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Unravel browning mechanism in making kue delapan jam

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Abstract

This research intended to study browning reaction mechanism in making kue delapan jam and its implication on nutritional value. Browning reaction among substance exist in raw material during processing influence the sensories quality and color of cake significantly. The study was based on the theoretical approaches relate to browning reaction, taking into account the experimental test, material balance, and the available information. The reaction pathway (Maillard reaction, caramelization, lipid oxidation) was determined by the changes in concentration of raw material (dough) and selected observable chemical marker according to their ability to indentify reaction stages. Research applied completely randomized design with steaming time as a treatment (0 h, and 8 hours) with 3 replications. Reaction pathway determined by observing changes of sucrose, lactose, glucose, fructose, hidroxy methyl furfural, acetic acid, formic acid, amino acid and fatty acid between the dough and the cake. Test results showed that there were no significant changes in sucrose concentration. No fructose, glucose, acetic acid, formic acid, and hidroxy methyl furfural indentified both in the dough and the cake. However there were significant decreasing on the concentration of lactose, amino acid and fatty acid. Browning reaction in making kue delapan jam likely occured via Maillard reaction between lactose and amino acids, lipids oxidation mechanism and reaction between amino acid and lipids oxidation product.

Introduction

The Kue delapan jam (KDJ) is a traditional cake in Palembang area which is brown in color. It is made from eggs, sugar, sweetened condensed milk and margarine which steamed for 8 hours. The brown color of KDJ formed during steaming process known as browning reaction (Agustini *et al.*, 2014). Browning reaction in food processing could be classified into two categories, namely enzymatic browning reactions and non-enzymatic browning reactions. In making KDJ enzymatic browning reactions do not occurred, because there is no addition of enzyme. Non-enzymatic browning reactions in food can be divided into four types, namely ascorbic acid degradation, lipid peroxidation, caramelization of sugars, and Maillard reactions (Hidalgo and Zamora, 2000; Nursten, 2005).

Ascorbic acid browning is the thermal decomposition of ascorbic acid in aerobic conditions according to mechanism of oxidation and non-oxidation. Decomposition produces dicarbonyl compounds that subsequently form brown compounds (Nursten, 2005). Based on the definition above, browning reaction in KDJ is not caused by ascorbic acid browning, because the materials used in making KDJ do not contain ascorbic acid and no ascorbic acid adding in processing.

Caramelization reaction is removal of water from sugar followed by isomerization and polymerization at high temperatures. The caramelization is start with melting of sugar at a high temperature and followed by the formation of foam (Simpson, 2012). The caramelization reaction may be occured in KDJ making especially on the surface, since there were abundant source of sucrose and lactose.

Another type of browning reaction is Maillard reaction. Maillard reaction occured between carbonyl compound (fructose and glucose) with amino acids, protein, and peptides (Saltmarch and Labuza, 1982; Ames, 1998; Martins *et al.*, 2001; Nursten, 2005; Yu and Zhang, 2010; Bastos *et al.*, 2013). Generally Maillard reaction consist of three stages. The first stage involves the sugar-amine condensation and the Amadori rearrangement (ARP). ARP then degraded to be furfural or hydroxymethylfurfural (HMF). At pH >7 it is degraded to be 4-hydroxy-5-methyl-2,3-dihydrofuran-3- one (HMFone), and a variety of

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fission products, including acetol, pyruvaldehyde and diacetyl. The second stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker degradation especially at high temperatures. At the end of second stage there is a begining of flavor formation. The final stage was formation of polymer through aldol condensation and heterocyclic nitrogen compounds to produce brown color compound (Martins *et al*., 2001).

Lipids peroxidation also cause browning in foods. Lipid peroxidation is occured by autoxidation of the fatty acids, especially unsaturated fatty acids to form aldehydes and ketones which then react with amino acids to form brown pigments, as in the Maillard reaction (Damodaran, 1996; Hidalgo and Zamora, 2000; Zamora and Hidalgo, 2005). In case of KDJ, changes in color indicate the existence of browning reaction during steaming. Since material used in KDJ making were rich in protein, sugar and lipids which were reactants for the browning reaction, it can be presumed that browning reaction was caused by at least one of three reactions, namely caramelization, Maillard reactions, and oxidation of lipids or interaction among them.

