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International
Journal
Of Modern Engineering Research (IJMER)

Optimal Conditionsof Enzymatic Treatment for Improvement of Total Soluble Solids Extraction and Antioxidant Capacity of Coffee Bean

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ABSTRACT:*Coffee is considered as a source of soluble solids and antioxidants traditionally consumed around the world. Study on the optimal conditions of enzymatic treatment to achieve the highest extraction of total soluble solids and antioxidants is important not only in laboratory scale, but also in pilot or industrial application, especially in the production of instant coffee. This research was designed to investigate optimal concentration of enzyme, duration and temperature of enzymatic treatment to achieve the highest extraction of those compounds from coffee. As the result, 1% of enzyme (volume of enzyme/weight of coffee sample), 50^{o}C, and 22 hours were optimal conditions of enzymatic treatment to achieve the highest extraction of soluble solids and antioxidants from coffee. The total soluble solids of coffee sample was increased from 2.43 to 4.9°Brix, which nearly doubled compared to the un-treated one, while the extraction of antioxidants significantly surged from 74.26 to 82.58% of the antioxidant capacity.*

Keywords:*antioxidants,coffee, enzymatic treatment, soluble solids.*

Date of Submission: 04-02-2019 Date of acceptance: 20-02-2019

I. INTRODUCTION

Coffee is considered as a source of soluble solids and antioxidants that traditionally consumed around the world. Soluble solids content are included sugars, acids and small amount of dissolved vitamins, proteins, pigments, phenolic compounds and minerals [1], while the antioxidant activity of coffee comes from various bioactive compounds in the bean, most well-known ones are caffeine, trigonelline, and chlorogenic acid… [2]. Those compounds are located deeply inside the bean and covered by complex structures constructed by pectin, pectic substances and cellulose[3]. The enzymatic treatment on coffee beans has a great impact on those structures. Enzymes hydrolyze pectin and pectic substances and cellulose to form tiny holes in the cell wall and leads to the weakness of the structure of the bean. This weak structure advances the liberation of contents from the beans, therefore, leads to the improvement of the extraction of soluble solids and antioxidants from coffee.

Enzyme-based extraction is the new and novel method, which draw attention of whole industry because of its friendliness to ecosystem. Enzymes are ideal catalysts to assist the process by region-selectivity, specificity, and ability to work at aqueous solutions[4].In this study, enzyme-based extraction would be applied as treatment on coffee beans for effective extraction of soluble solids and antioxidants in comparison with the conventional methods. Along with the treatment, parametersincluding concentration of enzyme, temperature and duration of treatment were investigated to enhance the quality of the product.

II. MATERIALS AND METHODS

2.1 Materials

Coffee beans whichused in this study, belongingto species of *Coffea canephora* (or normally called Robusta), was provided by Nguyen Huy Hung Coffee Company Ltd, at 427 Hung Vuong Street, Đắk Hà District, Kon Tum Province, Vietnam. Coffee beans were qualified in uniform size and shape, and were not contaminated by insects, strange objects, broken beans and moldy smell.

Chemicals: DPPH (2,2-diphenyl-1-picrylhydrazyl) was supplied by Sigma-Aldrich Corporation, Singapore.

3,5-Dinitrosalicylic acid (3,5-DNS) was bought from Himedia Company, India.
Enzyme: Viscozyme®L was purchased from Novozyme Corporation.

2.2 Methods
2.2.1 Preparation of samples
Each sample was contained 100g of coffee beans and immersed into 100ml of distilled water for three hours to ensure all the beans were well-absorbed of water.

2.2.2Investigation of the optimal concentration of enzyme in treatment for the highest extraction of total soluble solids and antioxidant capacity from coffee
Enzyme was transferred into coffee sample with the concentrationsranged from 0to 3% with the interval of 0.5%. The temperature and duration of treatment were fixed at 40°C and for 18 hours, respectively.

2.2.3Investigation ofthe optimal temperature of enzymatic treatmentfor the highest extraction of total soluble solids and antioxidant capacity from coffee
In this experiment,temperature was arranged from 20 to 60^{o}C with the interval of 10^{o}C. The concentration of enzyme was obtained from previous experiment, while duration of treatment was fixed at 18 hours.

2.2.4 Investigation of the optimal durationof enzymatic treatment for the highest extraction of total soluble solids and antioxidant capacity from coffee
This test used optimal concentration of enzyme and temperature from previous experiments. The duration was from 16 to 24 hours with the interval of 2 hours.

