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The Effect of External Conditions (Soaking Duration, Heat and Enzymatic Treatment) On Physiological and Biochemical Characteristics of Coffee Bean during Germination

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ABSTRACT: *The purpose of this study was to identify the effect of external conditions including soaking time, soaking temperature and Viscozyme® L treatment on germination process as well as the biochemical factors of coffee beans (Coffea robusta L. Linden). Besides that, the research was to investigate the optimal condition for coffee germination in order to develop a new product, called germinated coffee. Lipase activity, α-amylase inhibitory activity, antioxidant capacity and soluble solids content were measured by titrimetric, DNS, DPPH assay and refractometer, respectively. In 24 hours of soaking time and at 60°C of water temperature, the result illustrated the maximum value of germination rate, lipase activity, antioxidant capacity and soluble solids content while α-amylase inhibitory activity value was the lowest (19.95 ± 0.08% and 19.95 ± 0.07%, respectively) in the same conditions. Viscozyme® L application from 0.5 to 2% (v/w) performed bad result on germination rate (only control sample could reach state 3 of germination) and other biochemical characteristics, except α-amylase inhibitory activity (maximum 76.57% with 2% of enzyme treatment).*

Keywords: *α-amylase, antioxidant, germination, lipase, robusta, soaking temperature, soaking time, soluble solids content, Viscozyme® L.*

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I. INTRODUCTION

A huge problem in coffee industry is the prize of high-quality products because they came from digestive system of animals and had limited amount of product per year. As a result, a new kind of coffee should be developed with better quality, cheaper and higher rate of production. Based on previous studies, during the germination process, enzymes and chemical compositions of coffee were changed. In other words, the composition of low molecular weight carbohydrates and amino acids are different in different state of green coffees [1]. Moreover, the essential precursors of coffee flavors are amino acids and free sugars which could lead to cupping quality. Thus, the changing composition and these flavor differences of coffee must be the consequences of metabolic processes in the coffee bean during post-harvest processing and that related to germination [2]. Based on that theory, developing a new product called germinated coffee is essential and realizable.

The optimal conditions for coffee germination need to be carried out first for the success of this project. Indeed, coffee germination depends largely on external conditions such as water, temperature and enzyme treatment. Coffee seed is extremely dry and need to take in significant amounts of water, relative to the

dry weight of the seed, before cellular metabolism and growth are re-activated [3]. Besides, seeds often have a particular temperature range within which they will germinate, any temperature ranges fall outside this will not germinate. Furthermore, enzyme treatment is one of the essential laboratory factors for coffee germination. Viscozyme® L which is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase and xylanase, will be treated on green coffee bean in order to break down cellulose and hemicelluloses in the cell wall of the bean [4].

To evaluate the effects of the above external conditions on germination as well as coffee quality after this process, many parameters should be concerned such as the morphology of coffee seeds and several biochemical values. It is clear that the role of lipid hydrolysis in the behavior of coffee seeds during storage has been further confirmed by the negative correlation observed between coffee seeds viability and free fatty acid content. During storage, the off-flavors of green coffee are also produced by oxidation processes acting on the lipid fraction [5]. Therefore, lipase activity was characterized in coffee seeds to determine its involvement in lipid degradation during germination. In addition, α-amylase inhibitors are one member of the anti-diabetic drug families, which have a strong advantage and are suitable for healing type-2 diabetes. It acts in the prevention of α-amylase to reduce and slow down dietary carbohydrate digestion [6]. Antioxidants, another variable that should be mentioned, are the substances able to prevent or inhibit oxidation processes in human body as well as in food products. Antioxidants may act as a free radical scavenger, reducing agent, chelator and/ or singlet oxygen scavenger [7]. Last but not least, soluble solids content (SSC), especially sugar is also an important characteristic in coffee. There has been considerable diversity of opinion regarding the sugar of coffee. Many evidences have proved that this sugar is one of a peculiar species allied to sucrose [8].

From reasoning mentioned above, this study was performed to investigate the optimal soaking duration, soaking temperature and Viscozyme® L concentration for germination rate as well as chemical and biochemical characteristics related to coffee quality.