Research on browning reaction either caused by caramelization, Maillard reaction or lipid oxidation have been carried out widely. However, the research used a simple model involving one type of sugar, amino or lipids in aquos solution. The simple model is very helpful in providing information about the reaction. Research involving three types of reactants in highly heterogeneous systems such as in KDJ making will be more complex. The complexity of the reactants and its properties in the system will affect on the possibility of reactions that occured.

This research is intended to propose methodological approach to unravel interaction among reactant during steaming. It started with establishment reliable marker compounds, measurement it concentration both in the dough and the cake/KDJ and analyse their contribution in the browning reaction. Furthermore, research intended to study the impact of steaming on the nutrition value of the cake.

Materials and Methods

Materials and equipment

Materials used were hen eggs, sucrose, margarine, sweetened condensed milk and reagent for analysis. The apparatus used were steamer, basin, mixer, baking pan, desiccators, glasswares, incubator, analytical balance, Konica color reader, oven, HPLC, GCMS.

The KDJ was made by mixing 1,500 g of eggs, 600 g sugar, 400 g sweetened condensed milk, and 100 g margarine by using hand mixer to obtain homogeneous mixture. The mixture was then poured into 20 x 20 x 7 cm baking pan that has smeared with margarine. Then it was covered with aluminium foil to prevent water dropplet on the surface. Steaming was then performed according to treatment time (0 h, and 8 h). The cake was taken out and air cooled immediately after steaming time reached to stop further reaction.

The marker of each reaction have been developped based on their ability to identify reaction stages, such as acetic acid, formic acid, HMF (hydroxymethylfurfural), glucose, fructose, sucrose, lactose, and free amino groups as precursors for Maillard reaction, and caramelization. While fatty acid content were analysed as precursor for lipid oxidation.

The measurement of HMF content was made according to AOAC Official Method 980.23. Acetic acid, formic acid, sucrose, glucose, fructose, lactose and free amino groups measured by HPLC. Fatty acids content were measured by GCMS methods.

Research design

Research appliled completely randomized design with 3 replications. The treatment was steaming time consist of 0 h, and 8 h. Data were presented as a mean of three measurement.

Result and Discussion

The reaction that occured in a system which consist of carbohydrate, protein, and lipids can be classified as complex system which involved a series of chemical reaction. The reaction produces several of compounds which can be used as a reliable marker for reaction took place during steaming. The reliable markers were choosen based on the theoretical approaches, available test methods and analysis of available information. The occurence of reaction was also determined by calculating mass balance between reactant and product.

Hydroxy methyl furfural (HMF)

Test on the HMF content of KDJ and the dough is important in order to identify reaction involved in browning of KDJ. Since material that used were rich in carbohydrate, and amino acid, than it were subjected to Maillard reaction and caramelization. HMF is known as a marker which is used to identify thermal damage of carbohydrate-rich food (Capuano *et al*., 2008; Capuano and Fogliano, 2011). Normally

Table 1. Sugar and its decomposition product

Comp	t_0 (Dough)	Limit detection	t_8/KDJ
HMF	nd**	0.75 ppm	nd**
Acetic acid	nd**	15.7 ppm	nd**
Formic acid	nd**	10 ppm	nd**
Glucose	nd**	0.6 %	nd**
Fructose	nd**	0.6 %	nd**
Sucrose %	26.92 a	-	26.25 a
Lactose %	0.52a	-	0.26b

*Data represent mean values (n = 3) of the measurements
Means marked with the same letter in each batch are not significantly different (P>0.05)
**nd means not detected

HMF is not present in raw and fresh food. It may be formed when food or food products, that are contain carbohydrates and/or amino acids, cooked by thermal processing (Rufiàn-Henares *et al.*, 2006; Rufiàn-Henares and Delgado-Andrade, 2009).

