence by many factors such as temperature, pH, water activity, heating time, buffer concentration, reactant concentration and reactant source and sugar involved to the reaction (Ames, 1990; Wijewickrama, Krejpco & Kitts, 1999). Changing of any these products will alter reaction rate, reaction pathways and action end product.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for the improvement and optimization of complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions to provide sufficient information for statistical acceptable results (Lee et al., 2000). It has been successfully demonstrated that this technique can be used in optimizing process variables (Lee & Kwon, 1998; Gallagher et al., 2003; Shyu and Hwang, 2002; Zhang et al., 2007). Although the scientist have done lots of experiment on Maillard reaction between different type of reducing sugar and amino acids and its product little information is available on chemical structure of hundreds of

unknown products and its effect on the sensory character of foods. Especially the optimum conditions for the reaction have not discovered. This study involved two chemical compounds which are namely glucosamine and cysteine. It is very important to study Maillard reaction between these two compounds rather than study the reaction between reducing sugar and amino acids because glucosamine contain both an aldehyde moiety with electrophilic carbonyl group and a nucleophilic amino group and cysteine contain another nucleophilic amino group and sulfur atom. The overall objective of this work is to investigate the effect of heating time, temperature, initial pH and concentration ratio of glucosamine and cysteine in Maillard reaction to optimize the processing conditions using response surface method.

Materials and Methods

Materials

D-Glucosamine, L-cysteine, sodium hydroxide and hydrochloric acids were used. Sodium hydroxide and hydrochloric acid and L-cysteine were parched from Junsei Chemicals. Co. Ltd (Tokyo, Japan). D-glucosamine was purchased from Jiangsu Jiushoutang Organisms Manufacture Co. Ltd (Xinghua, China). All chemicals used were of analytical grade.

Experimental design for reaction

According to prior experimental findings, heating time (X_1), initial pH (X_2), temperature (X_3) and concentration ratio (X_4) are the most influential factors on the Maillard reaction between glucosamine and cysteine. In order to evaluate the effects and interactions of these four factors, Response Surface Method (RSM) was used in designing this experiment. The Box–Behnken factorial design with four independent variables (temperature, initial PH, heating time and concentration ratio) and absorbance, Hunter's Lab colour scale of L, a, and b values were used as a

response (dependent) variable. This design was constructed based on a 33 factorial design, three replications of the central run, leading to 15 sets of experiments, allowing each experimental response to be optimized.

Temperatures were achieved by using a dry oven (Dongwon Scientific System, Busan, Korea). The pH levels were achieved by using 0.1M NaOH and 0.1M HCl accordingly and verified by the using of pH meter (Metrohm 827 pH Lab, Herisau, Switzerland). The ranges of the concentration ratios were achieved by mixing 0.1M Glucosamine and 0.1M cysteine according to Table 1-1. The reactions were conducted in triplicate and each replicate were adjusted the total volume of 20 ml.

The ranges of independent variables, temperature (X_1), pH (X_2), Heating time (X_3) and concentration ratios (X_4) were set in five levels of code values of -2, -1, 0, +1, and + 2 according to Table 1.1.

Table 1-1. Coded levels of independent variables in the response surface design of Maillard reaction between glucosamine and cysteine.

Independent variable	Coded unit				
	-2	-1	0	+1	+2
Concentration ratio (glucosamine : cysteine)	0.6 (0.03:0.07)	0.8 (0.04:0.06)	1 (0.05:0.05)	1.2 (0.06:0.04)	1.4 (0.07:0.03)
Temperature (0C)	80	90	100	110	120
Time (hr.)	2/3hr	1 1/3hr	2hr	2 2/3hr	3 1/3 hr.
pH	6	7	8	9	10

Determination of degree of Maillard reaction

The degree of Maillard reaction was determined by absorption at the wavelength of 425 nm by using a spectrophotometer (Ultospec 2000, Pharmacia Biotech, Buckinghamshire, England). Distilled was used as the standard for reference.

Determination of degree of colour changes

The evaluation of colour of the heated Maillard reaction product of glucosamine and cysteine mixtures was carried out using a colorimeter

(Colour JC 801, Stable Micro Systems, Surrey, UK) according to the CIE Lab scale (CIE, 1974; McLaren and Rigg, 1976). The system provides the values of three colour components; L,* (black-white component, luminosity), and the chromaticness coordinates, a* (+ red to - green component) and b* (+ yellow to - blue component) (Hunter, 1942). The colour changes in the media were determined by reading the Hunter's Lab colour scale L, a and b values using a colour reader. Distilled water was used as the standard for reference.

Determination of headspace volatile compounds of Maillard reaction products.

Headspace volatile compounds produced by Maillard reaction were isolated by a dynamic headspace technique. Ten millilitres of each Maillard reaction products were placed in a dark color bottle and heated in a dry oven at 60^0C for 30 minutes before isolation the volatiles. Volatile compounds were absorbed to Tenax tubes for 5 minutes and the tubes were set to automatic thermal de-absorber (ATD 400, Perkin Elmer, USA). The volatile compounds were identified by using the combined system of

gas chromatography and mass selective detector (GC-MSD: QP-5050A, Shimadzu, Japan). The primary tube type of ATD400 is Tenax-TA and cold trap type is Tenax –TA 20 mg. First desorption at 350^0C for 4 minutes whereas second Cryogenic temperature is -30^0Cand second desorption is 350^0C for 1 minute. Desorb flow is 50.2 ml/min and outlet spilt is 11.5 l/min. The analytical conditions of gas chromatography and mass selective detector are, 35^0C -10min, 8^0C/min-120^0C-10 min, 12^0C/min-80^0C -7min, 15^0C/min-230^0C- 10min as oven temperatures and AT1-60m*0.32mm*1.0μm column. Interface temperature is 2300C and MS Detector temperature is 250^0C. Mass range is 20~350 m/z and column pressure is 15.9psi. Mass filter type is quadrupole and 99.9999% nitrogen gas is used as carrier gas.

Statistical analyses

Analysis of variance (ANOVA) was performed using statistical analysis software (SAS) (Version 9.2). ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The statistical significance of the

regression coefficients was determined by using the F-test and the applicability of the model was checked with significance coefficients of determination (R^2) and the coefficient of variation (CV) values. The optimum processing conditions were obtained by using graphical and numerical analysis based on the criterion of desirability. Data was subjected to multiple regression analysis using SAS (Version 9.2) to fit the following second order polynomial equation

$$Y = b_o + \sum_{i=1}^{4} biXi + \sum_{i=1}^{4} bii\, X_i^2 + \sum_{i<j=2}^{4} b_{ij} X_i X_j \quad (1.1)$$

Where b_o is the intercept, b_i, b_{ii} and b_{ij} are the linear, quadratic and interaction coefficients, respectively. The response surface plots were then plotted to present the individual, interactive and quadratic effects of the independent variables on the responses.

Results and Discussion

Response surface modelling for absorbance

The details of the real values of absorbance and Hunter's colour values of 30 experimental runs of Box – Behnken design for response surface methodology were summarized in Table 1-2.

The ANOVA confirms adequacy of the statistical models since their probability > F values are less than 0.05 and statistically significant at the 95% confidence level. The results show that the quadratic model equation is significant ($P<0.05$) on the account. The models present high determination coefficients (R^2) and low coefficients of variation (CV). These values are obtained as follows: $R^2 = 0.9472$ and CV = 18.976. This result indicates that a good precision and reliability of the experiments was carried out. The significance of each coefficient is determined by F value and Probability > F value. The smaller the magnitude of the Probability > F value the more significant is the corresponding coefficient. The fitted model equations are as follows:

Table 1-2. The Box–Behnken matrix and response dependent variables of Absorbance and Hunter's L, a and b for the response surface analysis of Maillard reaction between glucosamine and cysteine

Independent Variables (Coded)					Response variables				
					Absorbance	Hunter's Lab colour scale L, a and b values *			
Runs	X_1	X_2	X_3	X_4	Y_1	Y_2(L)	Y_3(a)	Y_4(b)	$Y_{5(\Delta E^*)}$
1	1	1	-1	1	1.37±0.03**	85.57±2.54	0.79±0.23	59.84±2.32	104.43
2	1	-1	-1	-1	0.71±0.10	96.19±0.53	-10.18±0.46	36.24±3.79	103.30
3	-1	1	-1	-1	0.18±0.01	98.11±0.46	-2.52±0.02	12.08±0.42	98.88
4	1	1	1	-1	0.58±0.05	95.15±1.41	-5.12±0.39	31.84±2.94	100.47
5	-1	-1	1	-1	0.73±0.07	95.92±0.55	-7.41±0.37	31.81±2.34	101.33
6	1	-1	1	1	1.95±0.02	88.23±0.96	-6.24±0.21	61.83±0.71	107.92
7	-1	1	1	1	1.04±0.01	90.94±0.23	-2.22±0.03	51.70±0.36	104.63
8	-1	-1	-1	1	0.76±0.03	96.12±0.73	-6.70±0.09	31.88±0.34	101.49
9	0	0	0	0	1.09±0.05	90.55±1.02	-3.80±0.12	50.97±0.95	103.98
10	0	0	0	0	1.27±0.18	89.20±1.37	-2.78±0.42	53.20±4.15	103.90
11	0	0	0	0	1.36±0.08	88.98±1.06	-2.63±0.38	56.32±2.09	105.34
12	1	1	-1	-1	0.37±0.03	97.16±0.80	-4.50±0.18	22.02±2.00	99.73
13	1	-1	1	-1	1.35±0.02	94.02±0.56	-10.66±0.04	51.81±0.88	107.88
14	-1	1	-1	1	0.45±0.00	95.60±0.36	-3.78±0.15	26.76±0.26	99.34
15	-1	-1	-1	-1	0.35±0.01	98.20±0.09	-4.39±0.07	17.80±0.20	99.90
16	-1	-1	1	1	1.38±0.01	92.58±0.92	-8.49±0.12	45.92±0.49	103.69
17	1	-1	-1	1	1.48±0.02	93.74±0.18	-9.93±0.36	48.63±0.45	106.07
18	-1	1	1	-1	0.37±0.02	96.83±0.43	-4.34±0.09	21.82±1.28	99.35
19	1	1	1	1	2.13±0.15	80.20±2.34	8.41±2.52	78.65±3.38	112.64
20	0	0	0	0	1.09±0.02	90.36±0.56	-3.24±0.21	50.46±0.08	103.54
21	0	0	0	0	1.30±0.00	88.70±0.16	-2.90±0.09	53.64±1.41	103.70
22	0	0	0	0	1.31±0.08	91.11±1.21	-3.49±1.25	54.80±3.12	106.37
23	-2	0	0	0	0.58±0.03	84.91±2.31	-0.43±1.24	33.91±0.93	91.44
24	2	0	0	0	2.29±0.18	80.74±1.08	6.03±0.19	79.40±0.80	113.40
25	0	-2	0	0	0.63±0.04	95.15±0.18	-7.45±0.05	24.98±0.61	98.65
26	0	2	0	0	0.45±0.03	96.35±1.29	-5.59±0.22	24.81±1.46	99.65
27	0	0	-2	0	0.39±0.04	89.99±2.36	-1.52±0.43	23.73±4.40	93.08
28	0	0	2	0	1.68±0.07	87.07±0.59	-1.25±1.09	64.29±2.20	108.24
29	0	0	0	-2	0.34±0.01	93.87±4.58	-4.70±0.27	18.95±0.87	95.88
30	0	0	0	2	2.45±0.03	77.61±0.93	9.05±0.14	78.60±0.68	110.83
R^2					**0.9472**	**0.7907**	0.7292	0.9588	0.7915

* $\Delta E=\sqrt{L^2 + a^2 + b^2}$

**The data represent means ± standard deviation of three replicate

The multiple polynomial regression analysis result was used to fit the data into the following second – order equation model.

$$Y_{1:}= -5.966181- 0.043483*X_1 + 2.719496*X_2 + 0.249484*X_3 - 8.640894*X_4 - 0.000045111*X_1^2 + 0.001956*X_1*X_2 - 0.211136*X_2^2 + 0.004095*X_3*X_1 - 0.009795*X_3*X_2 - 0.171370*X_3^2 + 0.060219*X_4*X1 + 0.368438*X_4*X_2 + 0.532183*X_4*X_3 + 0.365347*X_4^2 \quad (1.2)$$

Independent variables X_1, X_2, X_3 and X_4 denote temperature (^{0}C), PH, time (hr.) and concentration ratio respectively. The ANOVA results for the quadratic model for absorbance shows, the linear coefficient of pH and concentration ratio were significant ($P<0.05$). This signifies that the linear effects of initial pH ($p < 0.05$) and concentration ratio ($p<0.05$) were dominant over the quadratic and interaction terms. The interaction effects between heating time and concentration ratio was significant. The interaction effects between heating time, temperature and initial pH were not significant, but they slightly influenced the absorbance. Only the quadratic effect of pH was significant to absorbance, while others were not significant. The quadratic effects of time, temperature and concentration ratio were negative to absorbance.

Fig. 1-1(a) presents the variation of the absorbance with temperature and initial pH at a given heating time (2hr) and given concentration ratio (1). According to the graph A, absorbance increased rapidly with increasing temperature. Same results also observed in the reaction between reducing sugar and amino group of works of Labuza et al., 1994; O'Brien et al., 1998. Temperature and duration of heating were studied by Maillard and reported that the rate of the reaction increases with temperature (Maillard, L-C.1912). Reaction variation of glucosamine and cysteine according to the temperature, behave as same as the reaction variation of sugar and amino group but the kinetic of the reaction may be differ due to per attachment of amino group to carbonyl group. Further studies should be done on the kinetics of the reaction between glucosamine and cysteine. It may also be seen that the absorbance increased with the incensing of initial pH and after initial pH is around 8.0, the absorbance decreased with increasing initial pH.

