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# Synthesis and Characterization of Caramel from Simple Sugar for Brewing Color Application

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**Abstract :** Caramel is a brown to black liquid or solid having the characteristic odor of burn sugar with a pleasant bitter taste prepared by heat treatment of carbohydrate in a process called caramelization. The aim of this study is to synthesis a quality beer coloring caramel using a robust and reliable technique from a commercially available raw material. In this study, class III type caramel was prepared by adding ammonia to glucose at an optimized temperature of 145 °C and alkaline solution. The prepared caramel was characterized by measuring its absorbance using UV-VIS spectrophotometer at wavelength 510 nm and 610 nm and by color comparator. The results revealed that a higher alkaline pH give better results for ammonia caramel. It was also observed that a darker color value 42000 European Brewing Convention (EBC) and higher absorbance reading ammonia caramel were obtained with increasing the preheating temperature and contact time. In the experimental analysis, the caramel was produced within a shorter period of time (5 hours) at 145 °C with lowest viscosity (6.65 centipoises), lowest extract (68.44 ºP), lowest haze (0.10 EBC), highest shelf life (1.9 year), no detectable trace metals and free of oral toxicity. It was miscible with water and has a lowest percentage of nitrogen content (0.25%) and ash content (0.10%).

**Keywords** -Brewing Color, Caramelization, Characterization, Simple Sugar, Synthesis

## 1. Introduction

More than 6,000 additives are available in the food industry which include flavors, colorants, preservatives, antioxidants, emulsifiers, thickeners, acids, bases, anticaking agents, flavor enhancers, glazing agents, improvers, bleaching agents, sweeteners, solvents, and a miscellaneous category [1]. Among these, color has always played a vital role in food selection and acceptance. Colorants added to foods must also be proven safe, stable, legally permitted, and effective in a particular application [1, 2].

Caramelization is a serious of complex reactions that include hydrolysis, dehydration and polymerization of carbohydrates to form a colored constituent of brown pigment [3,4]. Caramel has been widely used for coloring and flavoring of foods and beverages like beer, soft drinks, soups and candies [5].

The carbohydrate raw materials used are commercially available food-grade nutritive sweeteners, which are the monomers glucose and fructose or polymers thereof (e.g., glucose syrups, sucrose or invert sugars, and dextrose). To promote caramelization, food-grade acids, alkalis as well as salts may be used in amounts consistent with good manufacturing practices [2, 5]. Caramel colors have been used so far long and in such a wide variety of food products that consumers tend to think of them as a single substance. However, in reality they are a family of similar materials with slightly different properties. Caramel color, from the palest yellows to the deepest browns, accounts for more than 80% (by weight) of all colorants added to the foods and drink [2].

There are four types of caramel based on the raw material used as reactants. Ammonium and sulphite compounds cannot be used as reactants for Class I caramel colors. Sulphite, ammonium and ammonium-sulphite compounds must be used as reactants for Class II, III and IV caramel colors respectively [6]. Each type of caramel color has specific functional properties that ensure compatibility with a product and remove undesirable effects, such as haze, flocculation, and separation [2,7]. Introduction of ammonia and ammonium salts in the manufacturing of caramel colors synthesis a positively charged caramel type III color compatible with non-acidic products like beer [8].

The ammonium compounds used are hydroxides, carbonates, bicarbonates, phosphates, sulphates, sulphites, and bisulphites. The sulphite compounds are sulphurous acid and sulphitesand bisulphites of potassium, sodium, and ammonium. Sulphuric and citric acid and sodium, potassium, and calcium hydroxide are compounds that can be used for all four types of caramel color [6, 7].

In Ethiopia, demands of the caramel quality improvement and controls as well as the markets are increasing. From the annual global consumption of 200,000 tones of caramel, the annual Ethiopian brewery caramel consumption exceeds 80,000 Kg [2]. In the production of caramel from carbohydrates several factors affect the formation of color viz., temperature, time, pH, reactant concentrations, nature of catalysts that may be used and nature of reactants (type of sugar, type of amino acid or protein) [9,10].

The main objective of this study was to establish a robust and reliable technique to synthesis standard and quality beer coloring caramel using a commercially available raw material and instrument. Besides, studies were conducted to determine the best condition of caramel color to get the high quality index of caramel color by analyzing the physicochemical properties of the caramel.