HMF is an intermediate compound formed during the Maillard reaction and by the degradation of hexoses at high temperatures at acid conditions (Martins *et al*., 2001; Arribas-Lorenzo and Morales, 2010). It is also known as a precursor of brown compounds in the caramelization reaction via enolization (Simpson, 2012). Test result showed there was no HMF neither in the dough nor in KDJ. The absence of HMF in the dough was in accordance to Rufian-Henares and Delgado-Andrade (2009) who stated that HMF ussually not present in raw product. This means that raw materials were used still fresh and not yet degraded.

The absence of HMF in KDJ means that decomposition or degradation of sucrose during steaming did not occur. It was due to sucrose trapped in matrix of food ingredients in KDJ, including lipids and protein. During steaming protein denaturated and interacted each other to form protein agglomeration in foods matrixes. In addition lack of available water in a solid system like KDJ make sucrose trapped arround and less mobile. This result was different from Capuano *et al.* (2008), Belitz *et al.* (2009), and Capuano and Fogliano (2011) who stated that protein and amino acid have role in the formation of HMF in foods via Maillard reaction mechanism. From the stand point of nutrition, the absence of HMF in KDJ was a positive point, because the existence of HMF in food have been studied extensively as a contaminant in food that could potentially have harmful properties (Capuano *et al*., 2008; Capuano and Fogliano, 2011; Kowalski *et al*., 2013).

Formic acid and acetic acid

Test on formic acid and acetic acid was important in order to identify Maillard reaction and caramelization. Formic acid and acetic acid are degradation product of Maillard reaction involving glucose and fructose (Brands and Van Boekel, 2001). Martins *et al.* (2001) found formic acid and acetic acid as intermediate Maillard products of primary reaction of glucose/ glycine model system.

Test result on the dough and the cake showed that there are no formic acid and acetic acid identified. The absence of formic acid and acetic acid in the dough described that reaction did not take place during mixing yet. The absence of formic acids and acetic acid in KDJ, showed that degradation of sugar were not occured. In the other words, browning in KDJ making did not occur via caramelization.

The caramelization reaction did not occur because the water content which trapped in the matrix of KDJ was still very high (48.9±0.18%), and the temperature which were applied for steaming not able to melt sucrose so that decomposition of sucrose into glucose and fructose did not occur. According to Nursten (2005) and Simpson (2012) caramelization is the process of removal of water in the sugar, followed by isomerization and polymerization at high temperatures. Caramelization is begun with melting of sugar at a high temperature. These results can be confirmed through testing of pH. The pH of KDJ was in the range of 7.44 ±0.3. This mean system was in the neutral condition tends to bases. Thus, the neutral or alkaline conditions may not occur if KDJ containing acetic acid or formic acid.

Glucose and fructose

Test on the glucose and fructose content in the dough and KDJ is important in order to identify hydrolysis of sucrose. Since sucrose hydrolysis is very slow, it can be accelerated by adding enzym or acid. Fructose and glucose also produced when sucrose heated until it decomposed.

Test result showed there were no glucose

Table 2. The concentration of amino acids (ppm)

Compound	Dough (C_0)	KDJ (C)	C/C_0
Aspartic	19907.11	13098.11	0.658
Glutamic	25029.53	14359.86	0.574
Serine	17500.85	8731.04	0.499
Glycine	8150.75	3659.23	0.449
Histidine	5825.8	2424.6	0.416
Arginine	13202.93	5212.59	0.395
Threonine	10979.19	5361.05	0.488
Alanine	11386.1	5813.18	0.511
Proline	8966.14	4421.16	0.493
Valine	14940.44	7562.87	0.506
Methionine	6603.8	2340.29	0.354
Isoleucine	19845.46	8525.23	0.430
Leucine	10402.25	4360.88	0.419
henylalanine	14775.78	6192.96	0.419
Lysine (Lysine HCl)	3362.09	925.54	0.275
Total	190878.22	92988.6	0.487