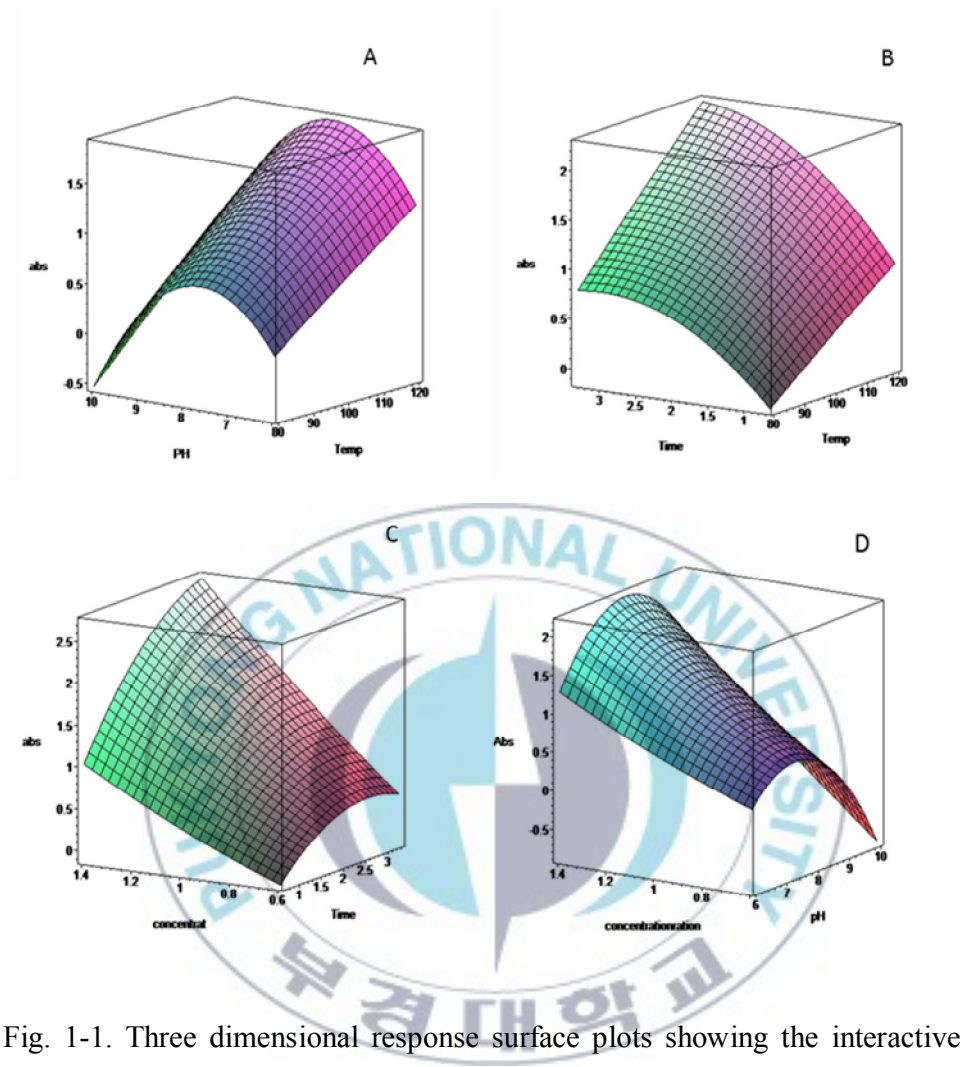

Fig. 1-1. Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and time, (C) time and concentration ratio and (D) concentration ratio and pH on absorbance at 425 nm. In each plot, the other two components are set at their central values (pH 8.00, temperature 100^0C, concentration ratio 1.00 and time 2 hour).

The variation of observance is curvilinear in nature with the increment of initial pH. Lerici and others (1990) described that intermediate stages of the nonenzymatic browning reaction produce more UV-absorbing compound and cause to higher absorbance. This observation is similar to the observation of Maillard reaction between fructose and lysine studied by Ajandouz et al (2001). According to his findings, higher the starting pH value causes higher absorbance if the fructose presence with lysine. The UV-absorbance quickly reached the maximum value at higher pH values and decrease thereafter because of almost complete degradation of sugar during the 1st stages in the heating period. The decrease of UV absorbance may result the transformation of some intermediate products into brown polymers. The reason for the hyperbolic curve of absorbance with the increase of pH was described by him. This curvilinear nature of absorbance according to the study of Ajandouz et al, 2001

Fig. 1-1(b) shows the effect of temperature and heating time on absorbance at a fixed initial pH of 8.0 and concentration ratio of 1. Fig. 1-1(b) shows that the absorbance increases rapidly and linearly with the increasing temperature. The absorbance increased rapidly with time at the first stage, while decreased slowly after 2.3 hr.

Fig.1-1(c) shows the effect of time and concentration ratio on absorbance at a fixed initial pH of 8.0 and temperature of 100^0C. The absorbance increased linearly with increasing of concentration ratios within the range of 0.6 to 1.4. Similar observations were explained by Benjakul et al. (2005) as the fluorescence intensity of the fructose–glycine model system increased with increasing reactant concentrations. According to his explanation "This might be due to transformation of intermediates to polymer compounds in the presence of reactants at high concentration". Benjakul et al. (2005) have found that fluorescence intensity increases when the concentration of sugar increases in Maillard reaction products from a porcine plasma protein–sugar model system. Moreover, Baisier & Labuza (1992) and Lerici et al. (1990) have discovered that concentration and ratios of reactants have significant impact on the reaction. In general, the variation of absorbance in Maillard reaction between glucosamine and cysteine can be explain as "fluorescent compounds formed prior to the generation of brown pigments are possibly the intermediate precursors of brown pigments" (Labuza & Baisier, 1992; Morales, Romeo, & Jimenez-Perez, 1996). The variation of absorbance with concentration ratio and initial pH at a constant temperature and constant time is presented in

Fig. 1-1(d). It is evident that at a fixed temperature and heating time, the absorbance increased rapidly with initial pH at the first stage, while decreased afterwards. At a fixed temperature, initial pH and time, concentration ratios affect to increase absorbance. The incensement is observed as linear relationship. The central composite design matrix showed the optimum absorbance can be achieved with the independent variable set at concentration ratio 1.30, initial pH 8.01, temperature 111^0C and reaction time of 2.47 hrs.

Response Surface Modelling for Hunter's colour scale L, a and b.

Table 1-2 gives the details of the actual Hunter's Lab colour scale L (Y_2), a (Y_3) and b (Y_4) values obtained from each of the 30 experimental runs. The values for the delta E (Y_5) were calculated by using the following equation (Giangiacomo and Messina, 1988).

$$\Delta E=\sqrt{L^2 + a^2 + b^2} \quad (1.3)$$

Among the response variables of L, a, b and ΔE, variable b (Y_3) has a higher R^2 value According to the ANOVA results in Table 1-2. The

ANOVA result of Hunter's Lab colour scale b value shows that the quadratic model equation is significant ($P<0.05$) on the account. The models present high determination coefficients (R^2) and low coefficients of variation (CV). These values are obtained as follows: $R^2 = 0.9524$ and CV = 13.4266. This result indicates a good precision and reliability of the experiments carried out. The significance of each coefficient is determined by F value and probability > F value. The fitted model equations are as follows. The multiple polynomial regression analysis result was used to fit the data into the following second–order equation model.

$$Y_3 = -312.687253 - 0.741541 * X_1 + 92.147287 * X_2 + 29.147287 * X_3 - 171.541892 * X_4 + 0.001668 * X_1^2 + 0.055562 * X_1 * X_2 - 7.773228 * X_2^2 - 0.049720 * X_3 * X_1 + 0.605410 * X_3 * X_2 - 6.794792 * X_3^2 + 1.071563 * X_4 * X_1 + 24.559375 * X_4 * X_2 + 10.191231 * X_4 * X_3 - 45.080702 * X_4^2. \quad (1.4)$$

Independent variables X_1, X_2, X_3 and X_4 denote temperature (^{0}C), PH, time (hrs.) and concentration ratio, respectively.

Hunter's (b) value has strong relationships with degree of color change of Maillard reaction according to R^2 (Table 1-2). The ANOVA results for the quadratic model for Hunter's 'b' value have a positive linear effect with the initial pH ($p < 0.05$). The interaction effects between pH and concentration ratio were significant while others were not significant, but they slightly influenced the Hunter's 'b' value. Only the quadratic effect of pH was significant to Hunter's 'b' value, while others were not significant.

Fig. 1-2 (a) presents the variation of the Hunter's 'b' value with temperature and initial pH at a given heating time and given concentration ratio. It can be seen from the figure that Hunter's 'b' value rapidly increased with increasing temperature. It may also be seen from in Fig. 1-2 (a) that Hunter's Lab colour scale b value increased with the incensing of initial pH. When initial pH is higher than 8.0, the Hunter's 'b' value decreased. The variation of 'b' value for pH was curvilinear in nature. Fig. 1-2 (a) shows the same pattern at the absorbance result of Fig.1-1 (a) and the 'b' value was increasing with the increasing temperature.

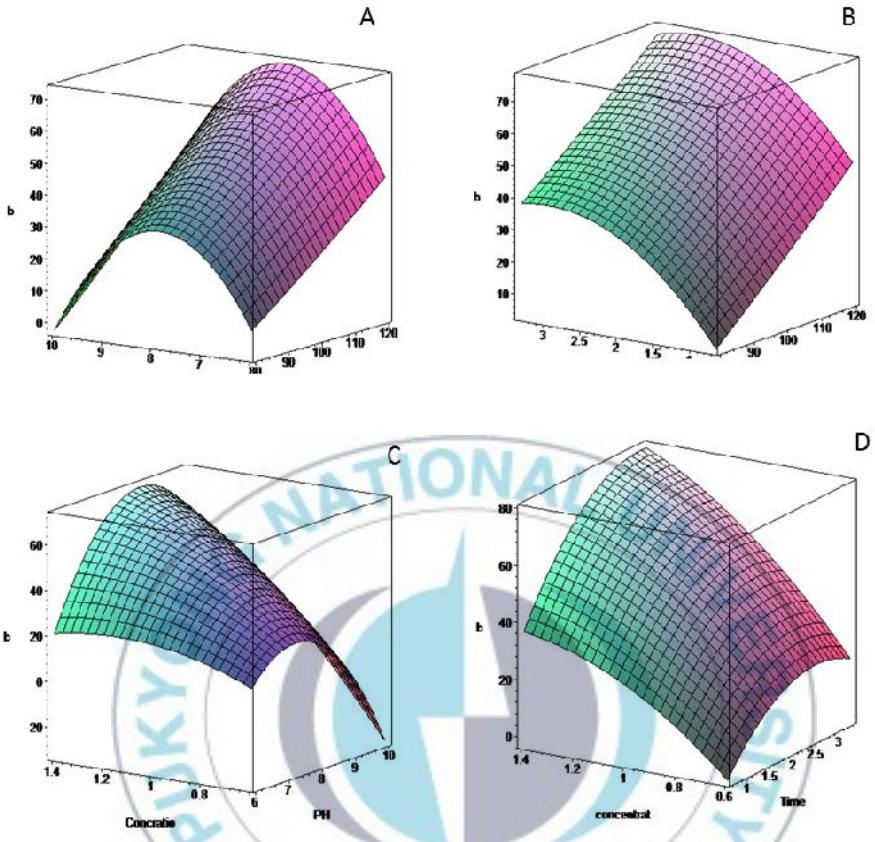

Fig. 1-2. Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and time, (C) concentration ratio and time, and (D) concentration ratio and pH on the Hunter's Lab color scale b value. In each plot, the other two components are set at their central values (pH 8.00, temperature 100^0C, concentration ratio 1.00 and time 2 hour).

Labuza et al., (1994) and O'Brien et al., (1998) described that the increasing temperature leads to an increase of reactivity of Maillard reaction. In casein and sugar model system, Morales and Boekel (1999) have observed that lightness (L*) of samples and the yellow and blue component (b*) have significant effect with severely heated samples. Lightness decreased significantly in severely heated samples which was indicated the increased darkness. The reduction of lightness is the result of formation of brown pigments in the sugar and casein mixtures in the advanced stage of the Maillard reaction. Results of yellow and blue component (b*) of glucose amine and cysteine can be explained as the same one as Morales and Boekel's(1998) findings. The blue component (b*) varied same as absorbance with the changing of initial pH of the reaction. The absorbance quickly reached the maximum value at higher pH values and decreased thereafter because of almost complete degradation of sugar during the 1st stages in the heating period. The decrease of absorbance may result the transformation of some intermediate products into brown polymers (Ajandouz, et al., 2001).

Fig. 1-2(b) shows the effect of temperature and heating time on Hunter's 'b' value at a fixed initial pH and fixed concentration ratio. Fig. 1.2(b)

shows Hunter's Lab 'b' value increased rapidly and linearly with the increased temperature. Hunter's 'b' value increased rapidly with the reaction time at the first stage, while decreased slowly after 2.3hr.

Fig.1-2(c) shows the effect of reaction time and concentration ratio on Hunter's 'b' value at a fixed initial pH and temperature. The 'b' value increased with the increased of concentration ratios within the range of 0.6 to 1.4. The variation of 'b' value with concentration ratio and initial pH at a constant temperature and constant reaction time is presented in Fig. 1-2(d). It is evident that at a fixed temperature and heating time, the 'b' value increased rapidly with an initial pH at the first stage, while decreasing afterwards. The central composite design matrix showed that the optimum color change can be achieved by the independent variable set at concentration ratio 1.30, initial pH 8.01, temperature 111^0C and reaction time 2.47 hrs.

The optimum processing parameters were determined to yield glucosamine-cysteine Maillard reaction product with higher absorbance and higher color change. The optimum estimated absorbance is 2.755 and it can be achieved at the condition of initial pH 8.01, 111^0C reaction temperature, 2.47 hr reaction time and 1.3 concentration ratio. The model

describes the optimum conditions for color change measured by Hunter's 'b' value as 2.41hr reaction time, 113.6°C reaction temperature, initial pH 8.3 and 1.26 concentration ratio. The responses, degree of Maillard reaction and degree of color change (Y_1 and Y_4) calculated from the final polynomial functions were 2.755 absorbance at 425 nm, and 90.24 'b' value.

Headspace volatile compounds of Maillard reaction products

Volatile compounds were isolated and, separated and identified at optimum condition of gas chromatography with mass sprectometory from Maillard product of glucosamine-cysteine in aqueous module system. Typical chromatogram of headspace volatiles produced during glucosamine-cysteine of sample number 19, 22, 26, 28, and 30 are presented in Fig.1-3. The main identified compounds are listed in Table 1-3, respectively.

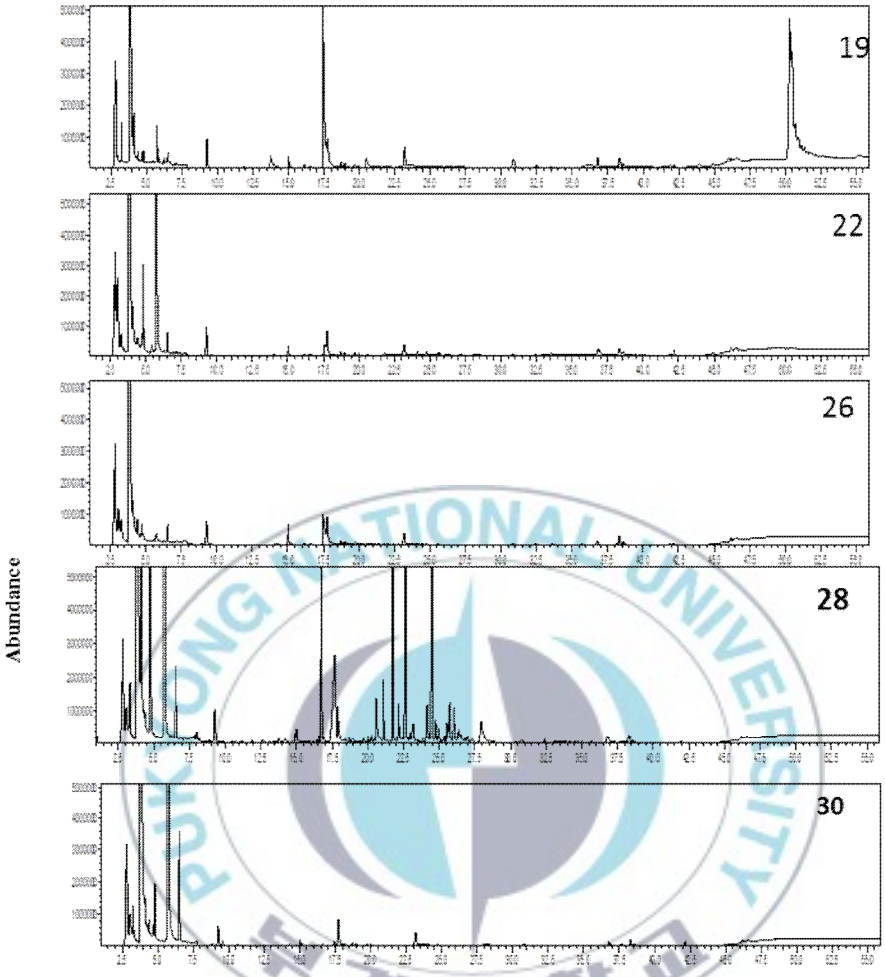

Fig. 1-.3. Chromatograms of volatiles isolated from sample number 19, 22, 26, 28 and 30 of glucosamine- cysteine Maillard browning model system and analyzed by means of capillary GC on DB5 column.