II. MATERIALS AND METHODS

2.1 Materials

Coffee fruits (*Coffea robusta* L. Linden) were purchased from Da Lat city, Vietnam. The seeds were removed for treatment at Food Engineering Laboratory at International University – Vietnam National University – Ho Chi Minh City.

All chemicals used for analytical test such as ethanol, methanol, DPPH, DNS, olive oil, gum Arabic, phenolphthalein indicator and α-amylase powder were purchased from Thinh Phat Company, Ha Noi Capital, Vietnam.

Other chemicals such as phosphate buffer, soluble starch, sodium hydroxide and distilled water were provided by Food Engineering Laboratory at International University – Vietnam National University – Ho Chi Minh City.

Viscozyme® L was supported by Assoc. Prof. Le Hong Phu - Department of Food Technology, International University – Vietnam National University – Ho Chi Minh City.

2.2 Methods

2.2.1 Sample preparation for survey of the effect of soaking duration

Each sample was collected with the same quantity (10g of coffee beans) which was immersed into 400mL tap water for 12, 24, 36 and 48 hours at 60^{o}C in different thermal cups [9]. After each 3 hours of soaking, water was replaced for temperature guarantee. Control sample was prepared by 0 hour of soaking duration. After each duration of soaking, 1g of each sample was taken out for moisture content measurement by moisture analyzer. Then, the remainders were labeled and keep at room temperature in the dark with watering three times per day for three weeks of germination.

2.2.2 Sample preparation for survey of the effect of soaking temperature

Each sample was collected at the same quality (10g of seeds) and soaked in different thermal cups which contained 400mL tap water at 40, 50, 60 and 70^{o}C and kept for 24 hours [10]. Control sample was prepared without soaking. After soaking, 1g of each sample was taken out for moisture content measurement by moisture analyzer. The rest 9g of these samples were kept at room temperature in the dark with watering three times a day for three weeks to germinate.

2.2.3 Samples for survey of the effect of Viscozyme® L concentration

After soaking each sample of 10g coffee seeds in 400mL tap water at 60^{o}C for 24 hours in thermal cups, Viscozyme® L was added with the ratio from 0 to 2%, with the interval of 0.5% (v/w) for the next 24 hours

[11]. These samples were kept for three weeks at room temperature in the dark with watering three times a day to germinate. Control sample was prepared without enzyme application.

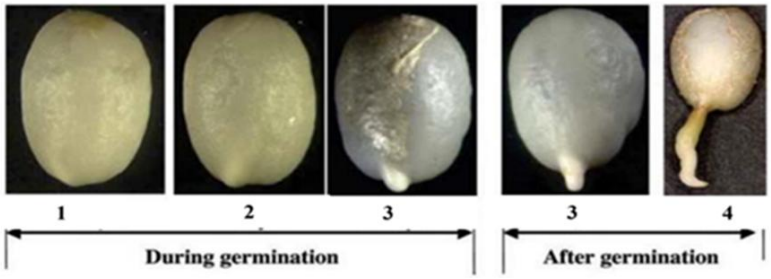
2.2.4 Extraction method

Germinated seeds were collected, dried in a dryer at 40^{o}C until they reached 12% of moisture content and finely ground by a grinner. The well ground sample (500 µm) was stored in a nylon-linear package which was then preserved in desiccators room temperature until being used for the next step. Then, 1g of well ground powder was weighed for 250mL Erlenmeyer flasks. Samples were extracted with 50mL distilled water for the determination of lipase activity and amylase inhibition activity. And 1g sample was extracted with 50mL of pure ethanol (99.7%) for determined soluble solids content and antioxidants capacity. The mixtures were then mixed in a shaking incubator under the certain temperature and rotation (150rpm) for 90 minutes. After the extraction process, samples were centrifuged at 10000 rpm. The extracts were stored at 4^{o}C in refrigerator for further analysis [12].

2.2.5 Determination of germination rate based on morphological observation

Samples after collected were measured by counting the number of germinated seeds. The standard of germination rate based on the morphology of seed in Figure 1. Analysis tool was used to determine the percentage of germination ability of coffee beans. The terms "state 1", "state 2", "state 3" and "state 4" were used to describe the germination state of coffee seeds according to Figure 1.

