Color development and antioxidant activity in honey caramel

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Abstract. Honey is a kind of food that used widely in culinary and health fields. Honey contains 50.90%–53.60% of glucose and fructose. The heating process in food would slightly convert sugars in honey to caramel. In food products, the caramel was used as a flavoring and coloring agent. Sugar degradation products formed during caramelization could contribute to antioxidants activity of honey caramel. Therefore, this study aims to identify the color development in honey caramel and antioxidant activity from honey caramelization. Antioxidant activity was analyzed with DPPH and color was analyzed by chromameter. The result showed that $L^*$, $a^*$, and $b^*$ value decreased as the temperatures increased. The inhibition result from light caramel 12.816%, medium caramel 29.098%, and dark caramel 33.533%. The antioxidant capacity increased as the color darkened and the temperature increased. Based on correlation analysis, there was significant value 0.058, $\alpha=0.1$ between $a^*$ and $b^*$ value and antioxidant activity. Blue-green low molecular weight compounds that formed during the caramelization process could contribute to dark color and antioxidant capacity in honey caramel.

Keywords: Antioxidant, Caramelization, Honey, DPPH, Browning

1. Introduction
Caramel is obtained by heating any kind of sugar until the color changes to a shade between brown and nearly black [1]. Caramel delivers three types of the product depending on its phase, they are solid, semi-solid, and liquid. Solid caramels are usually used to make candies and decorations of cakes, pastries, and other desserts, semi-solid syrups caramels usually used as flavoring sauces, gravies, and some beverages, and liquid caramels usually used as a food colorant and selected pharmaceuticals [2].

Process in caramel development called caramelization. Caramelization is a non-enzymatic browning reaction that involves the dehydration of sugars during heat treatment under specific conditions, either dry or in a concentrated solution, either alone or with additives [3]. Caramelization process for making light, medium, and dark caramels need heating temperatures respectively 180°C, 180–188°C, and 188–204°C [2].

Honey is viscous and aromatic products appreciated since ancient Grecian times. Traditionally, honey used as a sweetening agent in food products [4]. The use of honey increases in culinary worlds, especially for additive in cooking. Honey is commonly used in the cooking of foods such as cakes,
cookies, bread, and meat and fish dishes [4]. The uses of honey in cooking where the thermal process occurs will convert the honey into the caramel.

During caramelization, some compounds derived from sugar degradation. That compounds play a role to flavour, aroma, color, and antioxidant in caramel. Aldehydes and furans in caramel play roles as an antioxidant [5]. Some previous research found caramelization in sucrose increasing antioxidant capacity in food products [6–8]. Therefore the benefit of the antioxidant capacity of caramel in human health should be important and inquired by the consumer in the future [9]. Besides its antioxidant properties, some furans derivative like 5-sulfoxymethyl-2-furfural is of serious concern because of its genotoxicity. The formation of furan derivatives highly depends on the thermal load applied. Therefore limiting thermal input is one of the main strategies that could be applied [10].

Sucrose is disaccharides that formed from glucose and fructose as the monomers. Honey is a food product that consists of fructose and glucose for 50.90%–53.60% [11]. A lot of research has learned about caramelization and antioxidant capacity from dextrose, molasses, cane sugar, corn syrup, and glucose syrup, but there is no research about honey caramelization. Therefore, this research aims to identify the color development and antioxidant activity from honey caramelization.

2. Methods
This research was conducted at Food Processing Laboratory of Faculty of Medicine and Health Science Universitas Kristen Satya Wacana Salatiga. Antioxidant activity analysis was conducted at Chemical Faculty of Math and Science Universitas Kristen Satya Wacana Salatiga, and color analysis was conducted at Food Laboratory of Faculty of Agriculture Unika Soegijapranata Semarang. This research was experimental laboratory research that used a completely randomized design with three kinds of treatments. The honey (Apis mellifera) used in this research was obtained from Salatiga.

2.1. Honey caramelization
Three types of treatments used for this research (light caramel, medium caramel, and dark caramel). Caramels were made by heating 250 mL honey until 170˚C for light caramel, 180–182˚C for medium caramel, and 188–190˚C for dark caramel.

2.2. Color analysis
Caramel’s color was analyzed by chromameter (Konica Minolta CR-400). The chromameter calibrated with black and white before measure L “lightness”, a “redness” and b “yellowness”.