and fructose identified both in the dough and in KDJ. There were two possibilities to explain the phenomena. Firstly there was no sugar hydrolized during mixing and steaming, and no sugar melted during steaming. Steaming process was not able to melt sugar into fructose and glucose becaused temperature (100°C) applied in steaming process far below melting point of sucrose (186°C). In addition sugar was not hydrolyzed since the system in neutral condition tends to bases. Secondly, if there were sugar hydrolysis during steaming and mixing, then fructose and glucose formed would react with amino acids according to Maillard reaction to produce brown pigment known as melanoidin. This result consistent with the test results of acetic acid and formic acid. The absence of glucose and fructose in KDJ confirmed that acetic acid and formic acid were not produced during steaming.

Sucrose

Test on sucrose concentration in the dough and KDJ is important to identify whether sucrose undergo a series change during steaming. During steaming sucrose could be hydrolized, melted, degraded, and also experienced Maillard reaction and caramelization. The difference on sucrose concentration in the dough and KDJ, will reveal the contribution of sucrose in reaction series.

Test result showed that the concentration of sucrose both in the dough and KDJ almost the same. The concentration of sucrose changes only slightly. Statistically there was no significant difference on the sucrose concentration between the dough and KDJ. It can be said that there was no significant changes on sucrose concentration after steaming. This was means that steaming not able to hydrolyzed sucrose to be glucose and fructose. This result explained why there were no HMF, acetic acid, formic acid, fructose, and glucose were found in KDJ. The only possible explanation was becaused sucrose trapped in food matrix surrounded by protein crosslink and interaction between protein and lipids make it remain stable during steaming. Protein agglomeration caused hardening in texture. Lack of available water and solid system made it difficult to move and protected from hydrolyzed. This indicated that there was no sucrose contribution in browning of KDJ.

In terms of nutritional stand point, the stability of sucrose concentration during steaming of KDJ has a positive value to the quality of the cake. High sucrose content, make it potential to become a food that have high calories which is very good for growth period or for breaking off fasting, food appetizer or food for energy recovery period. This result also revealed that steaming can prevent degradation of sucrose by maintaining water content so the caramelization did not occur.

To ensure the contribution of proteins, sucrose, lactose and margarine in browning of KDJ then steaming of egg white, egg white + sucrose, egg white + milk, egg white + margarine were conducted. The results showed that steaming egg white for 8 hours resulted brown gel with a lightness (L) ranging from 44.7 ± 0.002. This means that during steaming protein denaturated, agglomerated, condensed, and polymerized (formed protein crosslinking) and

reacted with carbohydrate in egg white to produce brown color substance.

Steaming egg white with sugar resulted brown color gel with a lightness of 45.6 ± 0.003. The lightness of gel was lighter compared to egg white gel. This indicated that adding of sucrose inhibit browning reaction so the gel formed less brown compared to egg white gel. This result confirmed that browning mechanism in KDJ did not follow Maillard reaction between glucose/fructose derived from sucrose with amino acids or peptides nor caramelization. Alternatively another possibilities for browning in KDJ was reaction between lactose with amino acids. The reaction could be occured because lactose was a reducing disaccharide. Lactose containing mono saccharides glucose and galactose. Lactose has a carbonyl group which potentially free on glucose residue, so it could be react directly with the amine and amino, of the protein. According to Worlstad (2011) sucrose was more stable than lactose because sucrose was a non reducing disaccharide. Lactose immediately oxidized to be glyoxal (GO) or fructosilysin (FL). In the Maillard reaction, lactose may react with amino groups of proteins to form N-substituted glycosyl amine.

Steaming egg white with milk (source of lactose) resulted a gel that dark brown with a lightness of 41.2 ± 0.001. The resulted gel have a darker brown color than egg white gel. This showed the contribution of lactose in browning of gel formed. The adding of lactose in egg white may accelerate browning reaction so that the gel formed much darker.