2.2.5 Coffee extraction
After enzymatic treatment, samples were washed with water for around 5 minutes and dried for 12 hours at 50°C until moisture content of coffee beans reached 12%. Then, the beans were roasted at medium level (240^{o}C for 14 minutes). Then, roasted coffee beanwas blended and sieved with 212 µm sieve.
For the extraction, each sample with 3g of coffee powder was stirred well in hot distilled water (80^{o}C) for 10 minutes, then cooled down for 10 minutes and filtered through the filter paper to harvest coffee extract[5].

2.2.6 Determination of total soluble solids
The soluble solids content can be measured by using refractometer and reported as "degree of Brix" (^{o}Brix) or equivalent to %. "Degree of Brix" indicates the concentration of sugars, mainly sucrose in the solution. For example, 25^{o}Brix means that there is 25 gram of sugars in 100 gram of solution [6].

2.2.7 DPPH assay
The procedure for measuringantioxidant capacity of coffee was followed [7]with little modification. DPPH reagent (150 µmol in pure ethanol) was prepared along withsamples prepared for measurement, including 3.9 mL of DPPH solution and 0.1 mL of coffee extracts, then stand for 30 minutes in the absence of light. Those samples were measured at wavelength 517 nm using a UV-VIS Spectrophotometer.Antioxidant capacity was expressed as the percentage of DPPH scavenging relative to control using the following equation:
The DPPH scavenging activity (%) = $\frac{Absorbance\ \ of\ control\ -Absorbance\ \ of\ sample}{Absorbance\ \ of\ control}.100$
2.2.8 Statistical analysis
All collected data were statistical analyzed by using Excel and SPSS program.

III. RESULTS AND DISCUSSION

1. Investigation of optimal concentration of enzymefor the highest extraction of soluble solids and antioxidants from coffee

Concentration of enzyme is one of the most important factors in enzymatic treatment [4]. The insufficient amount of enzyme cannot give the optimal result, while the excessive quantity can waste in term of both materials and financial budget. In this study, concentration was varied from 0 to 3% with the interval of 0.5%. Enzyme could work at very small amount, therefore the experimental design was divided the interval to this low concentration.

Considering the soluble solids extraction, starting at 2.43°Brix at 0% of enzyme, the total soluble solids slightly increased to 2.45°Brix at 0.5% of enzyme, rising further and reaching the peak of 2.7°Brix at 1% of enzyme. Then, it gradually dropped and hit the bottom of 2.26°Brixand at 3% of enzyme in the treatment (Figure 1). For antioxidant capacity, it increased significantly from 74.26% of sample without enzymatic treatment to 79.20% at 0.5% of enzyme. It is proved that enzymatic treatment can enhance the antioxidant

activity of coffee effectively. From that point to 2% of enzyme, there was no significant difference in term of antioxidant capacity while at 2.5 and 3%, antioxidant activity suddenly declined (Figure 2). Those figures proved that the optimal concentration of enzyme to achieve the best total soluble solids extraction was 1%, but the optimal concentration of enzyme to have the highest antioxidant capacity could be varied from 0.5 to 2%. To achieve the best result in terms of both total soluble solids extraction and antioxidant capacity. The concentration of enzyme was chosen at 1% for the up-coming experiments.

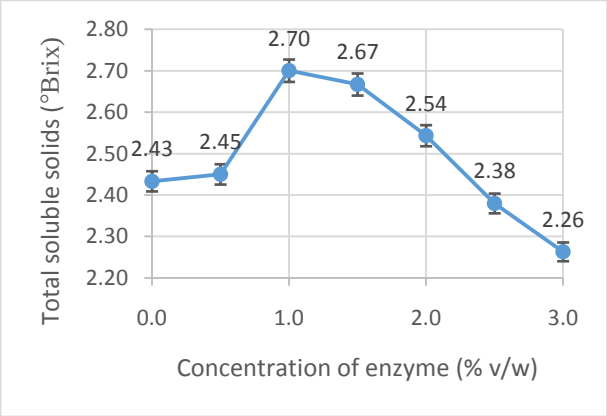

Figure1: Effect of concentration of enzymeon total soluble solids extraction of coffee.