## 2. Materials and Methods

### 2.1. Materials and chemicals

All chemicals used in this study were analytical grades; liquid ammonia solution with 25% concentration was used for Maillard reaction. To adjust the pH of the solution, (75%) phosphoric acid and 99% sodium hydroxide was used. As a source of carbohydrate, 98.5% D-glucose anhydrous was used.

The caramel analysis was taken according to the standards on European Brewing Convention (EBC) and other organizations. Samples in two replicas were taken from each run and the average value was taken for confidence. Accordingly, the physicochemical and nearby composition and functional properties of feedstock were recorded.

### 2.2. Experimental setup and description

The experiments were carried out in a stainless steel reactor. The feeds from the reactor containing D-glucose having with 98.5% of dextrose equivalent (D.E) mixed with of process water were reacted with 28% of anhydrous liquid ammonia and mixed with each other and entered in to the batch reactor at controlled temperature of 125 °C and 145 °C. The reaction mixtures were heated and continuously agitated to produce caramel. The product was kept at room temperature by adding hot water to prevent solidification and packed to prevent contamination.

### 2.2.1 Caramel preparation and effect of operating conditions

Six caramel preparation methods were applied in this particular study by varying the storage time and preheating temperature. In all the methods, 200 grams of solid D-glucose of 98.5% D.E mixed with 80 grams of process water were reacted with 5 mL of 28% of anhydrous liquid ammonia. The stirred syrup was preheated with a sealed hot oven at temperature of 125 °C for the methods one up to three. From method one up to method three the storage times at room temperature are three, six and seven hours respectively. Similarly, in the methods from four to six the preheating temperature was 145 °C and the storage times at room temperature are three, six and seven hours. The absorbance of the collected sample was first measured by UV-Vis spectrometer (model UV-1201) at wave length of 430 nm.

To study the effect of pH and catalysts, samples were taken from method one and analyzed at a three different pH values (3, 7 and 9) and adding five grams of each of the three catalysts (ammonium acetate, ammonium carbonate and anhydrous ammonium hydroxide) to a separate sample of method one. The products were analyzed by UV-Vis spectrophotometer at a wavelength of 430 nm and the color result units were recorded in EBC.

### 2.3. Characterization of Caramel

Before a caramel color is offered for sale, a complete evaluation must be made for the final product. Primarily a caramel color user looks for coloring strength and compatibility with other ingredients. The following characterization tests viz., color measurement (40 mm glass curvet, color disk D (19-27 EBC units), 25 mm curvet and color disk D(10-18EBC units)), trace metals (Cd, Cr, Cu and Pb) by Flame atomic absorption spectroscopy (Buck Scientific Model 210GP, USA), specific gravity (Density meter model DMA 38) and Viscosity (Ostwald viscometer) analysis were made. In addition the haze point, resinification, ash content, nitrogen content (back titration) and acute oral toxicity of the caramel were analyzed. The haze point is an indication of the resistance of the caramel color to a concentrated phosphoric acid solution. One part of the acid was added to two parts of caramel color and the mixture was heated in a boiling water bath. A drop is taken out every five minutes, placed in a tube of distilled water and the solution was observed for clarity by using Haz meter. The time at which the drop makes a turbid solution is recorded as the haze point.

The shelf-life of a caramel color is an important consideration for a buyer, and the resinification test gives an indication of the length of time the color will remain free-flowing. A small amount of caramel color is sealed in a glass ampule and held at 100 °C. The number of hours required for this sample to reach a point where it will not flow is the resinification value. Each 20 hours of resinification value is approximately equivalent to one year of storage under normal conditions [11]. For the analysis of acute oral toxicity 5 mice were weighted and numbered them by marking them on their tail. The mice were kept on a cage lid and grasping the loose skin behind the ears with our thumb and fore finger. As soon as the mice`s head is retrained, the mice were picked up and the tail was secured with our ring and little finger. Then, the feeding needle (oral gavage) was placed on the tongue and gently pushed the caramel solution in to the mouth. Acute toxicity test was done by administering single doses of caramel 10,000 mg/kg body weight. The caramel solution was given for seven consecutive days & their behavioral and biological changes were observed.