Test results on the lactose showed differences in concentration of lactose between the dough and KDJ. There was a remarkably decrease on lactose content. Approximately 50% of lactose in the dough disappeared during steaming. This indicated that lactose experienced a series reaction during steaming. Lactose may react with amine, amino acid, and peptides according to Maillard reaction to produce a brown color compound (melanoidin).

The reaction between lactose with lysine will produce Schiff base which further rearrange to be Amadori product (ARP) Lactulosyllysine –R. The ARP then degraded by oxidative cleavage or acid hidrolysis (Van Boekel,1998). In KDJ degradation of ARP was occured by oxidative cleavage since the pH was in neutral tend to bases. According to Ditrich *et al*. (2006) the reaction between lactose with lysine resulted lactulosillysine, wich further degraded to formed N-carboxymethyllysin (CML), pentosidine, pyrraline, or oxalic acid monolysinylamide (OMA).

Amino acids

Amino acid is one of important reactant which plays major role in food browning especially Maillard reaction. Amino acid able to reaction with carbonyl group either those derived from sucrose, lactose or derived from lipids oxidation such as aldehydes and ketones (Damodaran, 1996; Zamora and Hidalgo, 2005). Since test result did not identify either glucose or fructose in the dough and KDJ, then we proposed that browning reaction may be occurred between amino acid with lactose and lipids oxidation product.

Table 2 showed that all free amino acids were decreasing. This indicated that all amino acids were available in the dough experienced a series reaction during steaming. Since sucrose concentration only changed slightly, the decreasing in amino acids content were not caused by its reaction with carbonyl group derived from sucrose. Decreasing in amino acids were caused by its reaction with lactose and carbonyl compound derived from lipids oxidation to form brown colored compound. Since there were abundant of amino acids available in the dough, the reaction only consumed half of them. This result confirmed the existence of interaction between amino acids and lactose. Decreasing on amino content is in good agreement with those reported in many article (Saltmarch and Labuza, 1982; Martins and Van Boekel, 2001; Yeung *et al.,* 2006). Table 2 also showed that lysine concentration decreased the most compared to other amino acids. This result is in good agreement with Saltmarch and Labuza (1982) which stated lysine more reactive than other amino acids. Considering amino acids are able to reaction with carbonyl group those derived from lipids oxidation such as aldehydes and ketones, so we attempt to follow reaction by calculating mass balance of fatty acids in the dough and KDJ.

Fatty acid

Fat is one of important reactants which plays major role in food browning especially through fat peroxidation. Fat can be oxidized by reacting with oxygen to produce hydroperoxides and other oxygenated compound which is known as autoxidation. According to Zamora and Hidalgo (2005) the ability of fat to be oxidized, make it able to produce flavour compound, colour and texture. During processing fat will be oxidized and interacted each other to form brown color (Hidalgo and Zamora, 2002; Hutapea *et al*., 2004). Test on the fatty acid content in the dough and KDJ could reveal the contribution of fatty acid in browning reaction. Changes on the concentration of fatty acids in the dough and KDJ will be able to confirm role of fat in

Table 3. Fatty acid profile

Fatty acid	Dough (C_0) %	KDJ (C) %	C/C_0
Saturated fatty acid	4.3	4.27	0.993
Butyric (C4)	0.03	0.02	0.67
Kaproic (C6)	0.02	0.02	1.0
Kaprilic (C8)	0.01	0.01	1.0
Kapric (C10)	0.03	0.03	1.0
Lauric (C12)	0.05	0.05	1.0
Miristic (C14)	0.17	0.17	1.0
Palmitic (C16-0)	3.27	3.24	0.99
Stearic (C18-0)	0.72	0.73	1.0
Unsaturated faty acid	5.33	4.74	0.889
Oleic (C16-1)	3.99	3.61	0.90
Linoleic (C16-2)	1.28	1.07	0.83
Linolenic (C16-3)	0.06	0.06	1

browning of KDJ.