Table 1-3. Volatile compounds identified from sample number 19, 22, 26, 28 and 30 of glucosamine-cysteine Maillard browning model system and analyzed by means of capillary GC on DB5 column

Chemical name	Area (x10^6)						
	RT	RI	19	22	26	28	30
Hydrocarbons							
2-Methylpropene	3.32	<500	-	-	-	5.1	-
Diethoxyethane	3.82	<500	-	-	-	298.5	274.8
n-Hexane	6.54	600	1.8	3.1	2.6	10.8	16.7
4-Methyl-1,3-pentadiene	8.01	635	-	-	-	1.4	-
1-tert-Butoxy-2-methoxyethane	14.21	757	-	-	-	0.7	-
2,3,4-Trimethyl-oxetane	14.89	771	-	-	-	1.4	-
[(Hexyloxy)methyl]-oxirane	21.1	923	-	-	-	5.8	-
Sub total			1.8	3.1	2.6	323.7	291.5
Ethers							
Ethyl ether	4.39	510	-	-	1.6	-	1.2
Hexyl t-butyl, ether	27.95	1122	-	-	-	6.4	-
Sub total			-	-	1.6	6.4	1.2
Ketones							
Acetone	4.09	<500	3.1	2.4	1.4	69.9	1.0
2-Butanone	6.26	590	0.5	-	-	-	-
3-Pentanone	7.85	632	-	-	-	0.6	-
4-Methyl-4-penten-2-one	16.73	808	-	-	-	20.9	-
4-Hydroxy-4-methyl-2-pentanone	17.9	842	-	-	-	5	-
Subtotal			3.6	2.4	1.4	96.4	1.0
Nitrogen substances							
Pyrazine	13.74	747	3.2	-	-	-	-
Methyl-pyrazine	17.48	830	44.8	2.2	7.8	-	-
Ethyl-pyrazine	20.52	909	1.9	-	-	-	-
Subtotal			49.9	2.2	7.8	-	-
Alcohols							
Methanol	3.34	<500	0.2	-	-	-	-

Ethanol	3.86	<500	243.3	292.2	207.6	-	-
2-Methyl-2-propanol	4.72	527	-	-	-	28.3	-
2-Methyl-3-penten-2-ol	13.76	748	-	-	-	0.6	-
Hexylene Glycol	20.59	911	-	-	-	7.9	-
1,2-Heptanediol	21.74	937	-	-	-	32.2	-
1,2-Heptanediol	24.47	1031	-	-	-	48	-
4-Methyl-5-decanol	25.75	1069	-	-	--	7.8	-
Subtotal			243.5	292.2	207.6	124.8	-
Acids							
2,2-Dimethyl-propanoic acid	17.41	828	-	-	-	-	0.6
Subtotal			-	-	-	-	0.6
Aldehydes							
Acetaldehyde	3.26	<500	3	1.0	2.1	-	3.7
Subtotal			3	1.0	2.1	-	3.7
Aromatic compounds							
Methylcyclopentane	7.71	628	0.1	-	0.2	0.1	-
Benzene	9.27	660	4.3	4.1	3.6	5.9	3.2
Methylbenzene	15.02	773	1	1	2.2	1.2	-
p-Xylene	18.97	873	-	-	-	0.6	-
Pyrene	45.73	1601	0.2	-	-	-	-
Subtotal			5.6	5.1	6	7.8	3.2
Esters							
Formic acid, methyl ester	3.53	<500	-	-	-	0.1	-
Subtotal			-	-	-	0.1	-
Sulfur containing compounds							
Hydrogen sulfide	2.96	<500	-	6.5	-	-	-
Sulfur dioxide	3.05	<500	-	5.2	8.8	8.6	8.1
Methanethiol	3.46	<500	-	0.1	-	0.2	0.1
Carbon disulfide	4.81	531	0.9	9.8	-	-	5.9
Methanesulfonic anhydride	5.43	558	-	1	-	-	-
Ethylene sulfide	5.75	571	3.9	159.1	1.3	129.2	201.5
Sulfurous acid, decyl 2-pentyl ester	26.4	1088	-	-	-	2.5	-
[1,2,3,4]Tetrathiane	36.86	1324	-	1.1	-	0.9	-

Sulfurous acid, 2-ethylhexyl isohexyl ester	38.28	1349	-	-	-	1.6	-
Subtotal			4.8	182.8	10.1	143	215.6
Total			312.2	488.8	239.2	702.2	516.8

Total 42 volatile compounds were identified, which are 7 hydrocarbons, 2 ethers, 5 ketones, 3 nitrogen substances, 8 alcohols, 1 acid, 1 aldehyde, 1 ester, 5 aromatic compounds and 9 sulfur-containing compounds. Sample number 28 and 30 contained high amount of hydrocarbon and diethoxyethane and was most abundant in both samples. Comparing to other samples, these samples contained high amount of hydrocarbons and high amount of alcohols. Ethanol was most abundant and according to the findings of Karahadian and Lindsay (1989) alcohols are not greatly affected to food flavor because of high value of flavor threshold. Hydrocarbons contribute very low effect to food quality according to Heath and Reineccius (1986).

Sample number 28 shows higher amount of ethers, ketones, esters and aromatic compounds. Acetone found in all samples and sample number 28 contained relatively high amount of that. Nitrogen substances were not detected in sample number 28 and 30, but with the reaction condition of sample number 19 showed the highest nitrogen substances with

methylpyrazine. Ethanol was the most abundant alcohol in alcohols and it was not detected in sample number 28 and 30. Benzene was found in all samples and sample number 28 had the highest amount of aromatic compounds. Ethylene sulfide detected in all samples as sulfur contains substances and sample number 22, 28 and 30 showed high amounts of sulfur-containing compounds, compared to other two.

In this all samples, furfural was not detected, but in Maillard reaction between glucose-glycine aqueous systems, a large amount of furfural was detected. That result was similar to the finding of Hodge (1967) and Meynier & Mottram (1995). It is assumed that furfural is formed during the catalytic degradation of glucose in presence of amino compounds. According to the assumption, the enol form of 3-deoxy-D-erythrohexosulose appeared during the degradation that was followed by dehydration, oxidation and disproportionation (Olsson, Pernemalm, & Theander, 1978). In the case of glucosamine, the amino group is already attached to the glucose structure. It may be the reason for the undetected furfural.

Leahy and Reineccius (1989) reported that pyrazine in a glucose –lysine system had been found at high pH and total amount of pyrazines were more than 500 times higher than those at pH 9 than at pH 3. This study on glucosamine – cysteine system was conducted only in the high pH of pH 8, 9 and 10. Sample number 19 of pH 9 and sample number 26 of pH 10 showed high pyrazine contents.

Ethylene sulfide was detected in all samples and sulfur dioxide, carbon disulfide and methanethiol were prominent in these samples. Methanethiol also known as methyl mercaptan is a colorless gas with a smell like rotten cabbage or animal feces. Therefore, controlling of methanethiol is very important in food industry. According to McGorrin (2011), volatile sulfur compounds are important contributors to the characteristic flavors and off-flavors of many foods. As a class, sulfur-containing flavor volatiles have low sensory detection thresholds, are present in low concentration and are often chemically labile, which can present measurement challenges and sulfur flavor compounds are typically present in foods at extremely low levels, often at sub parts-per-billion concentrations. Some of these volatile components provide background sensory nuances to the flavor, while

others provide unique flavor characterizing identities to the foods they occur in. As he discussed, volatile sulfur compounds exhibit sensory potency at low concentrations due to their low aroma and taste thresholds. Sulfur-containing compounds contribute appropriate flavor character at low concentrations in most foods but at higher concentrations it produce sulfurous and objectionable taste and odder. Therefor these detected sulfur compounds play major role in foods and the concentration of that is very important.

Conclusion

The response surface methodology is a useful tool to investigate the optimum conditions of heating time, temperature, initial pH and concentration ratio for targeting measurement of degree of Maillard reaction and measurement of degree of color changes in glucosamine and cysteine Maillard reaction. The coefficients of determinations, R^2 values of the all parameters, show a good fit of the models with the experimental data at the 95% confidence level. The different conditions (heating time, temperature, initial pH, concentration ratio) for Maillard reaction revealed that initial pH and concentration ratio had the significant effect on degree of Maillard reaction, while other two variables had an optimum zone for degree of Maillard reaction. The different conditions (heating time, temperature, initial pH, and concentration ratio) for color change in Maillard reaction revealed that only the initial pH had significant effect. These results were well fitted with experimental data and obtained models can be used to the maximum values of the variables.

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CHAPTER 2

Optimization of Maillard Reaction between Glucosamine and Methionine using Response Surface Methodology

Abstract

This study is to optimize reaction condition of Maillard reaction between glucosamine and methionine. Response surface methodology was applied to optimize the independent reaction parameters of methionine and glucosamine in Maillard reaction. Box–Behnken factorial design was used with 30 runs of 16 factorial levels, 8 axial levels and 6 central levels. The degree of Maillard reaction was determined by reading absorption at 425 nm in a spectrophotometer and Hunter's L, a, and b values. Delta E was consequently set as the fifth response factor. According to the results of statistical analysis, initial pH, reaction time, reaction temperature and concentration ratio of reactants shows significant effect on absorbance in Maillard reaction between glucosamine and methionine. The optimal reaction conditions for absorbance are 113.9^0C reaction temperature, 1.76 hr reaction time, initial pH 8.4 and 1.26 concentration ratio of reactants. The expended optimum absorbance is 3.51. The optimal reaction conditions for colour development are 93.2^0C reaction temperature, 1.9 hr. reaction time, initial pH 6.18 and 0.9 concentration ratio of reactants. The expected optimum L value is 104.19.

Keywords: Glucosamine, Methionine, Maillard reaction, response surface methodology.

Introduction

Maillard reaction is a complex network of chemical reactions between reducing sugars and proteins or amino acids. It occurs during processing and storage of foods, and it produces a set of reaction products including volatile compounds of low molecular mass, non-volatile coloured compounds of intermediate molecular mass and brown substances of high molecular mass (Carpenter and Booth, 1973; Finot et al., 1981). The development of colour and volatile compounds are an extremely important in food processing. In the early stage of Maillard reaction, reducing sugar condenses with free amino group of amino acids or proteins to give a condensation product, N-substituted glucosamine, via the formation of a Schiff's base and Amadori rearrangement (Friedman, 1996; Van Boekel, 1998). This stage is characterized by the formation of unsaturated, brown nitrogenous polymers, copolymers and nitrogen-free polymers which are formed from condensation reactions from furfurals or dehydroreductones (Ames, 1992; Hodge, 1953). The produced colours range from pale yellow to very dark brown, depending on the type of food or the extent of the reactions. Strecker's pathway is one of source of off-flavors associated with Maillard browning (Namiki, 1988).

This non-enzymatic browning reaction product strongly affects to the quality of food. It influences food sensorial properties, such as flavor and colour and also influences nutritional and health aspects (Finot, Aeschbacher, Hurrell, & Liardon, 1990; Horiuchi, Taniguchi, Hayase, Kurata, & Osawa, 2002; Labuza, Reineccius, Monnier, OBrien, & Baynes, 1994; Waller& Feather, 1983). Colour formation can be due to both sugar caramelization and Maillard reaction. However, severe heating intensity, and extreme pH are necessary to cause sugar caramelization (Reyes et al., 1982; Walstra and Jenness, 1984). Many factors affect to the products of Maillard reaction and the velocity of reaction such as reaction temperature, reaction time, water activity, reactant source and concentration (Jing & Kitts, 2002), the type and ratio of reducing sugar (Naranjo, Malee, & Vigo, 1998; Yeboah, Alli, & Yaylayan, 1999), buffer type and concentration, presence of oxygen, light or metal ions (Ames, 1992), amino acids (Morales & Jimenez-Perez, 2001; Yeboah et al., 1999), pH (Ajandouz, Tchiakpe, Ore, Benajibas, & Puigserver, 2001) and food composition (Lerici, Barbanti, Manzano, & Cherubin, 1990; Tanaka, Chiba, Ishizaki, Takai, & Taguchi, 1994).

Thermally induced reaction between sulphur containing amino acids (cysteine/cysteine, methionine) and reducing sugars produces characteristic flavor compounds. Sulphur heterocyclic constituents that contribute aromas of coffee, toasted cereal grains, popcorn and roasted meats are products from Maillard reactions. Sulphur-containing heterocyclic compounds are associated with meaty character. There are two compounds identified that have more impact in cooked beef and chicken broth and in canned tuna fish. 2-methyl-3-furanthiol (1 ppt) and bis-(2-methyl-3-furyl) disulfide (0.02 ppt) identified by Boelens et al., (1992) and Withycombe et al (1988). Some of the sulphur volatiles contribute to off-flavors. 3-methyl-2-butene-1-thiol is act as off-flavor volatile in beer (Goldstein et al., 1993).

Investigations of the effect of sugar type in Maillard reaction are very important in two reasons of economic and technological aspects. In the first case, expensive sugars can be replaced by other cheaper ones without changing the resulting properties (Okazaki & Makino, 2004). The priority is technological expectations will goes to the ability of a sugar that limit to colour development of foods during storage and processing (Kwak & Lim, 2004). Finally, food industries have benefits from

scientific studies on the influence of sugar by reproducing artificial brown colour and flavor in food system.