## 3. Results and Discussion

### 3.1 Effect of operating condition on caramel preparation

### 3.1.1 Effect of temperature and contact time

The temperature sensitivity of the chemical reaction is dependent upon the activation energy. The longer the activation energy, the smaller the fraction of the activated molecules and the slower a reaction proceeds. Chemical reactions tend to go faster at higher

temperatures. As contact time and temperature of the reaction mixture increases, formation of caramel color increases (Figure 1). The progress of the color formation reaction was monitored by UV-VIS Spectrophotometer**.** In this study absorbance of wavelength 610 nm and 510 nm were used to measure the color intensity and the extent of formation colored stage of maillared reaction. The measurement of color changes due to the presence of soluble pre-melanoidins. The extent of browning (as indicated by measured absorbance at 610 nm and 510 nm) was directly proportional to heating time and temperatures of six prepared caramel.

**Table 1** Color Results of Caramel by using UV-VIS Spectrophotometer

| Method | Absorbance at 610 nm | Standard absorbance at 610nm | Absorbance at 510 nm | Hue Index | Color (EBC) | Contact Time (hr) | Temperature ($^0$C ) |
|---|---|---|---|---|---|---|---|
| 1 | 0.104 | 0.106-0.111 | 0.123 | 0.72 | 24000 | 3 | 125 |
| 2 | 0.108 | 0.106-0.111 | 0.140 | 1.13 | 30000 | 6 | 125 |
| 3 | 0.110 | 0.106-0.111 | 0.154 | 1.46 | 39000 | 7 | 125 |
| 4 | 0.105 | 0.106-0.111 | 0.130 | 0.93 | 28000 | 3 | 145 |
| 5 | 0.109 | 0.106-0.111 | 1.50 | 1.38 | 35000 | 6 | 145 |
| 6 | 0.111 | 0.106-0.111 | 1.59 | 1.56 | 42000 | 7 | 145 |