Table 3 showed that fatty acids in the dough and KDJ were dominated by palmitic, stearic, oleic and linoleic acid which were major fatty acids in egg and margarine. The amount of unsaturated fatty acid was much higher than saturated fatty acids. There was significant decrease on the fatty accid content. This indicated that fatty acid in the dough underwent a series reaction during steaming. The decreasing in fatty acid content was caused by the oxidation of fatty acids during steaming to produce lipids peroxidation and then interacted each others to form brown color. In addition, during steaming the lipids peroxidation product were able to react with amino acids to form brown pigment. This result is in accordance with Hidalgo and Zamora (2002), and Hutapea *et al.* (2004) which stated that lipids oxidation product are able to react with other component of foods such as protein. Further more Hutapea *et al.* (2004) found that oxidation product of dienoic acid (dienoic hydroperoxide) and its decomposition could react with amino acid to form dienoic imine which polymerized by aldol condensation to produce brown color substance.

According to Nawar (1996), thermodinamically fat autoxidation is difficult since it needs activation energy of 146 kJ/mol. A metal catalyst such as cobalt, copper, iron, manganese, and nickel needed to start reaction. The presence of metal catalyst even at 0.1 ppm, can decrease the induction period of oxidation. In KDJ making, oxidation could take place by metal catalysis especially iron. The egg, as a major substance in making KDJ containing 17.5 ppm iron. The amount of iron in KDJ will be able to catalyst the initiation oxidation so series of lipids oxidation could take place.

Decreasing in fatty acid, amino acids and formation of brown color in KDJ is in good agreement with those reported in many article (Hidalgo and Zamora, 2000; Hidalgo and Zamora, 2002; Hutapea *et al.*, 2004; Zamora and Hidalgo, 2005). A long with test on amino acids concentration, this result confirmed the existence of interaction between amino acids and lipids oxidation products in browning of KDJ.

The decreasing of fatty acid mainly oleic and linoleic indicated browning reaction were caused by lipids oxidation and interaction between lipids oxidation product with amino acids. If there were lipids oxidation, then browning mechanism in making KDJ was the oxidation of oleic and linoleic to form hydroperoxide. According to Frankel (1980) oxidation of oleic will produce hydroperoxide 8-, 9- , 10-, and 11- hydroperoxide allylic. Oxidation of linoleic results mixing of 9- dien hydroperoxide, 13-dien hydroperoxide. Propagation stage were decomposition of hydroperoxides to be aldehyde and alkyl radical and semi aldehyde or oxo-ester. According to Nawar (1996) decomposition of 8- hydroperoxide resulted dekanal, metil-8-oxooctanoic, 2-undekenal and methyl heptanoic. Decomposition of 9- hydroperoxide resulted nonanal, methyl-9-oxononeic, 2-decenal, and methyl octanoate. Decomposition of 10- hydroperoxide, resulted octane, metil-10-oxo-8-dekenoate, nonanal, and methyl-9-oxononanoate. Decomposition of 11-hydroperoxide resulted heptane, methyil-II-oxo-9-undekenoate, oktanal, and methyl-10-oxo-dekanoate. The termination stage consist of condensation and polymerization of oxidation product (carbonyl compound) resulting stabilized

polymer (Nawar,1996), and the reaction of lipids oxidation product with amine, amino to form brown color (termination stage analog to Maillard reaction).

Conclusion

The above result showed that the sucrose remain stable during steaming. There were no HMF, acetic acid, formic acid, glucose and fructose in KDJ. More over there were decreasing of lactose, amino acids and fatty acid content. The browning mechanism in KDJ did not follow Maillard reaction between glucose/fructose with amino acids or peptides nor caramelization. Browning reaction in KDJ making was occured via Maillard reaction between lactose with amino acid, lipids peroxidation, and interaction between amino acids and lipids oxidation product. Along with the test result of sucrose, amino acid and fatty acid, KDJ has a high nutritional value which potential as food for growth and energy recovery.

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