Figure 1: Coffee seed germination and subsequent radicle growth [13]



2.2.6 Determination of lipase activity

Lipase activity was measured by the titrimetric method based on the titrimetric determination of free fatty acids released from triacylglycerols by lipase catalysis [14]. The amount of fatty acid released during the reaction was determined by direct titration with NaOH to a phenolphthalein end point. The titration cocktail with 10mL of 95% (v/v) ethanol and 3 drops of 1% (w/v) phenolphthalein indicator was used to quench the reactivity of subsamples of the reaction mixture. The substrate was olive oil in a gum Arabic stabilized emulsion. It has been demonstrated that the increased oil/water interface leads to an increase in the enzymatic hydrolysis rate. At five suitable reaction intervals (e.g., 5, 10, 15, 20, and 25 minutes), removed 5mL of substrate to Erlenmeyer flask that contained enzyme solution and titration cocktail and swirled. Titrate the contents of each flask with 0.05N NaOH with burette until a light blue color appeared. The quantity of fatty acid liberated in each subsample based on the equivalent amount of NaOH used to reach the titration end point and was calculated by the formula below:

$$\textbf{µ fatty acid/mL sample} = \frac{(Vs - Vb) \times N \times 1000}{5\ mL}$$

In which:

Vs is the volume of 0.005N NaOH solution used for titration of the sample.

Vb is the volume of 0.05N NaOH solution used for titration of the blank.

N is the normality of the NaOH titrant used (0.05N).

A reaction curve was created by plotting the quantity of fatty acid liberated over time. From there, lipase activity was calculated by the following equation:

$$V_0 = \frac{y2 - y1}{x2 - x1}\ \textbf{mol/ (mL x min).}$$

2.2.7 Determination of α-amylase inhibitory activity

This assay was carried out based on the spectrophotometric method in triplicates [15]. The mixed of 0.5mL of extracts and 0.5mL of 20mm phosphate buffer pH = 6.9 containing 0.5mg/mL α-amylase was incubated at

25°C for 10 minutes. 0.5mL of 1% soluble starch solution prepared in 20mM phosphate buffer at pH = 6.9 was added into the mixture and then incubated at 25°C for 10 minutes. At the end of this process, in order to stop the reaction, 1mL DNS (3, 5- dinitro salicylic acid) was added into the mixture which was then incubated in water bath at 90°C for 10 minutes. After cooling down the mixture in room temperature, 10mL of distilled water was added. The blank sample was prepared by replacing 0.5mL extract with 0.5mL of phosphate buffer. After that, samples were moved on the next steps with adding starch solution and DNS. The absorbance was measured at 540nm. The percentage of α-amylase inhibition activity of the extract was calculated by the equation:

$$\textbf{\% α-amylase inhibition} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{Absorbance of control}} \times \mathbf{100}$$

2.2.8 Determination of antioxidant capacity

2mL of ethanolic DPPH 0.15mM is firstly mixed with 2 mL sample extracts and with 2mL 95% (v/v) ethanol as control. The mixed and control were vortexed for 1 minutes and kept in the darkness at room temperature for exactly 30 minutes. The absorbance of the mixture was then recorded at 517nm [16]. This analysis was determined the activity of antioxidant containing in each sample of coffee. Moreover, the method was not used standard solution because this test was compared the changing of antioxidant of sample coffee. The DPPH capacity was measured using the equation:

$$\textbf{\%DPPH radical} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{Absorbance of control}} \times \mathbf{100}$$

2.2.9 Determination of soluble solids content (SSC)

Refractometer was used to measure coffee extract. Calibrating with a drop of distilled water and drying with a soft tissue helped to collect the exact amount of solution.

2.2.10 Statistical analysis

The SPSS and Excel statistical program with One-way ANOVA method and Duncan standard ($\alpha \leq 0.05$) were used to analyze means, standard errors of triplications and significance of samples.