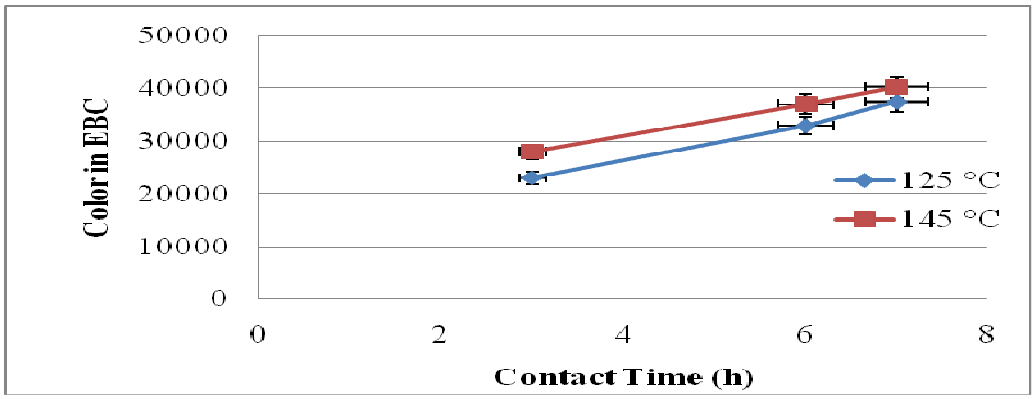


**Figure 1**. Color comparison at different temperature and time

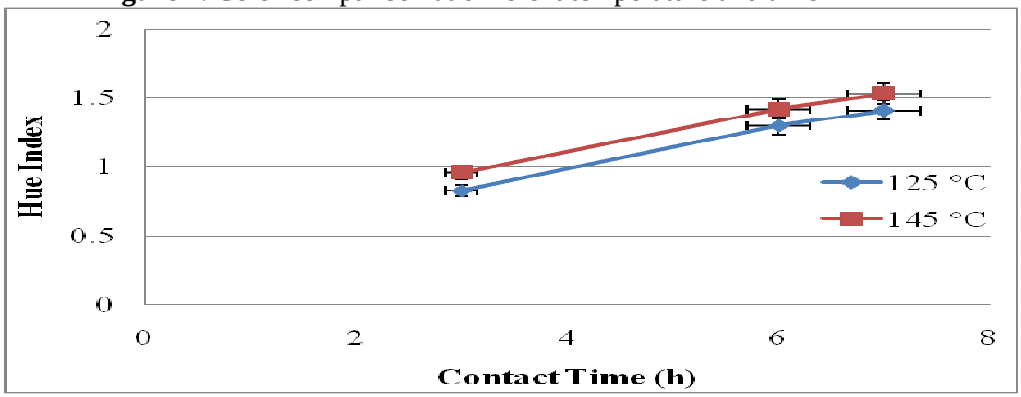


**Figure 2.** Hue index as a function of contact time and temperature

An increase in color intensity of the product is observed with increase of contact time for a fixed temperature (*Table* 1). The browning rate has also been increased after induction period. The plot of the logarithm of absorbance versus time keeps apparently its linearity till it reached a plateau, as shown by the linear plot of log of absorbance versus time for all three testing methods. At 125 °C preheating temperature but different three contact times (3, 6 and 7hours) and the second preheating temperature of 145 °C but by altering contact times (3, 6 and 7 hours). It was observed that the hue index increased with increased of contact time. It is also observed that the higher the temperature, the higher the Hue index recorded (Figure 2).Thus, for the production of caramel, method six is preferred to others because of the highest color values in European Brewing Convention and Hue index.

### 3.1.2. Effect of pH

The study on the effect of starting material discovered that monosaccharide exhibited much more rapid brown caramel formation in alkaline condition. Glucose was observed to develop browning pigments more significantly at pH 7 and 9 but very few of brown pigments were observed at pH 4.Glucose browning monitored at pH values ranging from 4 to 9 showed higher browning reactions in alkaline condition. The higher the basicity of solution, the higher the browning intensity observed. From the experimental data; as the pH of the reaction mixture increased (from acidic, neutral and basic),

the color formation of the caramel product increased (Figure 3).

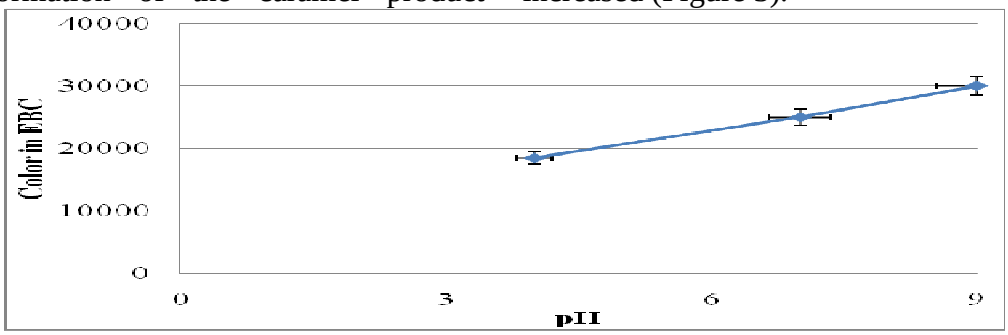


**Figure 3**. Effect of pH during caramelization at 125 ºC and 3 hours

### 3.1.3. Effect of catalyst

The caramel color prepared from monosaccharide (glucose) and the effect of ammonium salts and base on the formation of caramel color was studied. In the presence of ammonium acetate and ammonium carbonate, the development of a brown color was less than in the control samples where no ammonium salts were added. However, the presence of ammonium hydroxide resulted in some improvement in the development of caramel color. So, compared with other catalysts, ammonium hydroxide is the better catalyst for caramelization production.

## 3.2. Optimization of digestion procedure of caramel samples and metal analysis

Among the optimization procedures for FAAS analysis of trace metals, the acid mixture of 5 mL of $HNO_3$ (69-70%) and 3 mL of $HClO_4$ (70%), digestion time of 2 hours and digestion temperature of 200 °C were found the optimal condition for 10 mL caramel sample. These optimum conditions were selected based on clarity of digests, minimum reagent volume consumption, minimum digestion time, simplicity and minimum temperature applied for complete digestion of sample.