Glucosamine is the first precursor that produced in Maillard reaction between sugar and amino acids. It is one of the most abundant monosaccharide that produced commercially by the hydrolysis of crustacean exoskeletons. In this study, expensive sugars were replaced by cheaper glucosamine. This study involved with two chemical compounds of glucosamine and methionine. It is very important to study Maillard reaction between these two compounds rather than to study the reaction between reducing sugar and amino acids because glucosamine contains both an aldehyde moiety with electrophilic carbonyl group and a nucleophilic amino group, and methionine contains another nucleophilic amino group and sulphur atom.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for the improvement and optimization of complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions to provide sufficient information for statistical acceptable results (Lee et al., 2000; Simsek et al., 2007). It has been

successfully demonstrated that this technique can be used in optimizing process variables (Lee & Kwon, 1998; Gallagher et al., 2003; Shyu and Hwang, 2002; Zhang et al., 2007). Although the scientist had done lots of experiment on Maillard reaction and optimization of the reaction, very little information is available on the optimization of reaction between glucosamine and sulphur-containing amino acids. The overall objective of this work is to investigate the effect of heating time, temperature, initial pH and concentration ratio of glucosamine and methionine in Maillard reaction to optimize the processing conditions using response surface method.

Materials and Methods

Materials

D-Glucosamine, methionine, sodium hydroxide and hydrochloric acids were used. Sodium hydroxide and hydrochloric acid and methionine were purchased from Junsei Chemicals Co. Ltd (Tokyo, Japan). D-glucosamine was purchased from Jiangsu JJiushoutang Organisms Manufacture Co. Ltd (Xinghua, China). All chemicals used were of analytical grade.

Experimental design for reaction

According to prior experimental findings, reaction time (X_1), initial pH (X_2), reaction temperature (X_3) and concentration ratio (X_4) are more influential factors on the Maillard reaction between glucosamine and methionine. In order to evaluate the effects and interactions of these four factors, response surface method (RSM) was used in designing this experiment. The Box–Behnken factorial design with four independent variables (reaction temperature, initial PH, reaction time and concentration ratio) and absorbance, Hunter's Lab colour scale of L, a, and b values were used as response (dependent) variables. This design

was constructed based on a 33 factorial design, three replications of the central run, leading to 15 sets of experiments, allowing each experimental response to be optimized.

Reaction temperatures were achieved by using a dry oven (Dongwon Scientific System, Busan, Korea). The pH levels were achieved by using 0.1M NaOH and 0.1M HCl accordingly and verified by the using of pH meter (Metrohm 827 pH Lab, Herisau, Switzerland). The ranges of the concentration ratios were achieved by mixing 0.1M glucosamine and 0.1M methionine according to Table 2-1. The reactions were conducted in triplicate and each replicate were adjusted the total volume of 20 ml. The ranges of (X) independent variables, reaction temperature (X_1), pH (X_2), Heating time (X_3) and concentration ratios (X_4) were set in five levels of code values of -2, -1, 0, +1, and + 2 according to Table 2-1.

Table 2-1. Coded levels of independent variables in the response surface design of Maillard reaction between glucosamine and methionine

Independent variable	Coded unit				
	-2	-1	0	+1	+2
Concentration ratio (glucosamine: methionine)	0.6 (0.03:0.07)	0.8 (0.04:0.06)	1 (0.05:0.05)	1.2 (0.06:0.04)	1.4 (0.07:0.03)
Temperature (0C)	80	90	100	110	120
Time (hr)	2/3hr	1 1/3hr	2hr	2 2/3hr	3 1/3 hr
pH	6	7	8	9	10

Determination of degree of Maillard reaction

The degree of Maillard reaction was determined by absorption at the wavelength of 425 nm by using a sprectophotometer (Ultospec 2000, Pharmacia Biotech, Buckinghamshire, England). Distilled water was used as the standard for reference.

Determination of degree of colour changes

The evaluation of colour of the heated Maillard reaction product of glucosamine and methionine mixtures was carried out using a colorimeter (Colour JC 801, Stable Micro Systems, Surrey, UK) according to the CIE Lab scale (CIE, 1974; McLaren and Rigg, 1976). The colour changes in the media were determined by reading the Hunter's Lab colour scale *'L', 'a'* and '*b*The system provides the values of three colour components; L¸* (black-white component, luminosity), and the chromaticness coordinates, a* (+ red to - green component) and b* (+ yellow to-blue component) (Hunter, 1942). ' values using a colour reader. Distilled water was used as the standard for its reference.

Statistical analyses

Analysis of variance (ANOVA) was performed using statistical analysis software (SAS) (Version 9.2). ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The statistical significance of the regression coefficients was determined by using the F-test and the

applicability of the model was checked with significance coefficients of determination (R^2) and the coefficient of variation (CV) values. The optimum processing conditions were obtained by using graphical and numerical analysis based on the criterion of desirability. Data was subjected to multiple regression analysis using SAS (Version 9.2) to fit the following second order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{4} biXi + \sum_{i=1}^{4} bii\, X_i^2 + \sum_{i<j=2}^{4} b_{ij} X_i X_j \qquad (2.1)$$

Where $b_{o\ is}$ the intercept, b_i, b_{ii} and b_{ij} are the linear, quadratic and interaction coefficients, respectively. The response surface plots were then plotted to present the individual, interactive and quadratic effects of the independent variables on the responses.

Results and Discussion

Response surface modelling for absorbance

The details of the real values of absorbance and Hunter's Lab colour scales values of the 30 experimental runs of Box –Behnken design for response surface methodology were summarized in Table 2-2.

The ANOVA confirms adequacy of the statistical models since their probability> F values are less than 0.05 and statistically significant at the 95% confidence level. The results show that the quadratic model equation is significant ($P<0.05$) on the account. The models present high determination coefficients (R^2) and low coefficients of variation (CV).

These values are obtained as follows: $R^2 = 0.9445$ and CV = 15.16 This result indicate a good precision and reliability of the experiments carried out.

Table 2-2. The Box–Behnken matrix and response dependent variables of absorbance and Hunter's Lab colour scales L, a and b for the response surface analysis of Maillard reaction between glucosamine and methionine

Independent Variables (Coded)					Response variables				
					Absorbance	Hunter's Lab colour scale L, a and b values *			
Runs	X_1	X_2	X_3	X_4	Y1	Y_2(L)	Y_3(a)	Y_4(b)	$Y_{5(\Delta E^*)}$
1	1	1	-1	1	3.01±0.13**	46.47±1.79	37.42±0.54	77.65±2.62	97.93
2	1	-1	-1	-1	0.96±0.08	86.71±0.95	1.16±1.02	51.19±3.15	100.70
3	-1	1	-1	-1	1.02±0.06	87.92±0.98	0.74±0.68	55.70±2.03	104.08
4	1	1	1	-1	2.94±0.15	48.11±2.73	36.11±0.76	79.86±3.95	99.98
5	-1	-1	1	-1	0.80±0.03	87.48±1.48	-0.09±0.39	45.60±1.94	98.66
6	1	-1	1	1	2.56±0.09	62.27±1.41	23.44±1.52	81.49±0.85	105.20
7	-1	1	1	1	2.98±0.14	57.97±1.23	29.75±0.63	89.37±1.38	110.60
8	-1	-1	-1	1	0.70±0.06	91.83±0.69	-1.77±0.61	42.14±2.56	101.05
9	0	0	0	0	3.09±0.06	53.92±1.23	32.20±0.92	85.38±0.84	105.99
10	0	0	0	0	2.96±0.17	59.00±1.69	28.29±1.42	86.99±0.19	108.85
11	0	0	0	0	2.92±0.13	57.47±2.80	29.60±2.51	86.93±0.49	108.34
12	1	1	-1	-1	2.99±0.04	61.82±1.05	26.86±1.26	89.50±0.52	112.04
13	1	-1	1	-1	1.75±0.26	73.66±3.45	13.15±3.71	70.90±4.63	103.08
14	-1	1	-1	1	1.44±0.20	83.60±2.51	4.72±2.50	68.39±5.26	108.12
15	-1	-1	-1	-1	0.32±0.02	95.66±0.75	-2.64±0.19	21.61±0.78	98.11
16	-1	-1	1	1	1.41±0.05	81.10±0.90	6.03±0.79	64.00±1.21	103.49
17	1	-1	-1	1	3.03±0.09	51.26±1.74	32.55±0.96	82.04±1.53	102.07
18	-1	1	1	-1	2.30±0.02	72.86±1.11	16.01±0.38	83.38±0.63	111.88
19	1	1	1	1	3.00±0.14	27.71±3.97	41.00±0.79	47.54±6.73	68.62
20	0	0	0	0	3.14±0.07	51.70±2.02	33.43±1.46	83.53±1.76	103.76
21	0	0	0	0	2.94±0.08	60.59±1.21	27.54±1.11	87.76±0.33	110.14
22	0	0	0	0	2.96±0.17	59.03±1.74	28.48±1.48	87.01±0.12	108.94
23	-2	0	0	0	0.69±0.03	92.00±1.56	-2.90±0.17	42.71±0.76	101.47
24	2	0	0	0	3.08±0.09	29.97±2.34	40.85±0.10	51.35±3.91	72.14
25	0	-2	0	0	0.08±0.00	100.01±0.66	-1.33±0.05	6.74±0.79	100.25
26	0	2	0	0	3.12±0.06	52.37±0.57	35.75±0.14	86.08±0.77	106.91
27	0	0	-2	0	1.89±0.17	76.55±1.76	10.42±2.69	75.06±3.79	107.71
28	0	0	2	0	3.07±0.21	46.17±1.25	35.88±0.27	76.61±1.79	96.37
29	0	0	0	-2	1.72±0.02	78.10±2.41	9.14±1.47	70.75±2.01	105.78
30	0	0	0	2	3.07±0.03	42.52±1.30	39.00±0.35	71.64±1.99	91.98
R^2					**0.9445**	**0.9521**	**0.9351**	**0.8683**	**0.7838**

*** ΔE=$\sqrt{L^2 + a^2 + b^2}$**

****The data represent means ± standard deviation of three replicate.**

The significance of each coefficient is determined by F value and Probility > F value. The smaller the magnitude of the Probility > F value the more significant is the corresponding coefficient. The fitted model equations are as follows:The multiple polynomial regression analysis result was used to fit the data into the following second –order equation model.

$$Y_1 = -83.954574 + 0.748999*X_1 + 7.687561*X_2 + 4.719862*X_3 + 15.047802*X_4 - 0.003021*X_1^2 - 0.005369*X_1*X_2 - 0.372118*X_2^2 - 0.035047*X_3*X_1 + 0.116698*X_3*X_2 - 0.348160*X_3^2 + 0.026719*X_4*X_1 - 0.837813*X_4*X_2 - 0.342817*X_4*X_3 - 4.359209*X_4^2 \quad (2.2)$$

Independent variables of X_1, X_2, X_3 and X_4 denote reaction temperature (^{0}C), pH, reaction time (hr) and concentration ratio, respectively.

The ANOVA results for the quadratic model for absorbance shows, the linear coefficient of pH, reaction time, and reaction temperature and concentration ratio were significant ($p < 0.05$). The interaction effects of temperature * temperature, pH *pH, heating time* temperature and concentration ratio * concentration ratio were significant.

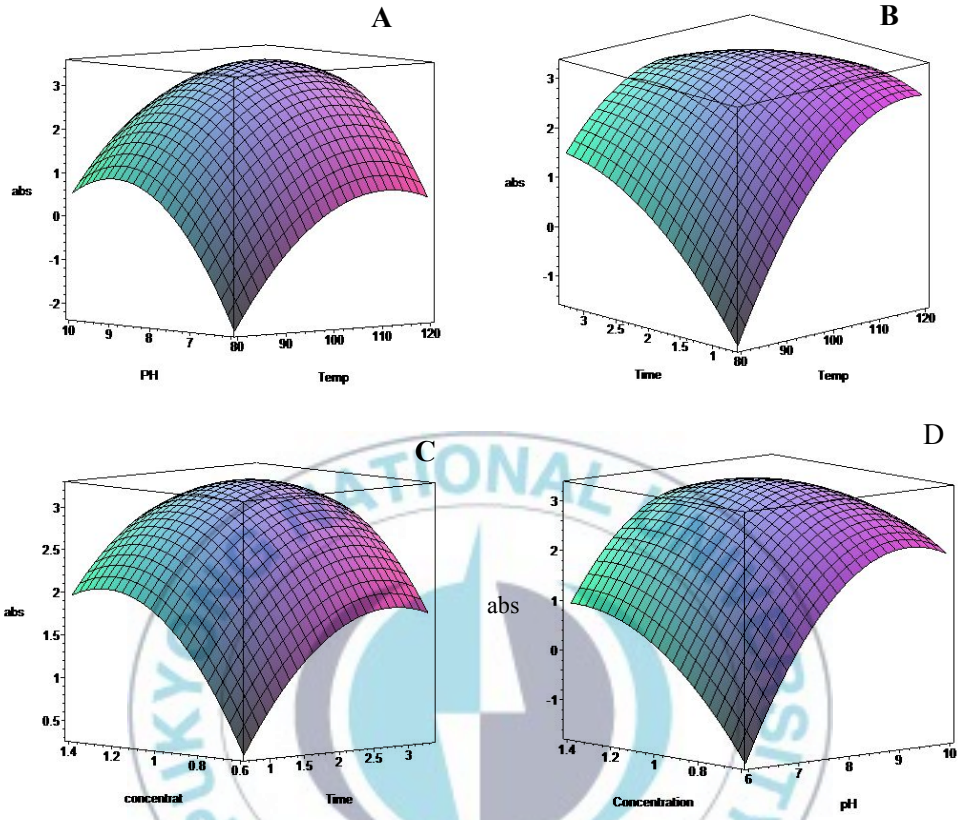

Fig. 2-1. Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and time, (C) time and concentration ratio and (D) concentration ratio and pH on absorbance at 425 nm. In each plot, the other two components are set at their central values (pH 8.00, reaction temperature 100^0C, concentration ratio 1.00 and time 2 hour)

Fig. 2-1(a) presents the variation of the absorbance with temperature and initial pH at a given heating time (2hr) and given concentration ratio (1). It can be seen that the absorbance increased rapidly with reaction temperature at a decreasing rate upto 113.9^0 and then, decrease slowly. But, the reaction between glucosamine and cysteine shows linearly increasing relationship between absorbance and reaction temperature according to Chapter 1. This observation deviates from the results of Maillard, L C., (1912) and the study of reaction between reducing sugar and amino group in Maillard reaction by Labuza et al., (1994) and O'Brien et al., (1998). described that reaction temperature effect on browning reaction in glucose–glycine system and galactose–glycine systems behaved the same as this finding. They have described both systems follow rapid increasment upto 45^0 C and after 60^0 C increasing rate decreases. They mentioned that their result follow the result of Baisier and Labuza (1992), in Maillard browning kinetics in an aqueous model system. It also can be seen that the absorbance increased at a decreasing rate with the increasing of initial pH and after initial pH is around 8.4 absorbance decrease gradually. The variation for pH is curvilinear in nature. Glucosamine–cysteine modal system in Chapter 1 and current

results show the same variation for absorbance and the results were similar as studies of Lerici, et al. (1990) and Ajandouz et al. (2001). They described that intermediate stages of the non-enzymatic browning reaction produce more UV-absorbing compounds and cause to higher absorbance and pH value causes higher absorbance. The UV-absorbance quickly reached the maximum value at higher pH values and decreased thereafter because of almost complete degradation of sugar during the first stages in the heating period. The decrease of UV absorbance may result the transformation of some intermediate products into brown polymers. The reason for the hyperbolic curve of absorbance with the increase of pH was described by them.