2.3. Antioxidant activity analysis
Antioxidant activity analyzed by Rahman method [12] with slight modification. 50 g sample extracted with 25 mL methanol 500 mL 1⁻¹ for 12h. The mixture filtrated with Whatmann paper No.4. 0.3 mL sample was added into 1.2 mL methanol and 1.5 mL DPPH reagent 0.5 mmol (in methanol). The solution was incubated in a dark place with a temperature 30˚C for 90 minutes. Absorbance was measured with a spectrophotometer (UV mini Shimadzu U-1240) with wavelength 517nm. Trolox was used as blank. The radical scavenging activity calculated with:

\[
\% \text{Antioxidant activity} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs control}} \times 100
\]  

(1)

2.4. Statistical analysis
Statistical analysis was done by the analysis of variance ANOVA with \( p<0.1 \). The analysis was done by SPSS ver2.0 for windows.

3. Result and discussion
Color change in honey during the heating process caused by caramelization reaction. Caramelization process happened when heating sugar without the absence of a nitrogenous compound [13]. During
caramelization, the sugars initially undergo dehydration and then condensation or polymerization into complex molecules of varying molecular weights. Lightly colored, pleasant-tasting caramel flavors are produced during the initial stages, but as the reaction continues higher molecular weight color bodies are produced, and the flavor characteristics become more bitter [13]. Some research has learned about caramelization and antioxidant activity in a food product such as soft drinks [14], coffee [15], brown sugar, cane sugar [16], and wine [17].

3.1. Color
Temperature difference for making honey caramel affected caramel color result. Light caramel made in temperature 107˚C shows a lighter color compared with medium caramel (180–182˚C). Dark caramel made with temperature 188–190˚C shows the darkest color. The change in color parameters of caramels is shown in table 1. The $L^*$ value from the Lab color scale was used to measure the color differences indicative of brown polymer as a result of sugar degradation. The $L^*$ value indicates the level of lightness or darkness on a 0–100 [18]. Based on the result, $L^*$ value decreases within the heating temperature increase. Browning in honey caramel evaluated by a decrease in $L^*$ value and brown color formation which expressed according to the heating temperature. Glucose and maltose thermal degradation products such as furfural, 5-methylfurfural, and HMF increase darkness in caramel [19].

| Sample          | Temperature | $L^*$  | $a^*$   | $b^*$   | $\Delta E^*$ | $C^{*ab}$ |
|-----------------|-------------|--------|---------|---------|--------------|------------|
| Light Caramel   | 107˚C       | 35.43±0.63 | 8.75±0.89 | 6.97±0.51 | 111.472      | 11.21      |
| Medium Caramel  | 180–182˚C   | 34.11±0.62 | 1.40±0.46 | 1.15±0.46 | 109.70       | 1.58       |
| Dark Caramel    | 188–190˚C   | 28.48±0.44 | 0.36±0.43 | 0.60±0.82 | 108.115      | 0.70       |

Notes:
- $L^*$ : Lightness/darkness (1–100)
- $a^*$ : Redness/greenness
- $b^*$ : yellowness/blueness
- $\Delta E^*$ : color difference
- $C^{*ab}$ : chromaticity

$a^*$ dan $b^*$ value also decreases within the heating temperature increase. $Cab^*$ and $\Delta E^*$ value also decrease as $a^*$ and $b^*$ value decrease. $\Delta E^*$ index (color difference) affected by the brightness value. The decrease in $\Delta E^*$ value shows that honey caramel lost the brightness within the heating temperature increase. The brightness value decrease probably caused by the accumulation of high molecular brown pigment produced from caramelization in dark caramel.

Honey contains a large number of carbohydrates about 80% (about 32.3% glucose and 38.6% fructose [4]. During the heating process, sugar in honey degradation. Monosaccharides in honey caramelize through an initial step called intramolecular rearrangements. The first step is initial enolization known as the de Bruijn van Eekenstein rearrangement [17]. This reaction resulting in the generation of enols intermediate and final dehydration products [4]. In this step, glucose and fructose molecules change their group into enediols form. Glucose turn to 1,2-enediol and fructose can turn to 1,2-enediol and 2,3-enediol. Through this step, glucose and fructose isomerize and epimerize via 1,2-enediol intermediate [10]. Besides isomerization and epimerization, the next step of enolization is dehydration reaction.

Dehydration mechanism in caramelization is indicated by water molecules release from enol intermediates. Dehydration via the β-elimination mechanism where the water molecules released from a carbohydrate carbon chain. The dehydration of the sugar via B-elimination is central in thermally induced sugar degradation under almost anhydrous conditions [17]. Kocadagli (2018) mention that
dehydration of sugars produces several reactive deoxyosones, and more stable furan and pyran derivatives, which are important for the formation of caramel color and flavor. Dehydration from C-4 of 3-deoxyglucosone produces 3,4-dideoxyglcososene-3-ene (3,4-dideoxy-D-glycero-hex-2-enos-2-ulose) [10]. Dehydration from a hemiacetal structure of 3,4-dideoxyglucosone-3-ene results in 5-cymethyl-2-furfural (HMF, 5-hydroxymethylfuranc-two-carbaldehyde). Dehydration from 1,2-enediol produces Hydroxymethylfuranol (HMF) through 3-deoxyhexosulose dehydration and 2,3-enediol dehydration produces Hydroxydimethylfuranone (HDF) and Hydroxyacetilfuran (HAF) through 4-deoxyhexosulose dehydration [17].