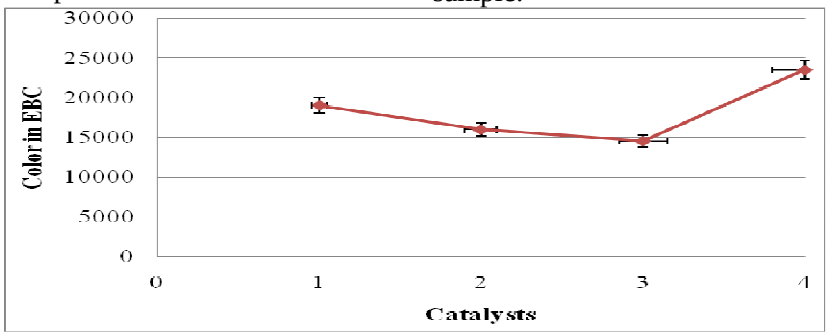


**Figure 4.** Effect of catalyst during caramelization at 125 ºC and 3 hours

**Table 2**. Optimization of digestion procedure for caramel samples

| **Optimization of reagent volume** | | | | |
|---|---|---|---|---|
| Reagents | Ratio (mL) | Temperature ($^0$C) | Time (hr) | Observation |
| $HNO_3$:$HClO_4$ | 3:3 | 200 | 2:00 | Deep brown |
| $HNO_3$:$HClO_4$ | 4:2 | 200 | 2:00 | Clear light brown |
| $HNO_3$:$HClO_4$ | 5:3* | 200 | 2:00 | Clear &colorless |
| $HNO_3$:$HClO_4$ | 4:1 | 200 | 2:00 | Clear brown |
| $HNO_3$:$HClO_4$ | 3:2 | 200 | 2:00 | Yellow |
| **Optimization for temperature** | | | | |
| Reagents | Ratio (mL) | Temperature ($^0$C) | Time (hr) | Observation |
| $HNO_3$:$HClO_4$ | 5:3 | 150 | 2:00 | Deep brown |
| $HNO_3$:$HClO_4$ | 5:3 | 180 | 2:00 | Clear light brown |
| $HNO_3$:$HClO_4$ | 5:3 | 200* | 2:00 | Clear & colorless |
| $HNO_3$:$HClO_4$ | 5:3 | 250 | 2:00 | Clear brown |
| $HNO_3$:$HClO_4$ | 5:3 | 300 | 2:00 | Yellow |
| **Optimization for time** | | | | |
| Reagents | Ratio (mL) | Temperature ($^0$C) | Time (hr) | Observation |
| $HNO_3$:$HClO_4$ | 5:3 | 200 | 1:00 | Deep brown |
| $HNO_3$:$HClO_4$ | 5:3 | 200 | 1:30 | Clear light brown |
| $HNO_3$:$HClO_4$ | 5:3 | 200 | 2:00* | Clear & colorless |
| $HNO_3$:$HClO_4$ | 5:3 | 200 | 2:30 | Clear brown |
| $HNO_3$:$HClO_4$ | 5:3 | 200 | 3:00 | Yellow |

** Indicate the optimal condition for the given parameter.*

**Table 3**. Working standards and correlation coefficients of the calibration curves for determinations of metals using FAAS

| Element | Conc. of standards in (mg/L) | Correlation coefficient |
|---|---|---|
| Cd | 0.02, 0.08, 0.32, 0.72 | 0.9995 |
| Cr | 0.5, 1.0, 2.0, 2.5 | 0.9988 |
| Cu | 0.5, 1.0, 1.5, 2.0 | 0.9999 |
| Pb | 0.2, 0.5, 1.0,1.5 | 0.9998 |

**Table 4**. Atomic absorption spectroscopy test result of caramel sample

| Element | Absorbance of Blank | Absorbance of sample | Sample value in ppm |
|---|---|---|---|
| Cd | 0.001 | 0.001 | Not detected |
| Cr | 0.001 | 0.001 | Not detected |
| Cu | 0.003 | 0.003 | Not detected |
| Pb | 0.000 | 0.000 | Not detected |