Fig. 2-1(b) shows the effect of reaction temperature and reaction time on absorbance at a fixed initial pH 8.0 and 1:1 concentration ratio. Fig. 2-1 (b) shows that the absorbance increased rapidly with time at the first stage, while decreased slowly after 1.8 hr. Fig. 2-1(c) shows the effect of time and concentration ratio on absorbance at a fixed initial pH of 8.0 and temperature of 100^0C. The absorbance was distributed curvilinear against both reaction time and concentration ratio of reactant variation. It shows that absorbance (X) decrease after the increasing concentration ratio to 1.3.

The variation of absorbance with concentration ratio and initial pH at a constant temperature and constant time is presented in Fig. 2-1(d). It is evident that at a fixed reaction temperature and reaction time, the absorbance increased rapidly with initial pH at the first stage, while decreased afterwards. After pH 8.4, it starts to decrease. At a fixed temperature, initial pH and reaction time, concentration ratios affect to increase absorbance. The increase is observed as curvilinear relationship. But the study of glucosamine cysteine in Chapter 1 shows the linear relationship. Even though the results are like that, kinetics of the glucose/glycine Maillard reaction pathways: Influences of pH and reactant initial concentrations studied by Sara I.F.S. et al (2004) reported that the concentration ratio did not significantly affect the reaction. But, Baisier & Labuza, (1992) and Lerici et al., (1990) have discovered that concentration and ratios of reactants have significant impact on the reaction. Benjakul et al. (2005) reported that concentration of sugar increased in Maillard reaction products from a porcine plasma protein–sugar model system. According to the ANOVA, it shows that all the four independent parameters significantly ($p < 0.05$) affect the reaction. The central composite design matrix showed that the optimum absorbance can

be achieved with the independent variable set at 1.30 concentration ratio, initial pH 8.4, 113.9 ^{0}C reaction temperature and 1.8 hrs reaction time of.

Response Surface Modelling for Hunter's colour scale L, a and b

Table 2-2, gives the details of the actual Hunter's Lab colour scale L (Y_2), a (Y_3) and b (Y_4) values obtained from each of the 30 experimental runs. The values for the delta E (Y_5) were calculated by using the following equation.

$$\Delta E=\sqrt{L^2 + a^2 + b^2} \quad (2.3)$$

Among the response variables of L, a, b and ΔE values, L, a and b variables have high R^2 value (Table 3). Because of low R^2 value shows in ΔE according to ANOVA, L value was selected for express the total colour variation of the reaction. The ANOVA result for Hunter's Lab colour scale L value shows that the quadratic model equation is significant ($P<0.05$) on the account. The ANOVA confirms adequacy of the statistical models since their probability > F values was less than 0.05 and statistically significant at the 95% confidence level. These values are

obtained as follows: $R^2 = 0.9524$ and CV = 9.0396. This result indicates a good precision and reliability of the experiments carried out. The significance of each coefficient is determined by F value and Probability > F value. The fitted model equations are as follows. The multiple polynomial regression analysis result was used to fit the data into the following second–order equation model.

$$\begin{aligned} Y_3 = {} & 568.652299 - 2.054571*X_1 - 68.734915*X_2 - 14.534171*X_3 \\ & + 33.224466*X_4 + 0.018498*X_1^2 - 0.225437*X_1*X_2 \\ & + 5.651039*X_2^2 + 0.234049*X_3*X_1 - 4.870336*X_3*X_2 \\ & + 4.423487*X_3^2 - 1.661562*X_4*X_1 + 0.653125*X_4*X_2 \\ & + 2.747201*X_4*X_3 + 42.025982*X_4^2 \end{aligned} \quad (2.4)$$

Independent variables of X_1, X_2, X_3 and X_4 denote reaction temperature (^{0}C), initial pH, reaction time (hrs.) and concentration ratio, respectively. Hunter's 'L' value has strong relationships with degree of color change of Maillard reaction according to the R^2 (Table 2-2.). The ANOVA results for the quadratic model for Hunter's 'L' value has a positive linear effect with the initial pH ($p< 0.05$). The interaction effects between pH*pH,

time*pH and concentration ratio* temperature were significant, while others were not significant, but they slightly influenced Hunter's 'L' value. Fig. 2-2(a) presents the variation of the Hunter's 'L' value with reaction temperature and initial pH at a given reaction time and given concentration ratio. It can be seen from the figure that the Hunter's 'L' value decreases rapidly with the increasing reaction temperature. It is obviously due to brown pigment formation. Labuza et al., (1994); and O'Brien et al., (1998) described that increasing temperature leads to an increase of reactivity of Maillard reaction. Morales and Boekel (1999) have observed that lightness (L*) of samples and the yellow and blue component (b*) significant effect with severely heated samples in casein and sugar model system. Lightness decrease significantly in severely heated samples which is indicating increased darkness

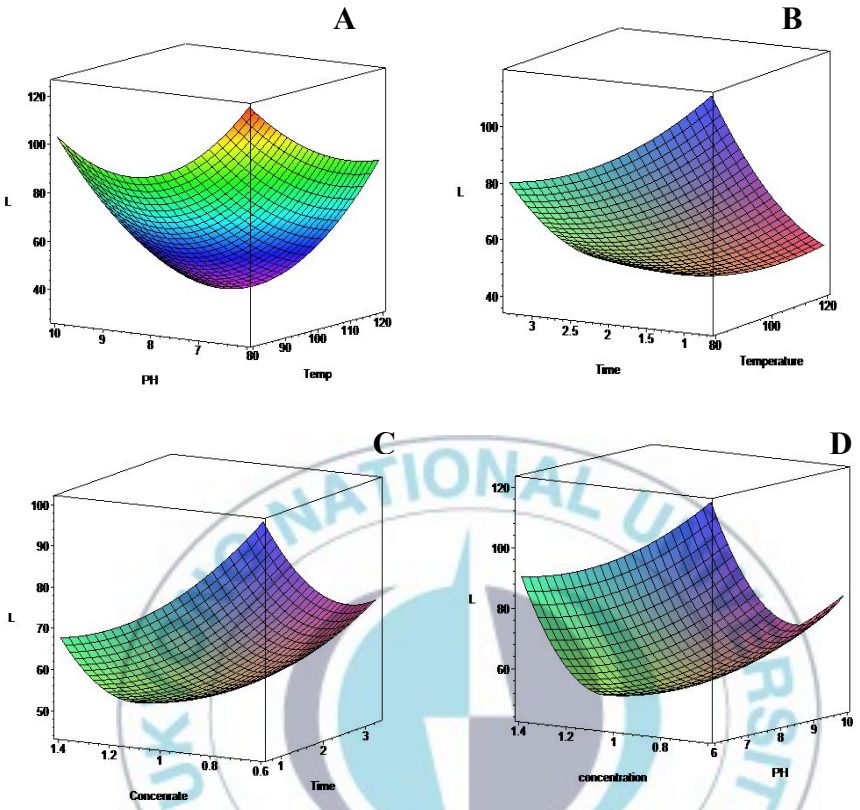

Fig. 2-2 Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and time, (C) concentration ratio and time, and (D) concentration ratio and pH on the Hunter's 'b' value. In each plot, the other two components are set at their central values (initial pH 8.00, 100^0C reaction temperature, 1.00 reaction concentration ratio and 2 hour reaction time).

It may also be seen from in Fig. 2-2(a) that Hunter's L value decreased with the increasing initial pH. When initial pH is higher than 8.5, the L value slightly increased again. The variation for pH is curvilinear in nature. It may be due to variation of brown colour pigment and flavor development and Strecker degradation compounds. Hodge (1953) explained that Maillard reaction at higher initial pH levels and lower initial pH levels produce more melanoidins than neutral and slightly alkaline pH levels. At neutral and slightly alkaline pH levels it produces more flavor compounds than pigments.

Fig. 2-2(b) shows the effect of temperature and heating time on Hunter's 'b' value at a fixed initial pH and fixed concentration ratio. Fig. 2-2(b) shows that Hunter's 'L' value decreased rapidly with the increasing temperature. Hunter's L value decreased rapidly with the reaction time. Both reaction curves are close to linear. The reaction between reducing sugar and amino group were studied by Labuza et al. (1994) and O'Brien et al. (1998). They explained that higher reaction temperature and longer reaction time allow to formation of melanoidins through the polymerization of the highly reactive intermediates that are formed during the advanced Maillard reaction (Mauron, 1981).

Fig. 2-2(c) shows the effect of time and concentration ratio on Hunter's Lab colour scale L value at a fixed initial pH and reaction temperature. The L value decreased with the increasing concentration ratios within the range of 0.6 to 1.4. The variation of 'L' value with concentration ratio and initial pH at a constant reaction temperature and constant reaction time is presented in Fig. 2-2(d). It is evident that at a fixed temperature and reaction time, the 'L' value decreased rapidly with the increasing initial pH at the first stage, while increased pH 8.5 afterwards. The central composite design matrix showed that the optimum lightness can be achieved by the independent variable set at 0.9 concentration ratio, 6.8 initial pH, 93.2^{0}C reaction temperature and 1.9 hr. reaction time.

Conclusion

RSM was utilized to investigate Maillard reaction between glucosamine and methionine by using central composite design. The optimal reaction conditions for absorbance are: 113.9 °C reaction temperature, 1.76 hr reaction time, initial pH 8.4 and 1.26 concentration ratio of reactants. The optimal expected absorbance is 3.51. The optimal reaction condition for colour development is 93.2 °C reaction temperature, 1.9 hr reaction time, initial pH 6.18 and 0.9 concentration ratio of reactants. The optimal expected L value is 104.19. It appears that linkage has been found between influencing factors and the synthesis of Maillard reaction compounds in the area of sensory evaluation. The transition from simplified model systems to real foods applications is very important to add commercial value food technology sector. This model can be refined further with additional experimentation and targeted software analysis to use for reduces the cost of production and achieve maximum quality product of foods through Maillard reaction in food processing industry.

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CHAPTER 3

The pH Effect on the Formation of Maillard Reaction Volatile Compounds between Glucosamine and Sulfur-containing Amino Acids Such As Cysteine, Methionine and Taurine.

Abstract

Volatile sulfur compounds are important contributors to the characteristic flavors and off-flavors of many foods. Cysteine, methionine and taurine are common sulfur-containing amino acids found in foods. Sulfur-containing amino acids play important roles in flavor generation in Maillard reaction. Maillard reaction mainly depends on reaction conditions and these conditions affect to the reaction rate and end product. Aqueous model systems for individual Maillard reactions of glucosamine and sulfur–containing amino acids such as cysteine, methionine and taurine were studied at fixed reaction temperature and fixed reaction time under different pH 5,7 and 9 levels. Final Maillard reaction products were tested for volatile flavour generation by using GC-MS detector. According to the results of Maillard reaction between glucosamine and cysteine, the products of pH 9 showed higher amount of volatiles and it showed higher amounts of sulfur-containing volatile compounds. Ethylene sulphide was found as the dominant among sulfur-containing compound in pH 9 sample. In alkaline medium, sulfur-containing volatile formation was prominent in glucosamine-cysteine and glucosamine-taurine systems of Maillard reaction. However, Acidic media facilitated glucosamine-methionine reaction. Sulfur-containing volatile compounds were not detected in neutral or basic medium. Carbon disulphide was

only sulfur-containing compound identified in pH 5. There is no pH effect on formation of sulfur-containing volatile compounds in Maillard reaction between glucosamine and taurine. The amounts of sulfur-containing volatile compounds formed were 84%, 82% and 79% at pH 5, pH 7 and pH 9, respectively

Keywords: Glucosamine, taurine, sulfur-containing volatile compound, Maillard reaction

Introduction

The reaction between carbonyls and amines, known as Maillard reaction, strongly affects the quality of foods. It plays a major role in food sensorial properties, flavor and color formation, as well as in its nutritional and health aspects. Maillard reaction can be affected by many factors including the temperature, chemical composition, type of buffer, water activity and pH. The type of sugar and amino group influence the rate of reaction as well as the products formed. The concentration and ratio of reactants also have a significant impact on the reaction. O.Brian, et al.,(1998). Maillard reaction has three stages. The initial stage starts with a condensation between an amino group and a reducing sugar, leading to an N-glycosylamine in the case of an aldose sugar that rearranges into the so-called Amadori product or Heyns product, if the reducing sugar is a ketose. The intermediate stage starts from the Amadori or Heyns products that lead to sugar fragmentation products and release of the amino group. The final stage leads to all kinds of dehydration, fragmentation, cyclization and polymerization reactions in which amino groups participate again. Especially in relation to flavor formation, the Strecker degradation is of utmost importance, in which amino acids are degraded by dicarbonyls

formed in the Maillard reaction that leads to deamination and decarboxylation of the amino acid. The reaction path way varies according to reaction condition. It strongly depends on reaction temperature, pH and nature of the reactants. Fig. 1 shows a general overview of reaction. It represents that in the case of proteins or peptides, the reactive amino group is the- amino group of lysine, because the α-amino groups are tied up in the peptide bond and are available neither for Maillard reaction nor Strecker reaction. The results were in a different behavior of amino acids compared to proteins and peptides. In the case of proteins, Maillard reaction often leads to crosslink formation, while brown pigments are for a large part covalently attached to proteins (Brands et al., 2002).

Flavor compound formation in Maillard reaction depends on the type of sugars and amino acids involved, and on reaction temperature, reaction time, pH and water content (Jousse et al., 2002).