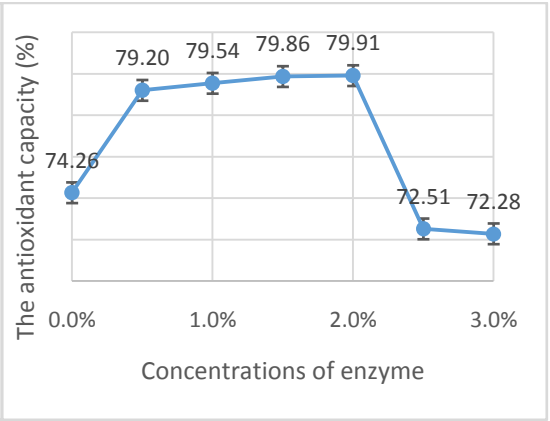

Figure2: Effect of concentration of enzymeon antioxidant capacity of coffee.

2. Investigation of optimal temperature of enzymatic treatmentfor the highest extraction of soluble solids and antioxidants from coffee

Temperature plays a crucial role in enzymatic treatment, simply because enzyme is characterized as protein and sensitive to heat[8]. If the temperature of treatment is too low, enzyme cannot be activated. On the other hand, very high temperature can cause denaturation of protein and lead to the loss of enzyme activity[9]. In large scale application, there is difficult to control temperature of treatment, especially in bulk or tank which does not have temperature controller. Therefore, a study on effect of temperature on enzymatic treatment is needed to be carried out. This experiment was designed to answer that question and to suggest an adequate temperature of enzymatic treatment which can be applied in large scale of production to obtain the best result in the extraction ofsoluble solids and antioxidants from coffee. In this study, temperature of treatment was varied from 20 to 60^{o}C with the interval of 10^{o}C. The result was shown in figure 3 and 4.

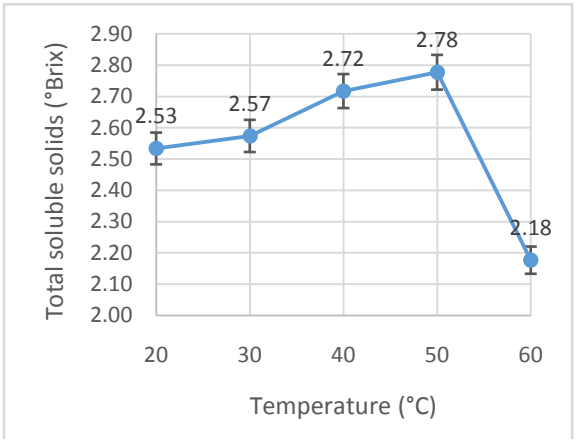

Figure3: Effect of temperature of treatment on total soluble solids extraction of coffee.

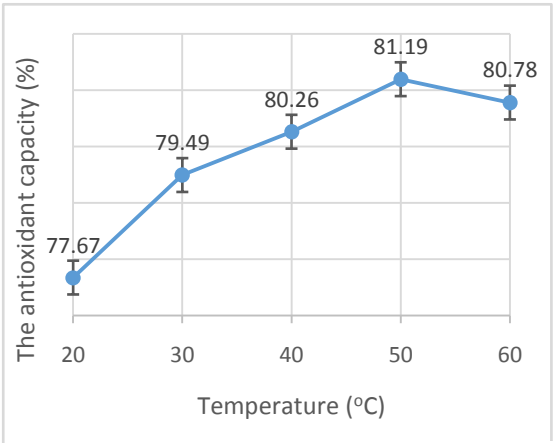

Figure4: Effect of temperature of treatment on antioxidant capacity of coffee.

At 20^{o}C, the total soluble solids was 2.53°Brix, then it steadily went up and reached the highest point of 2.78°Brix at 50°C. At the increasing temperature, the total soluble solids suddenly decreased to 2.18°Brixat 60°C. Concerning percentage of scavenging activity (or antioxidant capacity), it was 77.67% at 20°C, while achieved the peak of 81.19% at 50^{o}C. Then, it also declined to 80.78% when the temperature increased to

60°C.This data indicated the optimal temperature for extraction of both soluble solids and antioxidants was 50°C. In practical situation on farm or in industry, this temperature is naturally occurred inside the heap or tank of fermenting coffee due to the increasing of temperature inside the bulks during fermentation.