**Table 5**.Correlation Table of Extract Determination

| Density | Extract | Density | Extract | Density | Extract | Density | Extract |
|---|---|---|---|---|---|---|---|
| 1.02400 | 6.077 | 1.02600 | 6.572 | 1.02800 | 7.066 | 1.03000 | 7.558 |
| 5 | 6.089 | 5 | 6.584 | 5 | 7.078 | 5 | 7.570 |
| 10 | 6.101 | 10 | 6.597 | 10 | 7.091 | 10 | 7.583 |
| 15 | 6.114 | 15 | 6.609 | 15 | 7.103 | 15 | 7.595 |
| 20 | 6.126 | 20 | 6.621 | 20 | 7.115 | 20 | 7.607 |
| 25 | 6.139 | 25 | 6.634 | 25 | 7.127 | 25 | 7.619 |
| 30 | 6.151 | 30 | 6.646 | 30 | 7.140 | 30 | 7.632 |
| 35 | 6.163 | 35 | 6.659 | 35 | 7.152 | 35 | 7.644 |
| 40 | 6.176 | 40 | 6.671 | 40 | 7.164 | 40 | 7.656 |
| 45 | 6.188 | 45 | 6.683 | 45 | 7.177 | 45 | 7.668 |
| 1.024 | 6.200 | 1.026 | 6.696 | 1.028 | 7.189 | 1.030 | 7.681 |
| 55 | 6.213 | 55 | 6.708 | 55 | 7.201 | 55 | 7.693 |
| 60 | 6.225 | 60 | 6.720 | 60 | 7.214 | 60 | 7.705 |
| 65 | 6.238 | 65 | 6.733 | 65 | 7.226 | 65 | 7.717 |
| 70 | 6.250 | 70 | 6.745 | 70 | 7.238 | 70 | 7.730 |
| 75 | 6.263 | 75 | 6.757 | 75 | 7.251 | 75 | 7.742 |
| 80 | 6.275 | 80 | 6.770 | 80 | 7.263 | 80 | 7.754 |
| 85 | 6.287 | 85 | 6.782 | 85 | 7.275 | 85 | 7.767 |
| 90 | 6.300 | 90 | 6.794 | 90 | 7.287 | 90 | 7.779 |
| 95 | 6.312 | 95 | 6.807 | 95 | 7.300 | 95 | 7.791 |
| 1.025 | 6.325 | 1.027 | 6.819 | 1.029 | 7.312 | 1.031 | 7.803 |
| 5 | 6.337 | 5 | 6.831 | 5 | 7.324 | 5 | 7.816 |
| 10 | 6.350 | 10 | 6.844 | 10 | 7.337 | 10 | 7.828 |
| 15 | 6.362 | 15 | 6.356 | 15 | 7.349 | 15 | 7.842 |
| 1.032 | 8.048 | 1.034 | 8.537 | 1.036 | 9.024 | 1.038 | 9.501 |
| 5 | 8.061 | 5 | 8.549 | 5 | 9.036 | 5 | 9.526 |
| 10 | 8.073 | 10 | 8.561 | 10 | 9.048 | 10 | 9.534 |
| 15 | 8.085 | 15 | 8.574 | 15 | 9.060 | 15 | 9.542 |
| 20 | 8.093 | 20 | 8.586 | 20 | 9.073 | 20 | 9.556 |
| 25 | 8.11 | 25 | 8.598 | 25 | 9.085 | 25 | 9.573 |
| 30 | 8.122 | 30 | 8.61 | 30 | 9.097 | 30 | 9.582 |
| 35 | 8.134 | 35 | 8.622 | 35 | 9.109 | 35 | 9.596 |
| 40 | 8.146 | 40 | 8.634 | 40 | 9.121 | 40 | 9.601 |
| 45 | 8.159 | 45 | 8.647 | 45 | 9.133 | 45 | 9.615 |

Based on the analysis of the caramel by FAAS, no trace metal was detected in the final product (*Table* 4) that revealed good character of the caramel and free from these metal toxicity.

## 3.3. Synthesized Caramel characterization

### 3.3.1. Extract Determinations

The specific gravity of measured values was 1.03205, 1.03200, 1.03000, 1.03050, 1.02800 and 1.02710. The extracts determined from the correlation (*Table* 5) were 8.061, 8.048, 7.558, 7.681, 7.066 and

6.844 °P respectively. From the caramel preparation methods, method six has lower extract value and is the best and easy operation for brewing since it has lower viscosity. The experimental test result shows that the value of extract decreased when the temperature and the contact time increases.