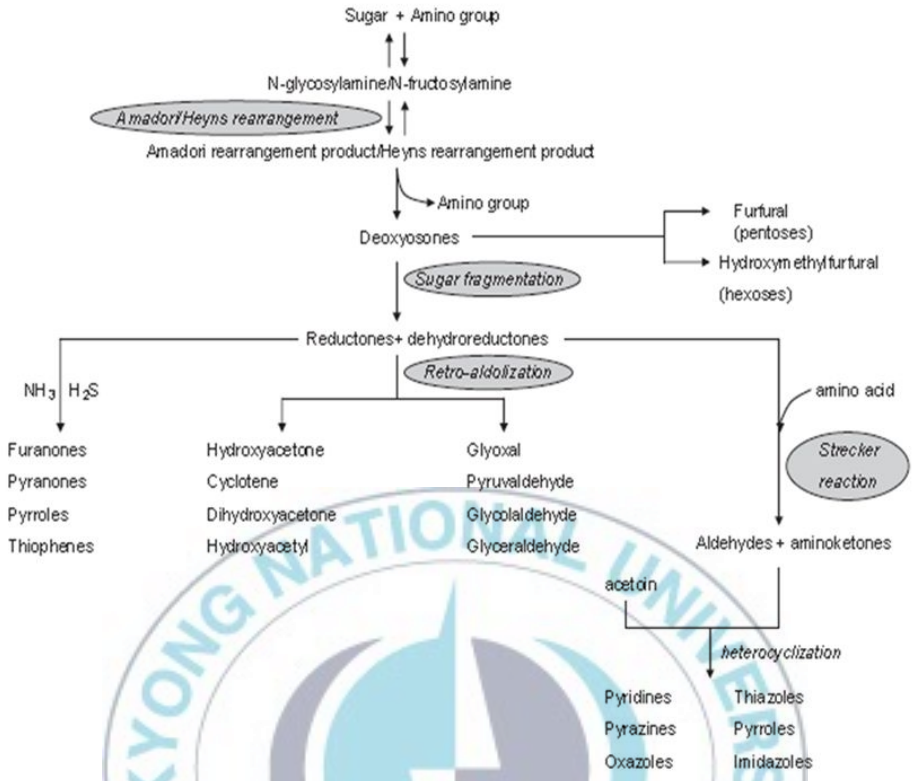

Fig .3.1 General overview of Maillard reaction showing compounds as end product (Ho., 1996)

In general, sugars and amino acids determine the type of flavor compounds formed, while the other factors influence the kinetics. Some examples of the first factor are that meat-related flavor compounds are mainly sulfur-containing compounds, derived from cysteine and ribose,

while the amino acid of proline gives rise to typical bread, rice and popcorn flavors. Table 3.1 gives an overview of some important classes, while Fig. 3.1 indicates where they are formed in Maillard reaction.

Table 3-1. Overview of some class of Maillard – derived flavor compounds (Ho., 1996)

Compound class	**Associated flavor/aroma**	**Food examples**	**Remarks**
Pyrazines	Cooked, Roasted, Toasted, Baked cereals	Heated foods in general	
Alkylpyrazines	Nutty, Roasted	Coffee	
Alkylpyridines	Green, bitter,	Coffee, Barley,	Generally regarded
Acylpyridines,	Astringent, Burnt	Malt Cereal	as unpleasant
Pyrroles		products Cereals, coffee	
Furans, furanones, pyranones	Cracker-like	Heated foods in general	
Oxazoles	Cereal-like	Cocoa, coffee, meat	
Thiofenes	Sweet, burnt, pungent, caramel-like	Heated meat	Typical for heated meat, formed from ribose and cysteine

Amadori reaction product or Heyns reaction product are relatively stable intermediates and have been detected in various heat processed foods (Eichner et al., 1994). And it can be easily synthesized (Van den Ouweland and Peer, 1970; Yaylayan and Sporns, 1987), their potential as

flavor precursors has been evaluated in several studies. Doornbos et al. (1981). These intermediate compounds such as 4-hydroxy-5-methyl-3(2H)-furanone (norfuraneol)and 3-mercaptomethylfurans are used to produce meat-like aromas by reacting with hydrogen sulfide or cysteine (Vanden, & Peer (1968). Vanden & Peer (1975) and Whitfield & Mottram (1999) also identified sulfur-containing aroma compounds derived from the reaction of norfuraneol and hydrogen sulfide. Shu and Ho (1989) and Zheng et al. (1997) studied the reaction of the methyl homologue 4-hydroxy-2,5-dimethyl-3-furanone (Furaneol) with hydrogen sulfide or cysteine, which also gave rise to meat-like aromas. Fig 3-2 shows the formation of main odorant by using Maillard intermediate. Cysteine is the favored amino acid that contains sulfur to produce meat-like flavors, both on heating with reducing sugars (Lane and Nursten, 1983).

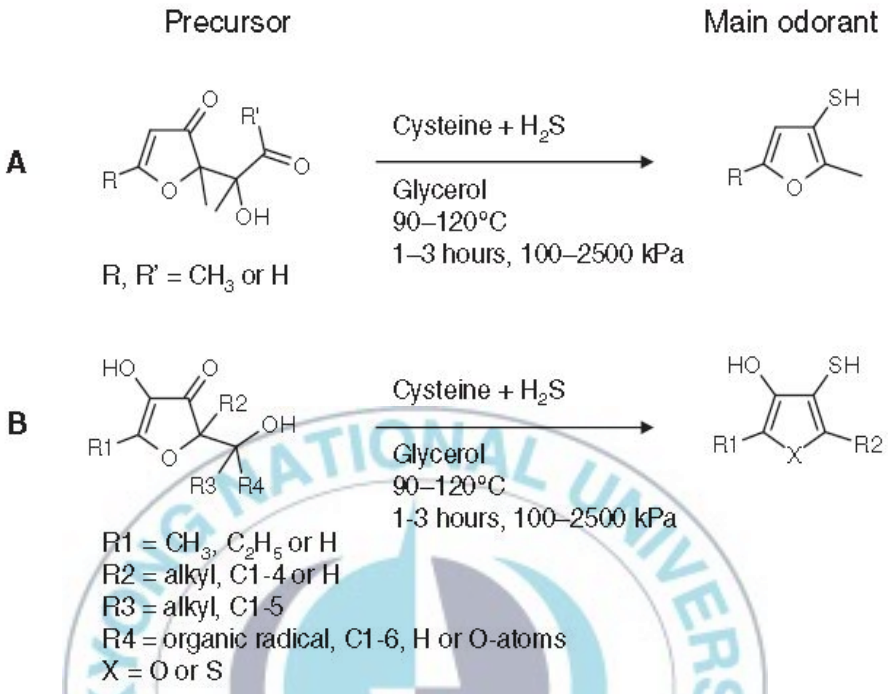

Fig. 3-2. Precursors, reaction condition and main aroma compounds of two savoury process flavours (Kerler, J., Winkel, C., Davidek, T. & Blank, I. 2010)

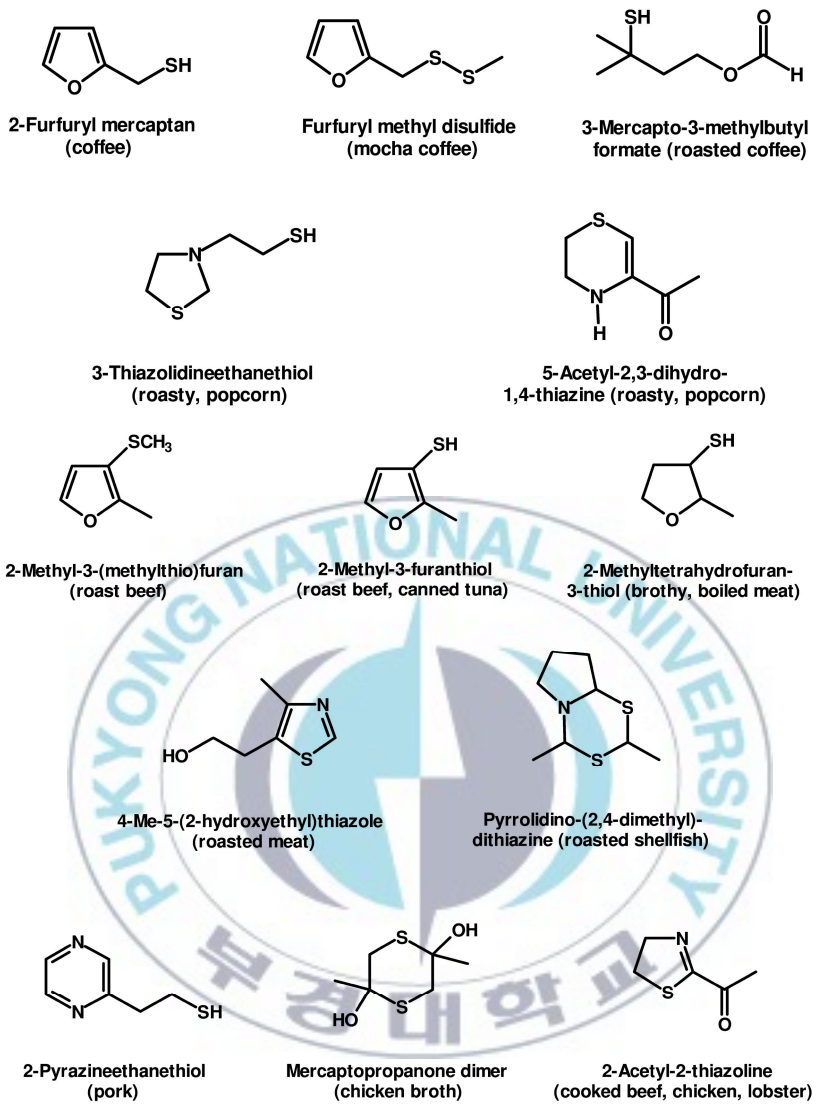

Fig 3-3. Representative character-impact sulfur-containing flavor compounds in cooked foods. (McGrrin, R. J., 2011)

Thermally induced reaction between sulfur amino acids of cysteine and methionine with reducing sugars have typical characteristic flavor compounds arise through Maillard reaction.

Different flavors and off-flavors are produced in non-enzymatic Maillard browning reaction. The composition of Maillard volatiles are affected by various factors, such as temperature, pH, formation of reactive chemicals, e.g. Strecker aldehydes. Various groups of researchers analyzed composition of odor compounds produced by thermal reactions in heat-treated food products and model systems. The model system was used for studies of an influence of various parameters (e.g., temperature, pH) on the volatile products of the browning reaction. The effect of the pH on the volatile components was investigated by Madruga and Mottram (1995) in cooked meat system and by Meynier and Mottram (1995) in the meat related model system.

Although the scientists had done lots of experiment on Maillard reaction between different type of reducing sugars, amino acids and reaction parameters and its volatile products, little information is available on the reaction between glucosamine and sulfur-containing amino acids and its

volatile formation according to pH variation. The overall objective of this work is to investigate the effect of initial pH on Maillard reaction between glucosamine and sulfur- containing amino acids (cysteine methionine and taurine) in the formation of odor in model system

Materials and Methods

Materials

D-Glucosamine, L-cysteine, Taurine, sodium hydroxide and hydrochloric acids were used. Sodium hydroxide and hydrochloric acid, Taurine and L-cysteine were parched from Junsei Chemicals. Co. Ltd (Tokyo, Japan). D-glucosamine was purchased from Jiangsu JJiushoutang Organisms Manufacture Co. Ltd (Xinghua, China). All chemicals used were of analytical grade.

Methods

Preparation of Maillard reaction mixtures

Different pH levels of pH 5, 7, 8 and 9 of each 0.1M glucosamine , 0.1 M cysteine , 0.1 M methionine and 0.1M taurine solutions were prepared by dissolving their respective quantities in deionized water. The pH levels were achieved by using 0.1M NaOH and 0.1M HCl and verified by the using of pH meter (Metrohm 827 pH Lab, Herisau, Switzerland). Ten ml of glucosamine solution at pH levels 5, 7, 8 and 9 were mixed with each

amino acid separately at pH levels 5, 7, 8 and 9, respectively. The reactants were heated at 120 ^{0}C reaction temperature for 2 hr reaction time. Reaction temperatures were achieved by using a dry oven (Dongwon Scientific System, Busan, Korea).

Determination of headspace volatile compounds of Maillard reaction products

Headspace volatile compounds produced by Maillard reaction were isolated by a dynamic headspace technique. Ten milliliters of each Maillard reaction products were placed in a dark color bottle and heated in a dry oven at 60^{0}C for 30 minutes before isolation the volatiles. Volatile compounds were absorbed to Tenax tubes for 5 minutes and the tubes were set to automatic thermal de-absorber (ATD 400, Perkin Elmer, USA). The volatile compounds were identified by using the combined system of gas chromatography and mass selective detector (GC-MSD: QP-5050A, Shimadzu, Japan). The primary tube type of ATD400 was Tenax-TA. The first desorption was 350^{0}C for 4 min, whereas the second cryogenic temperature was -30^{0}C and the second desorption was 350^{0}C for 1 min.

Desorb flow was 50.2 ml/min and outlet spilt was 11.5 l/min. The analytical condition of gas chromatography and mass selective detector was 35^0C – 10 min, 8^0C/min - 120^0C - 10 min, 12^0C/min - 80^0C - 7 min, 15^0C/min - 230^0C - 10min as oven temperatures and AT1 (60m * 0.32mm * 1.0μm) column. Interface temperature was 230^0C and MS Detector temperature was 250^0C. Mass range was 20~350 m/z and column pressure was 15.9 psi. Mass filter type was quadrupole and 99.9999% nitrogen gas was used as carrier gas. Two replicates were tested for volatiles.

Result and Discussion

Headspace volatile compounds were isolated, separated and identified at optimum condition of gas chromatography with mass sprectometory from Maillard product of glucosamine and sulfur- containing amino acids of cysteine, methionine and taurine in aqueous module systems. Reaction temperature, concentration ratio, initial pH and reaction time may affect flavour formation during Maillard reaction. Reaction temperature, concentration ratio and reaction time were therefore fixed, while pH was varied accordingly. The pH variation was pH 5, pH 7 and pH 9. Typical chromatograms of headspace volatiles produced from Maillard reaction between glucosamine-and sulfur containing amino acids were analyzed and major volatile compounds were identified and discussed in section 3-1, 3-2 and 3-3 for cysteine, methionine and taurine model systems, respectively.

Section 3-1. Aqueous model systems of Maillard reaction of glucosamine and cysteine

Maillard reaction between glucosamine and cysteine at constant 120^0C reaction temperature for 2 hr fixed reaction time. Glucosamine and cysteine reactant ratio was set at 1:1 and the Maillard reaction products were checked for volatiles compounds by using GC-MS ditector. Fig. 3-4 shows the chromatograms of volatiles isolated from Maillard reaction. Isolated and identified volatile compounds are shown in Table 3-2.

The numbers of headspace volatile compounds identified from Maillard reaction product in glucosamine –cysteine model systems at pH 5, pH 7 and pH 9 were 21, 23 and 17, respectively. The reaction products of pH 9 showed higher amount of volatiles. It showed higher amounts of sulfur-containing volatile compounds, while pH 5 and pH 7 showed half of that. The amount of sulfur-containing volatile compounds was 43% pH 9, while pH 5 and pH 7 were 6.6% and 6.4%, reapectively. In alkaline medium, sulfur- containing volatile formation is prominent in glucosamine- cysteine systems of Maillard reaction.