III. RESULTS AND DISCUSSION

3.1 Effect of soaking duration and temperature on moisture content

The effects of soaking duration and temperature on moisture content of coffee seeds are illustrated in Figure 2. Moisture content (%) increased gradually which was proportional to the expansion of soaking duration (h). In the first 12 hours, moisture content of seed immersed in water rose rapidly from 10.98 ± 0.53% (control sample) to 49.32 ± 1.74% (12-hour sample). Water absorption process become more stable and slower over the next 36 hours. More remarkable, from 24 to 36 hours of soaking duration, the difference in moisture content between these samples was observed hardly from the graph. Moisture content reached the peak after 2 days, in other words, absorption was still ongoing within the range studied.

The result of moisture content related to the change of water temperature during soaking process was slightly fluctuate. It was predictable that after 24 hours of soaking at 40°C, this value went up significantly. Temperature improvement from 40 to 60°C slightly increased water absorption process of coffee seeds but this trend was no longer accurate when the temperature reached 70°C. At this point, moisture content of beans noticeably declined due to several reasons, including thermal inhibition of absorption and thermal damage of seeds [17]. Although in some cases with the interruption of temperature, the seed germinative capacity rose with increasing moisture content, the direct relationship between them is still unclear [18].

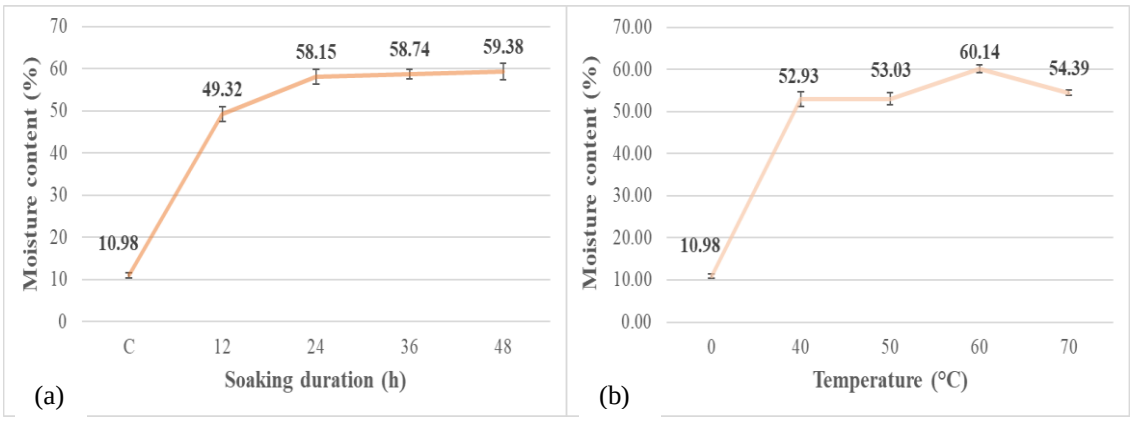


Figure 2: Moisture content (%) of coffee beans over (a) different soaking durations and (b) different soaking temperatures

3.2 Determination of germination rate based on morphological observation

The results below based on morphological observation of coffee seeds following Figure 1. Obviously, soaking duration had significant effect on the germination rate of coffee beans. In comparison to control sample, all samples which had been soaked in water for 12 hours or more could reach at least state 2. According to the percentage of beans that reached the third state, highest performance of germination was recorded from seeds soaked for 24 hours. Sample soaked in 12 hours gave the second-best number of third stated seeds, which was much better than the other two models. Generally, the germination rate peaked after 24 hours of soaking and then slightly descended. In fact, hydration starts initially as a consequence of matric forces of cell walls and cell contents of the seed at imbibition phase [19]. Such initial uptake might be delayed or prevented by hard seed coats. Thus, water uptake by intact seeds was delayed until 24 hours of soaking duration at 60°C. Furthermore, the harmful effect of excessive soaking on coffee seeds (more than 24 hours) could be a reason of the decrease of germination rate. In other words, an optimal level of moisture should be applied rather than the fall saturation to activate cell division, differentiation and multiplication process of embryo [20].