HMF can be degraded to other products, including furfural, via fragmentation reaction (with indirect subsequent H+ release) and 5-methylfurual, via reduction reaction. Some research found that fructose produces more HMF intermediate that contributes to brown color development [10] because it has a reactive fructofuranosyl group [20]. Low molecular weight like diacetyl, 2,3-pentanedione, acetoin, furan, 2-methylfurualan, 3-methylfurualan, furfural, 2-acetylfrualan, and 2- hydroxyacetylfrualan are the constituents of the volatile fraction in thermally treated foods also can be directly formed from sugar backbone during caramelization [21]. Low molecular weight compound (Mr< 3,500) also resulting blue-green pigment beside of giving caramel aroma [18]. When the reaction continue higher molecular-weight color bodies are produced, and the flavor characteristics become more bitter [13]. HMF and furfural are considered to be precursors of such regular polymers [17]. Brown pigment caramel formed during polymerization of low molecular weight compound at intermediate step resulting higher molecular weight compound (yellow-red) [18]. The polymeric material from the plain caramel is generated from the condensation reactions of the aldehydes and ketones formed by heating the sugar with bases or acids [22]. This reaction occurs without the presence of amines. Caramel C_{12}H_{16}O_{1}; and two polymers, caramel C_{20}H_{30}O_{25} and caramelin C_{26}H_{102}O_{51}, known as the polymer that formed during sucrose caramelization [13]. Caramel, described as a brown, brittle solid of a bitter taste. It is very deliquescent, and very readily soluble in water. A loss weight of 12% gave mostly caramelan. Caramelen is described as a brown substance much darker in colour than caramel and not deliquescent. A loss weight of 15% gave mostly caramelen. Caramelin is a substance of high molecular weight and colloidal character that much darker than carbalmealen and caramelen. A loss weight of 22% gave mostly caramelin [23]. High temperature cause the honey lost the weight because dehydration of water molecules. Higher temperature produce more caramelin, a dark molecular weight that make a* and b* value decrease.

3.2. Antioxidant activity
Antioxidant activity result of methanol extract with DPPH show that honey caramels have antioxidant activity as shown on table 2. The inhibition values increase with the heating temperature increase. Light caramel has the brightest color from other samples shows less antioxidant activity 12.816%, followed by medium caramel 29.098%, and dark caramel 33.533%.

| Sample               | Inhibition %          |
|----------------------|-----------------------|
| Blank                | 0                     |
| Light Caramel 1000 ppm | 12.816 ± 0.0005       |
| Medium Caramel 1000 ppm | 29.098 ± 0.0005       |
| Dark Caramel 1000 ppm | 33.533 ± 0.0005       |

3.3. Trolox response
The concentration response curve of trolox to dphh assay was obtained (the concentrations ranging between 10–80 ppm r^2=0.9922). Trolox at 10 ppm inhibited DPPH oxidation by 11.86% and Trolox at 80 ppm, by 94.55%. Methanol extract of honey caramel exhibit weak free radical scavenging capacity.
to DPPH assay dark caramel inhibited DPPH oxidation by only 33.533% at 1000 ppm equivalent with 28.27 ppm Trolox.
Antioxidant activity in caramel produced from sugar degradation compound. Several compound play a role as antioxidant are colorless low molecular weight reductones [24]. HMF specifically, and caramelization products in general, is that they have an antioxidant effect [25]. Several volatile compounds in caramel also potential as antioxidant. Pyrroles, furans, and thiophenes exhibited antioxidant activity by inhibiting hexanal oxidation at a concentration range of 50–500 g/mL [26]. Aldehydes and furans in caramel also reported for its contribution as antioxidant [5]. Antioxidant potential compounds inhibition in caramel are low molecular weight produced from sugar dehydration stage. At higher temperatures, more dehydration occurs due to the release of water molecules. High dehydration causes the amount of both colorless intermediate and final brown caramel formation is increasing as heating time increases [9]. More colorless intermediate like HMF and furfural increase in higher temperature [9], makes the antioxidant capacity of dark caramel higher than light and medium caramel. When the reaction continues, some intermediate derivative that has stronger antioxidant activity like 2-ethylpyrrole, 2-acetyl-1-methylpyrrole, 2-pyrrole-carboxaldehyde, 2-acetylpyrrole, 2-thiazole carboxaldehyde, 2-methylthiophene, and 2-ethylthiophene also formed [26].