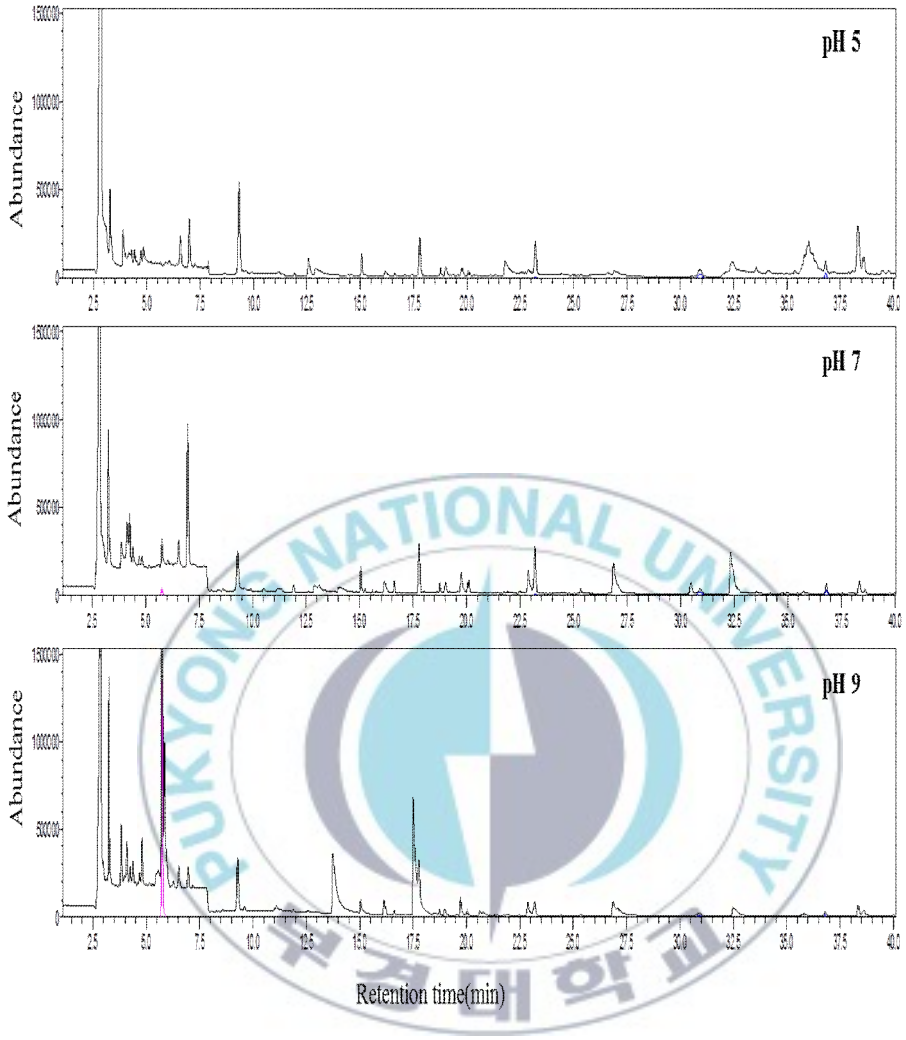

Fig. 3-4. Chromatograms of volatiles isolated from Maillard reaction products of glucosamine and cysteine at pH 5, pH 7, and pH 9 in Maillard browning model system and analyzed by means of capillary GC of DB5 column.

Table 3-2. Volatile compounds identified from Maillard reaction products between glucosamine and cysteine at pH 5, pH 7, and pH 9 in Maillard browning model system and analyzed by means of capillary GC of DB 5 column

Glucosamine And Cysteine					
Compounds	**RT**	**RI**	**Area x 10^6**		
			pH5	**pH7**	**pH9**
Sulfur-containing compounds					
Carbon disulfide	4.82	532	0.5	0.3	0.7
Ethylene sulfide	5.76	572	-	0.8	15.2
Isothiocyanato cyclohexane	33.52	1236	0.3	-	-
Benzothiazole	33.86	1247	0.2	-	-
Subtotal			**1.0**	**1.1**	**15.9**
Aldehyde					
Formaldehyde	2.98	<500	-	0.7	-
Acetaldehyde	3.26	<500	1.2	2.6	3.0
2-Propenal	4.04	<500	0.6	0.3	-
2-Methyl-propanal	5.16	547	-	0.1	-
n-Butanal	5.98	580	-	0.3	-
Benzaldehyde	21.75	957	0.9	-	-
Octanal	22.85	992	0.4	1.0	-
n-Nonanal	26.84	1099	0.7	1.9	-
n-Decanal	32.36	1198	0.8	2.7	-
Subtotal			**4.7**	**9.5**	**3.0**
Alcohol					
Methanol	3.34	<500	0.3	-	-
Ethanol	3.86	<500	1.1	1.0	0.7
2-Ethylcyclobutanol	6.04	582	-	0.2	-
2,4-Dimethyl-3-pentanol	21.10	936	0.0	-	-
Subtotal			**1.4**	**1.2**	**0.7**
Ketone					
Acetone	4.09	<500	-	-	0.9
2-Propanone	4.20	502	0.9	1.2	1.0
2,3-Butanedione	5.86	576	-	-	3.5
2-Butanone	6.29	591	-	0.2	0.1
Cyclohexanone	19.52	887	0.2	-	-
Subtotal			**1.0**	**1.4**	**5.6**
Ester					
Acetic acid, ethyl ester	7.14	615	1.6	0.3	-

Ethyl Acetate	7.17	616	-	-	0.1
2-Methyl-propanoic acid, 1-methylethyl ester	7.25	618	-	0.1	-
Carbonic acid, allyl pentadecyl ester	35.44	1298	0.1	-	-
Eicosanoic acid, methyl ester	36.01	1320	0.8	-	-
Octadecanoic acid, phenyl ester	37.68	1386	0.0	-	-
Subtotal			**2.6**	**0.4**	**0.1**
Ether					
Ethyl ether	4.40	512	-	0.2	0.3
Diethyl ether	7.65	627	-	0.1	-
Subtotal			**0.0**	**0.3**	**0.3**
Furan					
Furan	4.25	504	-	1.0	0.4
4-Phenyldibenzofuran	55.06	1956	0.7	-	-
Subtotal			**0.7**	**1.0**	**0.4**
Pyrazine					
Pyrazine	13.70	747	-	-	3.9
Methylpyrazine	17.50	831	-	-	4.4
Subtotal			**0.0**	**0.0**	**8.3**
Aliphatic compounds					
Hexane	6.54	600	-	0.7	0.6
n-Nonane	20.04	900	0.1	-	-
Subtotal			**0.1**	**0.8**	**0.6**
Aromatic compounds					
Benzene	9.29	661	2.9	1.6	1.5
Cyclododecane	38.08	1401	0.7	-	-
Propylcyclopropane	6.23	589	-	0.1	-
Subtotal			**3.6**	**1.7**	**1.5**
Total			**15.1**	**17.4**	**36.4**

The formation of sulfur –containing volatile compounds was not observed in pH 5. Ethylene sulphide found as the dominant among sulfur-containing compound in pH 9 sample. Pyrazines were not found in both acidic and neutral PH samples, but they were found in 23% in alkaline condition. In neutral pH, aldehyde formation is higher than that in other

conditions. High quantities of acetaldehyde and decanal were observed. Benzene was observed in all samples as an aromatic compound. According to observation, pH did not critically affect the formation of aromatic compounds and aliphatic compounds.

Section 3-2. Aqueous model systems of Maillard reaction of glucosamine and methionine

Maillard reaction between glucosamine and methionine was studied at the fixed conditions of 120^0C reaction temperature, 2 hr fixed reaction time and 1:1 glucosamine and methionine reactant ratio. The headspace volatile compounds of model system were isolated, separated and identified by using the combine systems of purge and trap, ATD, GC and MSD.

Fig. 3-5 shows chromatograms of volatiles isolated from Maillard reaction of glucosamine and methionine model systems. Isolated and identified volatile compounds are shown in Table 3-3. The numbers of headspace volatile compounds identified from Maillard reaction product in glucosamine– methionine model systems at pH 5, pH 7 and pH 9 were 37, 14 and 12, respectively.

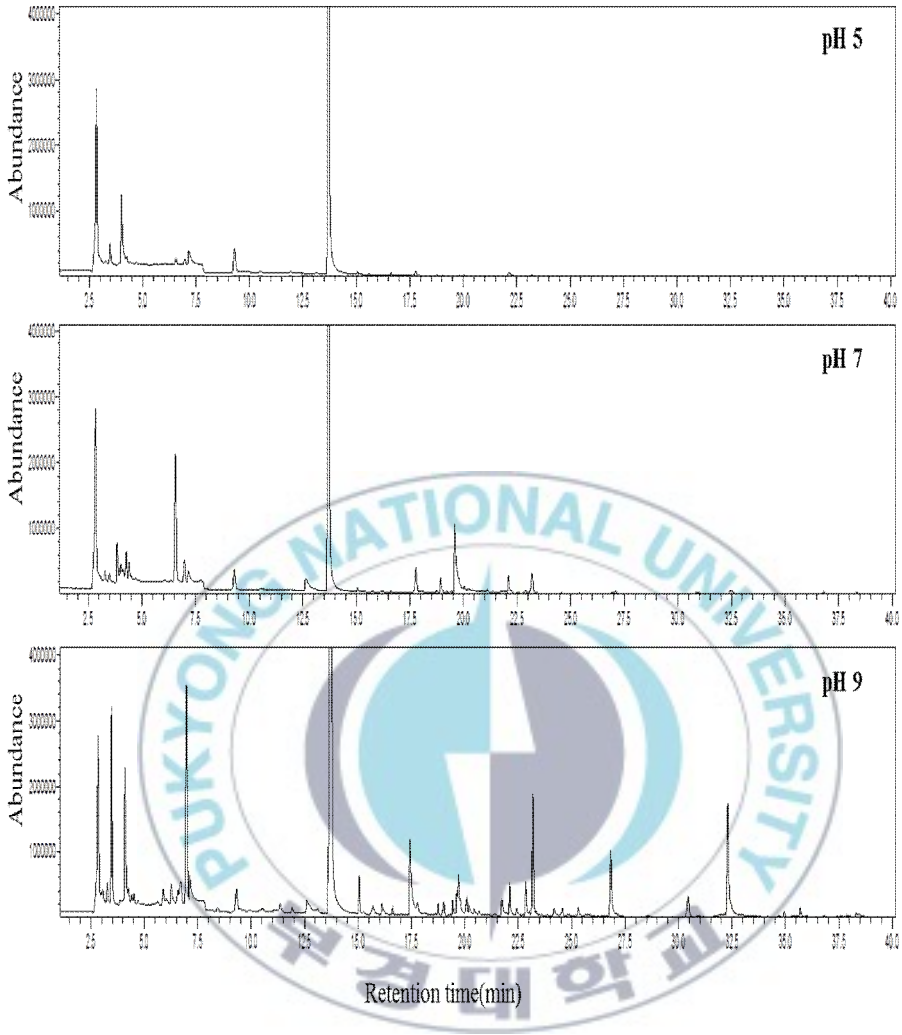

Fig. 3-5. Chromatograms of volatiles isolated from Maillard reaction products of glucosamine and methionine at pH 5, pH 7, and pH 9 in Maillard browning model system and analyzed by means of capillary GC of DB5 column.

Table 3-3. Volatile compounds identified from Maillard reaction products of glucosamine and methionine at pH 5, pH 7, and pH 9 in Maillard browning model system and analysed by means of capillary GC of DB5 column

Glucosamine and Methionine					
Compounds	**RT**	**RI**	**Area x 10^6**		
			pH5	**pH7**	**pH9**
Sulfur-containing compounds					
Carbon disulfide	4.80	531	0.2	-	-
Subtotal			**0.2**	**0.0**	**0.0**
Aldehyde					
Acetaldehyde	3.26	<500	1.3	1.2	1.1
2-Propenal	3.98	<500	0.4	-	-
Benzaldehyde	21.71	956	1.1	-	-
n-Octanal	22.83	991	0.7	-	-
n-Nonanal	26.80	1098	2.6	-	-
n-Decanal	32.29	1197	1.1	-	-
Undecanal	35.66	1306	0.8	-	-
Hexadecanal	38.37	1411	1.6	-	-
Subtotal			**9.6**	**1.2**	**1.1**
Alcohol					
Ethanol	3.86	<500	1.4	0.3	0.2
1-o-Decyl-lyxitol	34.74	1276	0.3	-	-
Subtotal			**1.7**	**0.3**	**0.2**
Ketone					
2-Propanone	4.12	<500	-	0.5	5.7
Acetone	4.10	<500	0.7	-	-
2,3-Butanedione	5.88	576	-	-	1.6
2-Butanone	6.31	592	0.7	0.1	0.2
1-hydroxy- 2,6-piperidinedione	6.55	600	-	0.7	-
1,3-Isobenzofurandione	36.02	1321	0.4	-	-
Subtotal			**1.9**	**1.3**	**7.4**
Ester					
Acetic acid, ethyl ester	7.11	614	11.5	5.1	4.3
Octadecanoic acid, 4-hydroxybutyl ester	26.63	1093	1.9	-	-
Pentanoic acid, propyl ester	37.57	1382	1.5	-	-
4-tert-Butylcyclohexyl acetate	37.77	1389	0.7	-	-
Subtotal			**15.6**	**5.1**	**4.3**
Ether					

Ethyl ether	4.40	512	0.3	0.2	0.1
Subtotal			**0.3**	**0.2**	**0.1**
Acid					
Benzoic acid	31.65	1186	3.2	-	-
Subtotal			**3.2**	**0.0**	**0.0**
Furan					
2,3-Dihydro furan	3.50	<500	0.5	-	-
Furan	4.26	505	0.9	2.7	-
2-Methylfuran	6.65	603	-	-	1.2
Subtotal			**1.4**	**2.7**	**1.2**
Pyrazine					
Pyrazine	13.73	746	-	-	1.9
Methyl-pyrazine	17.47	827	-	-	4.3
Subtotal			**0.0**	**0.0**	**6.1**
Aliphatic compounds					
Ethylene	1.28	<500	0.3	-	-
3-Cyclopropyl-1-butyne	5.34	555	0.2	-	-
2-Methylpentane	5.61	566	0.2	0.1	-
3-Methyl-hexane	6.00	581	0.4	-	-
3-Methyl-pentane	6.04	582	-	0.1	-
5-Methyl-1-hexene	6.06	583	-	0.2	-
n-Hexane	6.55	600	1.3	0.7	0.4
1,2,4-Triazine	6.66	603	-	0.1	-
Methylcyclopentane	7.72	628	0.9	-	-
n-Octane	16.62	800	0.9	-	-
3-Methyl-octane	19.21	877	0.4	-	-
n-Nonane	20.04	900	1.6	-	-
Pentadecane	41.06	1500	0.5	-	-
Subtotal			**6.6**	**1.2**	**0.4**
Aromatic compounds					
Benzene	9.29	661	3.0	4.5	2.2
Ethylbenzene	18.73	864	0.8	-	-
1,2-Dimethyl benzene	18.99	871	1.9	-	-
1,3-Dimethyl benzene	19.78	893	0.6	-	-
Subtotal			**6.2**	**4.5**	**2.2**
Others					
o-Isopropylaniline	37.57	1382	1.9	-	-
Subtotal			**1.9**	**0.0**	**0.0**
Total			**48.7**	**16.4**	**23.1**

The reaction products of pH 5 showed higher amount of volatiles and sulphur-containing volatile compounds were identified only in pH 5. Carbon disulphide was the only sulphur-containing compound that found in pH 5. Its amount was 0.004% from total volatiles of Maillard reaction. According to the results, acidic media facilitate the reaction. The amount of total volatile products was 48.7 $*10^6$ (peak area) while other amounts were $16.4*10^6$ and $23.1*10^6$.le. Relativly high amount of esters were formed in the Maillard reaction between glucosamine and metioneine. Its amount was increased with the increase pH. with the increased pH it showed decling. Pyrazine and methylpyrazine were detected only in alkaline reaction media.its amount was 26% from total volatiles formed in pH 9.