Soaking temperature was also an effective factor for coffee germination rate. After 24 hours of soaking in hot water, all samples could reach at least state 2. The percentage of beans performed state 3 went up gradually while the water temperature increased from 40^{o}C to 60^{o}C and declined substantially at 70^{o}C. In particular, the temperature of 70^{o}C might have caused inhibition to the synthesis of gibberellin (GA) and stimulation to abscisic acid (ABA) synthesis process, which were lower the germination percentage [21]. Besides that, between 40^{o}C to 50^{o}C was also not the optimal condition for the changes of metabolic processes of coffee seeds, thus 60^{o}C was the optimal temperature for germination within the studied range.

Different from the two factors above, Viscozyme® L concentrations from 0 (control sample) to 2.0 (% v/w) performed negative effect on germination process, in particular, no sample could reach state 3 except the control. According to Figure 3, increasing enzyme ratio reduced the amount of beans that attained state 2. At high Viscozyme® L concentration, it is clear that there might have many active sites to compete the substrate and enzyme related to germination process. Moreover, the hydrolysis of cellulose and pectin was strong due to the present of a huge amount of cellulose and pectinase in Viscozyme® L may damage the cells of coffee beans.

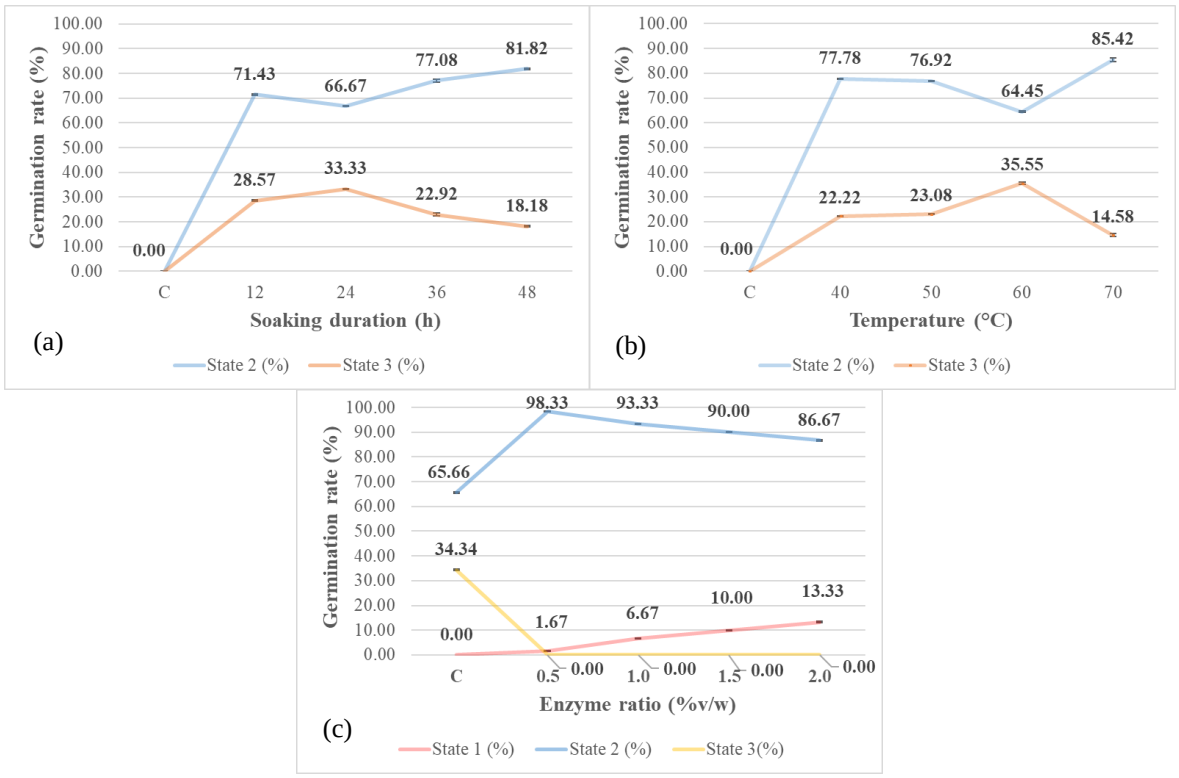


Figure 3: Germination rate of coffee beans over different (a) soaking durations; (b) soaking temperatures and (c) Viscozyme® L concentrations

3.3 Determination of lipase activity

Lipase activity in coffee seeds evaluated by titrimetric method. In such seeds contain a high amount of lipid, the lipolytic activities could play a regulative role during the first steps of germination and lipase was responsible for the catalysis of these reactions [22].