### 3.3. 2.Viscosity Determination

The study on the effects of starting materials demonstrated that monosaccharide's exhibited much more rapid decrease in viscosity of caramel formation with increase in contact time and temperature. In this research, glucose was observed to lowest viscosity most significantly at a temperature of 145 °C and contact time of seven hours. On the other hand very high viscosity of caramel was observed at a temperature of 125 °C and lowest contact time of three hours.

In this study, the result of the prepared caramel viscosity is 8.35, 8.13, 7.03, 7.26, 6.91 and 6.65 centipoises (*Table* 10) respectively. Despite the fact that similar reactions of mixtures were used for all the six treatment, the viscosity value obtained was minimum with the higher heating and reaction time. This shows that the higher the viscosity, the higher difficulty in operational process of brewing when we add the caramel in to pre-run tank. Thus, the prepared caramel viscosity 6.65 centipoises is suitable for brewing because the flow resistance is low and it is easily poured to pre-run tank for color application.

### 3.3.3. Resinification determination

The synthesized caramel was characterized to determine its shelf life. Caramel preparation via method one which was sealed in a glass ampule and held at 100 °C, was started to flow after 30 hours. This is equivalent to one and half year of shelf life in the measurement of international shelf life. But international standard of caramel shelf life is less or equal to two years [12]. The actual result is far from the international standard. For caramel prepared by methods 2-5, were started to flow after 32, 36, 30 and 34 hours respectively. These are equal to 1.6, 1.8, 1.5 and 1.7 years of caramel shelf life. However, caramel prepared by method 6 was started to flow after 38 hours which is equal to 1.9 years of caramel shelf life (Table 6). This suggested that, as heating contact time and temperature increases, caramel shelf life increase significantly with direct proportion.

**Table 6** . Result of the prepared caramel shelf life

| Method | Time not flow (hr) | Shelf Life (year) |
|---|---|---|
| 1 | 30 | 1.5 |
| 2 | 32 | 1.6 |
| 3 | 36 | 1.8 |
| 4 | 30 | 1.5 |
| 5 | 34 | 1.7 |
| 6 | 38 | 1.9 |

### 3.3.3. Ash Determination

About 0.12 gram, 0.10 gram, 0.048 gram, 0.056 gram, 0.050 gram and 0.04 gram of ash were obtained after cooling in the desiccators, which are equivalent to 0.50%, 0.15%, 0.12%, 0.14%, 0.11% and 0.10% of ash content. The permissible limit of ash content of caramel was less or equal to 0.3% [12]. In this study, the prepared caramel percent ash content result was below the international standard which indicates good quality of caramel.

**Table 7**.Percent of Ash content of prepared caramel

| Method | mass of crucible (g) | mass of caramel (g) | mass of crucible & Caramel before heating (g) | mass of crucible & Caramel after heating (g) | % of Ash |
|---|---|---|---|---|---|
| 1 | 50.0 | 40 | 90.0 | 50.120 | 0.30 |
| 2 | 50.2 | 40 | 90.2 | 50.100 | 0.25 |
| 3 | 50.1 | 40 | 90.1 | 50.148 | 0.12 |
| 4 | 50.0 | 40 | 90.0 | 50.056 | 0.14 |
| 5 | 50.3 | 40 | 90.3 | 50.350 | 0.13 |
| 6 | 50.1 | 40 | 90.1 | 50.140 | 0.10 |

### 3.3.5. Percent nitrogen determination

In back titration, ammonia gas was reacted with 25 mL of 0.05 M of sulphuric acid acid .The result of consumed 0.1 M of sodium hydroxide after titration of the blank was 23.5mL and for caramel sample 21.6 mL, 21.1 mL, 20.5 ml, 20 mL, 20.6 mL and 21 mL.These results 0.20%, 0.24%, 0.28%, 0.32%, 0.27% and 0.25% of nitrogen were obtained respectively (*Table* 8). All methods showed low concentration nitrogen percent. The permissible limit of nitrogen content is less than or equal to 0.6% N [12].