3. Investigation of optimal duration of enzymatic treatment for the highest extraction of soluble solids and antioxidants from coffee

This experiment was designed to investigate the optimal duration of enzymatic treatment on coffee bean. Enzymes are able to degrade or break down cell wall, leading to the liberation of bioactive compounds and effective extraction of antioxidants. Enzymes need necessary time to activate and work under specific conditions. If the duration was too short, enzyme did not have enough time for working. On the other hand, if the treatment was over treated, the cell wall of the beans might be under excessive impact and caused the loss of antioxidant activity[10]. Figure 5 shown the data on the extraction of soluble solids, while figure 6 pointed out the changes of antioxidant capacity of coffee samples during the time of enzymatic treatment. In figure 5, standing at 2.69°Brix at 16-hour treatment, the total soluble solids climbed dramatically and reached the top of 4.9°Brix for 22 hours of treatment, which gave a growth of more than 50% in term of the extraction. After this point, total soluble solids slipped to 3.31°Brix when the test was lasted for 24 hours. Regarding figure 6, the antioxidant capacity was also remarkably changed from 70.43% of 16-hour treatment to 82.58% of the treatment lasted for 22 hours. When the treatment continued for 24 hours, antioxidant capacity slumped to 69.04%. The results suggested that the optimal for enzymatic treatment to achieve the highest extraction of both total soluble solids and antioxidants was 22 hours. This duration was significantly shorten compared to traditional fermentation of coffee which usually lasted for around 2 to 3 days at usual conditions[11]. Moreover, the washing step in the process also played a crucial role. In this step, after undergoing enzymatic treatment, coffee bean was washed under running-tap-water for around five minutes to remove the silver skin and any remaining enzyme on the bean. However, this step could also wash away the dissolved soluble solids and antioxidant which led to the reduction of those compounds in the final product of coffee.

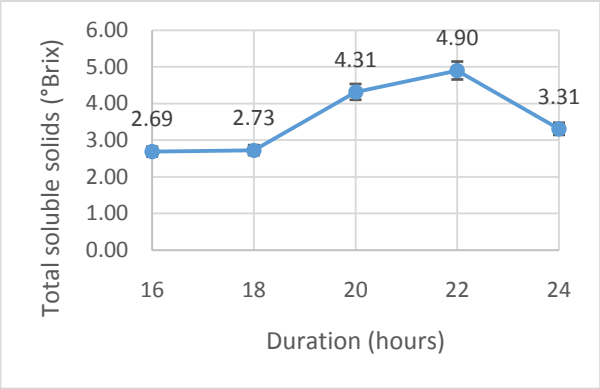

Figure5: Effect of duration of treatment on total soluble solids extraction of coffee.

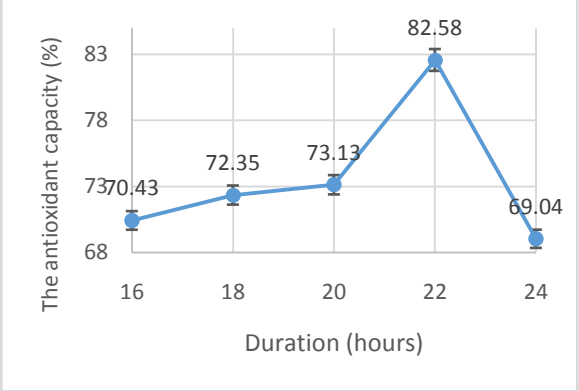

Figure6: Effect of duration of treatment on antioxidant capacity of coffee.

IV. CONCLUSION

The optimal conditions of enzymatic treatment to obtain the highest extraction of total soluble solids and antioxidantsfrom coffee was successfully investigated. Those conditions were concentration of enzyme, temperature and duration, which reported at 1%, 50°C and for 22 hours, respectively. The total soluble solids of coffee sample was increased from 2.43 to 4.9°Brix, which nearly doubled compared to the un-treated one. The antioxidant capacity rose from 74.26 to 82.58%, which also proved that the enzymatic treatment can enhance the extraction of both soluble solids and antioxidants from coffee. Those results can be used for further studies or applied in large scale, such as pilot or industrial production.

ACKNOWLEDGEMENTS

I would like to express my faithful thanks to Food Technology Department of International University which provided valuable supports in both facilities and spiritual perspectives during the accomplishment of this project.

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Phu H. Le" Optimal Conditionsof Enzymatic Treatment for Improvement of Total Soluble Solids Extraction and Antioxidant Capacity of Coffee Bean " International Journal of Modern Engineering Research (IJMER), vol. 09, no. 1, 2019, pp 17-21