Three factors which applied in this study: Soaking time, soaking temperature and enzymatic treatment had significant effect on lipase activity, which related to the germination process. In particular, after reaching the peak at 24h, 60°C and 0% (control sample) in Figure 4, all charts tent to decrease slightly. In the comparison to those graphs in Figure 3, these changes of lipase activity had the same tendency to germination rate. In other words, this result agreed with the idea that there was a strong relationship between lipase activity and germination rate.

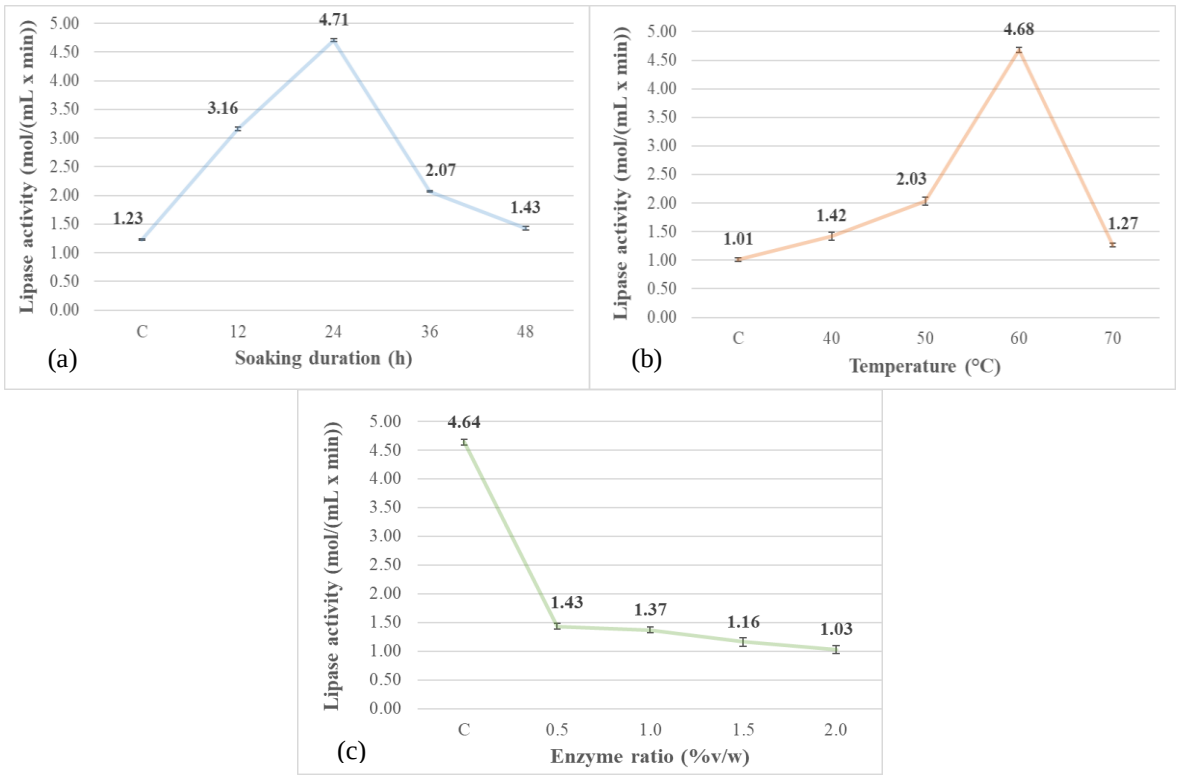


Figure 4: Lipase activity of coffee beans over different (a) soaking durations; (b) soaking temperatures and (c) Viscozyme® L concentrations

3.4 Determination of α-amylase inhibitory activity

Figure 5 below illustrates that soaking duration significantly affected α-amylase inhibitory activity. As can be seen, control sample had the highest value (73.59 ± 0.13%), then, the graph gradually decreased in the order of samples that soaked in 48h, 36h, 12h and 24h, respectively. Considering the effect of soaking temperature and Viscozyme® L, the change of α-amylase inhibitory activity went against the trend of germination rate in comparison to Figure 3.