4. Correlation between Lab* and antioxidant activity
Some research suggested a relationship between antioxidant capacity and brown pigment, instead of the colorless intermediate of caramel [9]. Correlation test result between Lab* values and antioxidant activity in honey caramel shows significant correlation for a* and b* 0.058 with α=0.1. It showed that there was a significant relationship between antioxidant activity with the change of color.

| Table 3. Correlation test between L* and antioxidant activity. |
|-------------|------------|---------|-------|----------|
| Df | SS | MS | F | Significance F |
| Regression | 1 | 148.0691 | 148.0691 | 1.640587 | 0.422002 |
| Residual | 1 | 90.25374 | 90.25374 | |
| Total | 2 | 238.3229 | |

| Table 4. Correlation test between a* and antioxidant activity. |
|-------------|------------|---------|-------|----------|
| Df | SS | MS | F | Significance F |
| Regression | 1 | 236.2943 | 236.2943 | 116.4801 | 0.058819 |
| Residual | 1 | 2.028624 | 2.028624 | |
| Total | 2 | 238.3229 | |

| Table 5. Correlation test between b* and antioxidant activity. |
|-------------|------------|---------|-------|----------|
| Df | SS | MS | F | Significance F |
| Regression | 1 | 236.2943 | 236.2943 | 116.4801 | 0.058819 |
| Residual | 1 | 2.028624 | 2.028624 | |
| Total | 2 | 238.3229 | |

The value of a* showed redness/ greenness (+red to –green components) and b* value show yellowness/blueness (+yellow to – blue components). Based on test result a* and b* values decrease with the antioxidant activity increase. Decrease in a* and b* value show lot of formation green-blue compound at higher temperatures than red-yellow compound. That blue-green compound may potential as antioxidant activity. During reaction, the colorless intermediate compound like HMF, furan, aldehyde, thiophene, and pyrrole also degraded and produce coloured derivative compounds like 1-methylpyrrole, 2-ethylpyrrole, pyrrole-2-carboxaldehyde, 2-acetyl-1-methylpyrrole, 2-methylfuran, 2-ethylfuran, 2-furaldehyde, 2-acetylfluran, 2-methylthiophene that have more antioxidant properties...
The derivative antioxidant compound give light yellow-orange to brown color [27–29]. Beside enhance antioxidant activity, that compound may contribute to the decrease in Lab* value. Based on correlation test result there is no significant value for L* value and antioxidant activity 0.42, with α=0.1. It shows that there no correlation between the decrease of L* and antioxidant activity in honey caramel. L* value used to evaluate the lightness of caramel. Based on correlation test, it is no that there no correlation between the decrease lightness in honey caramel with antioxidant capacity even the variable of a* and b* have significant value. This probably because the antioxidant activity has more impact on the formation of dark color than with the lightness in honey caramel even the L* value decrease.

Caramel color classified into four classes they are plain caramel (type I), caustic sulfite caramel (Type II), ammonia or beer caramel (type III) and sulfite-ammonia (Type IV). Each type of caramel color has specific functional properties that ensure compatibility with a product and eliminate undesirable effects, such as haze, flocculation, and separation [30]. Every type of caramel also has a different color based on its hue and color intensity. According to international standards, color intensity is defined as the absorbance of a 0.1% (w/v) solution of caramel color solids in water in a 1-cm cell at 610 nm [30].

Honey caramel classified in plain caramel (type I) because the absorbance is 0.01–0.05 at 601 nm. Plain caramel usually used for cereals, nutrition bars, rice cakes, croutons, and stuffing [31].

5. Conclusion
Browning in honey caramel increase with the temperature increase. It indicated by the decrease in Lab* values. The antioxidant capacity in honey caramel increase as the temperature increase. The antioxidant capacity of honey caramel for light caramel 12.81%, medium caramel 29.09%, and dark caramel 33.35%. The higher temperature increases antioxidant activity and browning on honey caramel due to high dehydration which causes the formation of more antioxidant and brown compounds. There is a significant correlation between the a* and b* value and antioxidant activity probably because of several dark-coloured compounds that have antioxidant activity cause decrease of a* and b* value.

6. Suggestion
Further analysis is needed to ensure compounds that contribute to browning in honey caramel color with Thin Layer Chromatography (TLC). Additional analysis also needs to do to find out the compounds formed during caramelization and ensure that antioxidant capacity in honey caramel derived from low molecular weight compound with GCMS analysis.

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