### 3.3.6. Acute oral toxicity

The food constituents participating in Maillard reactions represent major dietary constituents; the safety evaluation of the products formed presents special problems. It is particularly difficult to incorporate the same margins for dosage in animal experiments that can be employed in the safety evaluation of the synthesized caramel because of different weights in each rat's. From the experimental result no acute oral toxicity data are observed on the synthesized caramel in mice. The prepared caramel that is administered at single doses of up to 10,000 mg per kg of body weight did not result in any biological and behavioral changes on the five rats. The only change was average weight gained by 3.16 gram for each rat.

**Table 8.**Percent of ammonium nitrogen on synthesized of caramel

| Method | Blank 0.1N NaOH Consumption (mL) | Caramel 0.1N NaOH Consumption (mL) | Difference (mL) | % N |
|---|---|---|---|---|
| 1 | 24.5 | 21.6 | 2.9 | 0.20 |
| 2 | 24.5 | 21.1 | 3.4 | 0.24 |
| 3 | 24.5 | 20.5 | 4.0 | 0.28 |
| 4 | 24.5 | 20.0 | 4.5 | 0.32 |
| 5 | 24.5 | 20.6 | 3.9 | 0.27 |
| 6 | 24.5 | 21.0 | 3.5 | 0.25 |

**Table 9.**Acute oral toxicity result

| Rats code | Weight (g) | | Doses at different days (mg) | | | | | Remark |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | 1 | 2 | 3 | 4-6 | 7 | |
| 1 | 26.6 | 29.2 | 266 | 266 | 266 | 266 | 266 | No death |
| 2 | 22.2 | 25.4 | 222 | 222 | 222 | 222 | 222 | No death |
| 3 | 18 | 21.0 | 180 | 180 | 180 | 180 | 180 | No death |
| 4 | 18.9 | 22.5 | 187 | 187 | 187 | 187 | 187 | No death |
| 5 | 20.9 | 24.3 | 199 | 199 | 199 | 199 | 199 | No death |

**Table 10**. Physicochemical test result of prepared caramel

| Parameter | unit | IS | Method 1 | Method 2 | Method 3 | Method 4 | Method 5 | Method 6 |
|---|---|---|---|---|---|---|---|---|
| pH(10%w/v) | - | 3.5-5 | 3.95 | 4.00 | 4.10 | 4.45 | 4.85 | 5.00 |
| Extract | °P | - | 80.61 | 80.48 | 75.58 | 76.81 | 70.66 | 68.44 |
| Haziness | EBC | ≤1 | 0.13 | 0.12 | 0.11 | 0.14 | 0.11 | 0.10 |
| Ammonical $N_2$ | % | ≤0.6 | 0.20 | 0.24 | 0.28 | 0.32 | 0.27 | 0.25 |
| Solubility in $H_2O$ | - | Soluble | soluble | soluble | soluble | soluble | soluble | soluble |
| Visual Inspection | - | Ok | Ok | Ok | Ok | Ok | Ok | Ok |
| Odor | - | BS | BS | BS | BS | BS | BS | BS |
| Ash content | % | ≤0.3 | 0.2 | 0.15 | 0.12 | 0.14 | 0.11 | 0.1 |
| Viscosity at 27 °C | cP | - | 8.35 | 8.13 | 7.03 | 7.26 | 6.91 | 6.65 |
| Shelf life | Year | ≤2 | 1.5 | 1.6 | 1.8 | 1.5 | 1.7 | 1.9 |
| 4-methylimidazol | ppm | ≤300 | | | | | | |

*Where BS: burnt sugar; ND: not detected, IS: International Standard*

## 4. Conclusion

The major parameters that affect the production and quality of the caramel were temperature, pH, contact time and type of catalyst feed.

From this study it is concluded that preparation of caramel type III color at high temperature and long contact time yield the best quality caramel with a yield of 42,000 EBC of color and lowest viscosity. In addition, the caramel type III is miscible with water with lowest extract and lowest haze. The study also confirm that the caramel has highest shelf life without biological toxicity and detectable trace metals with lowest percentage of ammonical nitrogen and lowest ash content compared with the international standard. This quality issues are very important in operational condition of brewing and the final product of the intended beer flavor.

In conclusion, the results revealed that this method used to synthesis internationally acceptable beer coloring caramel from a commercially available raw material and instrument.

## 5. References


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