Section 3.3. Aqueous model systems of Maillard reaction of glucosamine and taurine

Maillard reaction between glucosamine and taurine was investigated at fixed 120^0C reaction temperature and 2 hr reaction time. Glucosamine and taurine reactant ratio was set at 1:1. Maillard reaction products were studied for measuring headspace volatile compounds by using GC-MSD.

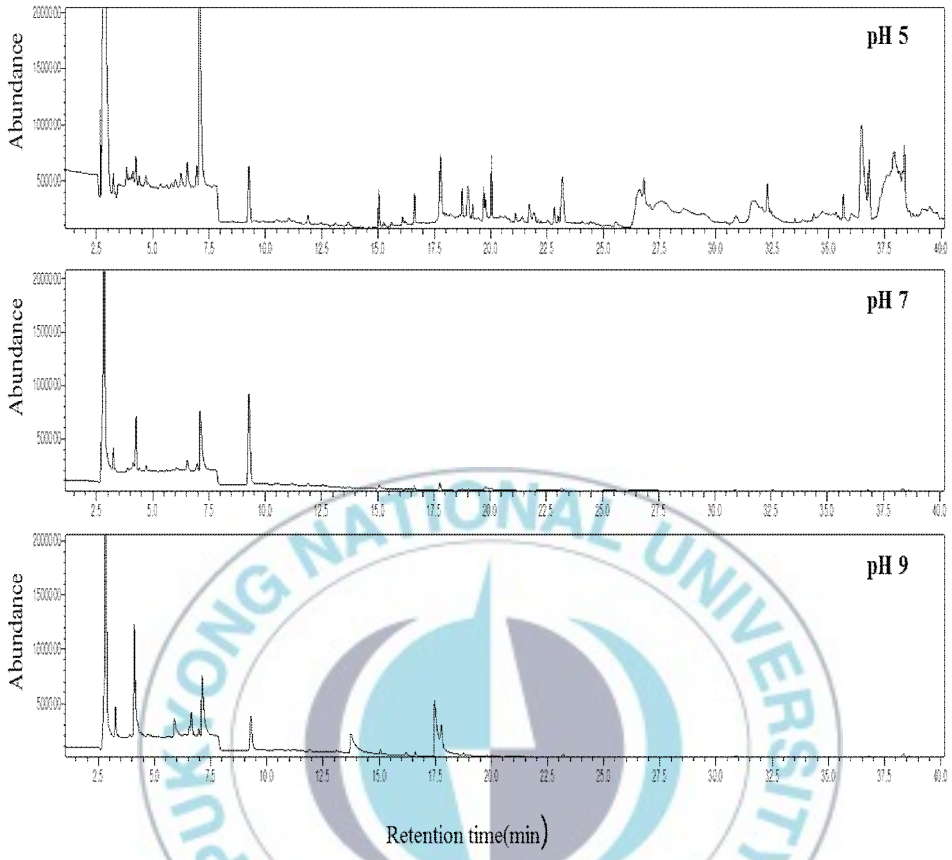

Fig. 3-6. Chromatograms of volatiles isolated from Maillard reaction products of glucosamine and taurine at pH 5, pH 7, and pH 9 in Maillard browning model system and analyzed by means of capillary GC of DB5 column.

Table 3-4. Volatile compounds identified from Maillard reaction products of glucosamine and taurine at pH 5, pH 7 and pH 9 in Maillard browning model system and analyzed by means of capillaryGC of DB5 column

Glucosamine And Taurine					
Compounds	**RT**	**RI**	**Area x 10^6**		
			pH5	**pH7**	**pH9**
Sulfur-containing compounds					
Sulfur dioxide	3.05	<500	-	-	1.6
Methanethiol	3.47	<500	1.3	-	5.7
Thiobis-methane	4.51	517	-	0.2	-
Dimethyl sulfide	4.52	518	-	-	0.5
Methyl vinyl sulfide	6.70	604	-	-	2.5
Methyl thiolacetate	11.30	694	-	-	0.6
Dimethyl disulfide	13.69	747	36.1	79.6	209.3
3-Methylthio-propanal	19.61	889	-	6.4	1.3
Dimethyl trisulfide	22.08	968	-	1.1	2.0
Subtotal			**37.4**	**87.3**	**223.5**
Aldehyde					
Acetaldehyde	3.27	<500	-	-	1.1
2-Propenal	4.02	<500	3.3	1.5	-
Benzaldehyde	21.73	957	-	-	1.6
n-Octanal	22.85	992	-	-	2.2
n-Nonanal	26.82	1098	-	-	5.8
n-Dodecanal	30.44	1166	-	-	1.4
n-Decanal	32.30	1197	-	-	9.6
Subtotal			**3.3**	**1.5**	**21.8**
Alcohol					
Ethanol	3.83	<500	-	2.7	4.9
Subtotal			**0.0**	**2.7**	**4.9**
Ketone					
2-Propanone	4.11	<500	-	0.8	10.1
2,3-Butanedione	5.89	576	-	-	1.1
2-Butanone	6.32	592	-	0.1	0.9
3-Methyl-hexan-2-one	20.10	902	-	-	0.7
Subtotal			**0.0**	**0.9**	**12.8**
Ester					
Acetic acid, ethyl ester	7.15	615	1.4	1.2	2.1
1-Methoxy-2-propyl ester of acetic acid	18.93	871	-	1.1	-

Ethyl ether	4.41	512	-	1.0	0.4
Subtotal			**1.4**	**3.3**	**2.6**
Acid					
2-Methyl-propanoic acid	15.70	786	-	-	0.7
Subtotal			**0.0**	**0.0**	**0.7**
Furan					
Furan	4.27	505	0.3	1.8	-
2,5, dihydro furandione	7.73	628	-	-	0.5
Subtotal			**0.3**	**1.8**	**0.5**
Pyrazine					
Methyl pyrazine	17.39	828	-	-	7.2
Subtotal			**0.0**	**0.0**	**7.2**
Aliphatic compounds					
2-methyl butane	4.27	505	-	-	1.1
2-Methyl pentane	5.64	567	-	0.1	0.3
3-Methyl-pentane	6.06	583	-	0.2	-
4-Methyl-1-hexene	6.03	581	-	-	0.6
n-Hexane	6.56	600	0.3	5.0	0.7
Methylcyclopentane	7.74	629	-	0.7	-
Subtotal			**0.3**	**6.0**	**2.7**
Aromatic compounds					
Benzene	9.30	661	1.7	1.9	2.3
Ethylbenzene	19.01	873	-	-	1.1
Subtotal			**1.7**	**1.9**	**3.5**
Others					
Pyridinium Perchlorate	5.38	556	-	-	0.1
2,4-Dithiapentane	19.44	885	-	-	0.7
Subtotal			**0.0**	**0.0**	**0.9**
Total			**44.3**	**105.3**	**280.7**

Fig. 3-6 shows chromatograms of volatiles isolated from Maillard reaction of model system. Isolated and identified volatile compounds are shown in Table 3-4. Total 39 volatile compounds were identified in Maillard reaction between glucosamine and taurine. The numbers of identified volatile compounds at pH 5, pH 7 and pH 9 were 7, 16 and 32 respectively.

The model system at pH 5 showed the lowest amount of headspace volatile compounds. Various kinds of sulfure-containing volatiles were observed in Maillard reaction between taurine and glucosamine. The result showed that alkaline medium facilitated the reaction between to form more sulfur-containing volatiles. All the pH conditions reflect high amount of dimethyl disulfide. All these acidic, neutral and basic reaction condition showed high amount of sulfur containing volatiles. Their amounts were 84%, 82% and 79% at pH 5, pH 7 and pH 9, respectively.

Table 3-5. The summary of the percentages of total sulfure containing volatiles found in Maillard reaction between glucosamine and sulfur- containing amino acids at different pH levels

Reaction	**pH 5**	**pH 7**	**pH 9**
Glucosamine - Cysteine	6.6%	6.4%	43%
Glucosamine –Methionine	0.4%	0.0	0.0
Glucosamine –Taurine	84%	82%	79%

According to this results taurine react with glucosamine easily and produce more sulfure containing volatiles. All the samples were exposed to same temperature (120^0C) for same reaction time (2 hr.). So the given

energy for Maillard reaction was equal. This observation shows the kinetics of the reaction combinations are different and depend on the reactants. It has been noted that most of the research on the formation of Maillard-based flavour compounds is on mixtures of sugar and free amino acids, and hardly on sugar–protein or sugar–peptide mixtures (Izzo and Ho., 1992). They have reported that peptides and proteins, and in the absence of free amino acids, the Strecker reaction cannot take place, and this has consequences for flavor generation. With the help of their findings, it can be assumed that the reaction rate of the taurine and glucosamine is higher due to high streaker degradation facilitated by the taurine. The chemical structure of taurine is simpler and it does not contain carbonyl carbon group. Moreover, the sulfure atom is present as sulfonic group that contain two electrophilic sulfonic oxygens which can attack by nucleophilic nitrogen atom. At the same time, it contains NH_2^+ group also. This structure and the product from degradation of Amadori rearrangement may influence to the rate of the reaction and the end product of the reaction. Therefore It need to do more research on glucosamine and taurine thermal reaction and need to study more on chemical reaction and the kinetics of the reaction.

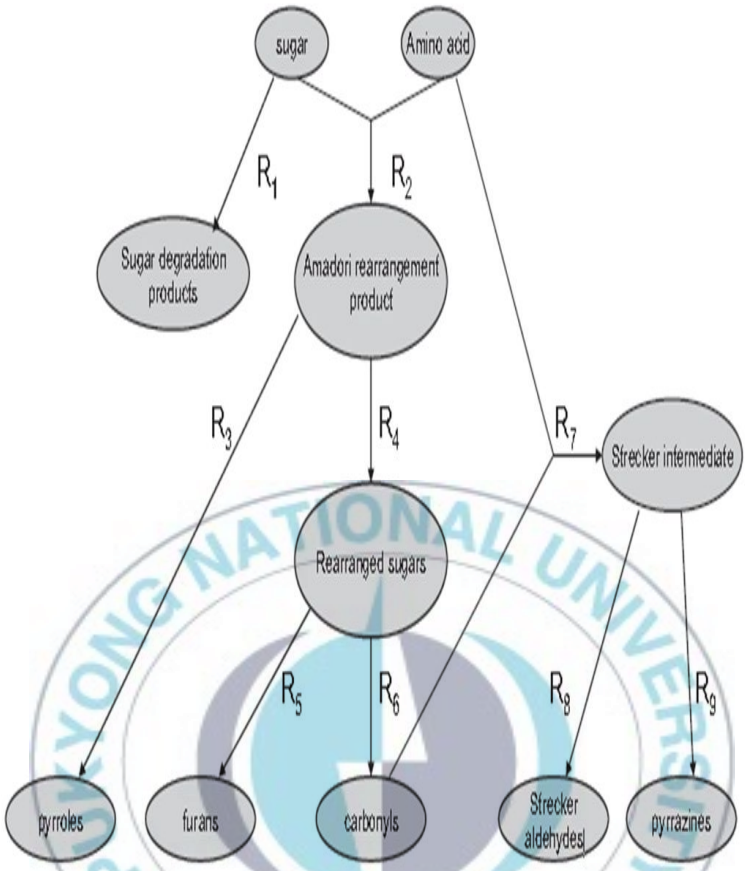

Fig. 3-7. Kinetic model with essential rate-determination steps R1-R9 describing the formation of the most important Maillard reaction flavor compounds (Jousse et al., 2002).

Maillard reaction is very complicated due to the many reaction paths and effect of processing conditions. But Jousse et al. (2002) has proposed a model to study the flavor formation in Maillard reaction (Fig. 3.7) and it

can be used to identify the kinetics of glucosamine and taurine thermal reaction. Study on the reaction rate of R1- R9 with glucosamine and taurine is suggested to further findings.

Aldehyde formation is highest in acidic medium in methionine, highest in neutral medium in cysteine and highest in basic medium in taurine. Pyrazine were detected only in alkaline medium of each cysteine, methionine and taurine Maillard reaction system. Pyrazine and methyl-pyrazine were detected and this finding is tally with the finding of Leahy and Reineccius (1989). He observed that the amount of pyrazine formation in glucose –lysine system at pH 9 is more than 500 times higher at pH 3.

In glucosamine –cysteine Maillard product, benzothiazole was found as sulfure containing volatile and it cause gasoline or rubber odder (Moio, et al., 1994; Schreyen, et al., 1979). It was found only in acidic media that this odder can be controlled by controlling initial pH of reaction in food processing. Furan substances were found in acidic and neutral media of each Maillard reaction and that substances are caramel or spice in odder according to Moio, et al.,1994; Takeoka, et al., 1996.

It is easy to increase this beneficial odor by controlling pH at below pH 7.

Pyrazine and methyl pyrazine were found in all kind of glucosamine and sulfure containing amino acids reaction. But it was observed only in alkaline reaction medium. These compounds are lead to popcorn and roasted potato flavor (Yong, et al., 1989). Maillard reaction between glucosamine and taurine at higher pH levels shows higher volatile formation than neutral and acidic pH. Especially formations of sulphur containing volatiles are prominent. Methanethiol which cause sulfur, gasoline, garlic odder (Rychlik, et al., 1998), dimethyl sulphide which cause cabbage, sulfur, gasoline odder (Tamura, et al., 1995), Dimethyl disulphide which cause onion, cabbage, putrid odder (Rychlik, et al., 1998; Tamura, et al., 1995) and dimethyl trisulfide which cause sulfur, fish, cabbage odder (Tamura, et al., 1995) were detected in pH 9 sample as off-flavor odorant. Therefore this knowledge is very important to maintain quality of the food in food processing industry which is used taurine in heat treatment.

Conclusion

The development of Maillard reaction products mainly depend on the initial pH of the reaction. Maillard reaction between glucosamine - cysteine and glucosamine-taurine induce by basic pH levels. Glucosamine and methionine reaction was induced by acidic pH. The reaction between glucosamine and cysteine showed high amount of total volatile formation and high amount of sulfur-containing volatiles formation at pH 9. Ethylene sulphide was the most abundant sulfure volatiles found at pH 9. Total volatiles and sulfure-containing volatiles formation was low at acidic and neutral pH levels.

Glucosamine–methionine Maillard reaction favour acidic initial pH and carbon disulfide was most abundant. Total volatile formation from Maillard reaction between glucosamine and taurine also depend on initial pH of the reaction and basic medium induced the reaction. Formation of sulfur- containing volatiles was not depend on the initial pH of reaction. Very high percentages of sulfur-containing volatiles were found at all pH. Dimethyl disulphide was most abundant sulfure – containing volatile at all tested pH levels.

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