The direct obstruction of α-amylase inhibitors on α-amylase activity might be the key reason for these results. During seed germination, α-amylase plays an important role in hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots [23]. Obviously, α-amylase inhibitory activity is an important factor for anti-diabetes in coffee beans but its development has negative effect on the germination process.

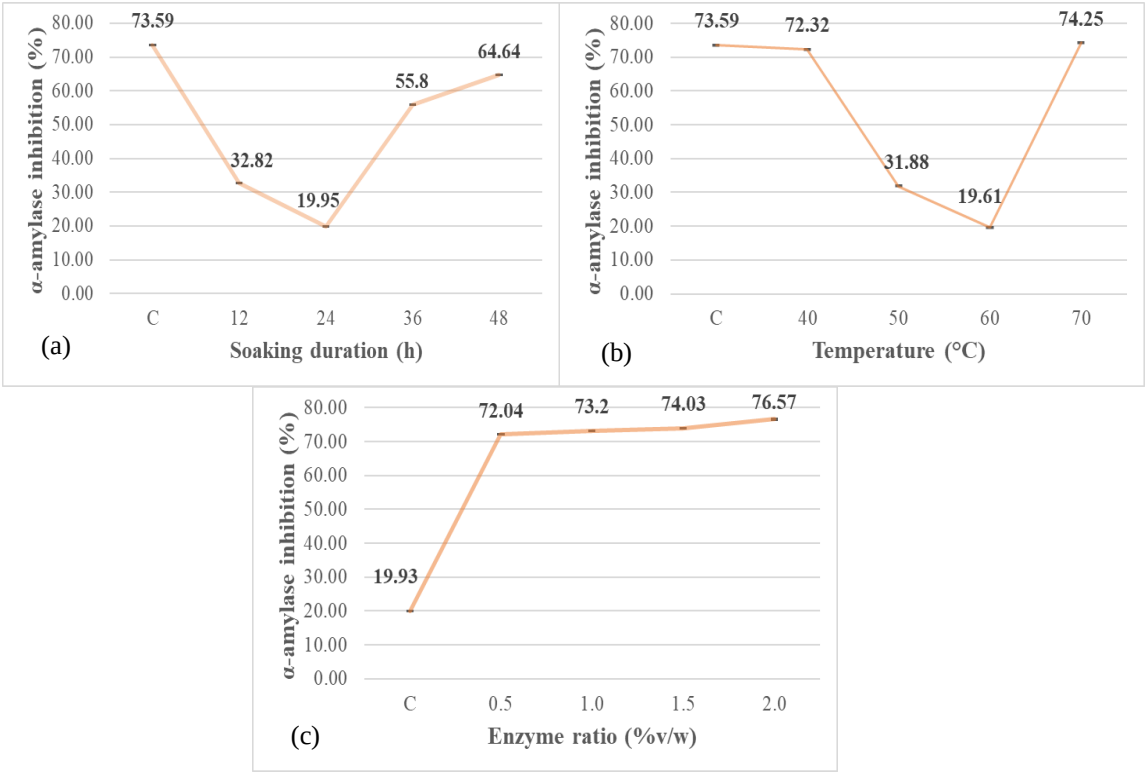


Figure 5: α-amylase inhibitory activity of coffee beans over different (a) soaking durations; (b) soaking temperatures and (c) Viscozyme® L concentrations

3.5 Determination of antioxidant capacity

Antioxidant activity evaluation of germinated coffee extract was based on their ability of scavenging DPPH radical capacity. According to Figure 6, concerning the effect of soaking duration and soaking temperature on antioxidant activity, 24 hours and 60°C were the best conditions, respectively. Besides, the highest result from control sample proved that enzymatic treatment performed negative effect on DPPH radical scavenging capacity. In other words, the variation of three graphs in Figure 6 had the same trend as those in Figure 3, which illustrate the influence of factors studied on germination rate. In fact, previous research has shown that the antioxidant activity increases in beans and peas during germination. In coffee, antioxidant is highly concentrated and it has been linked to a huge amount of health benefits, including protection against heart disease and cancer [24].

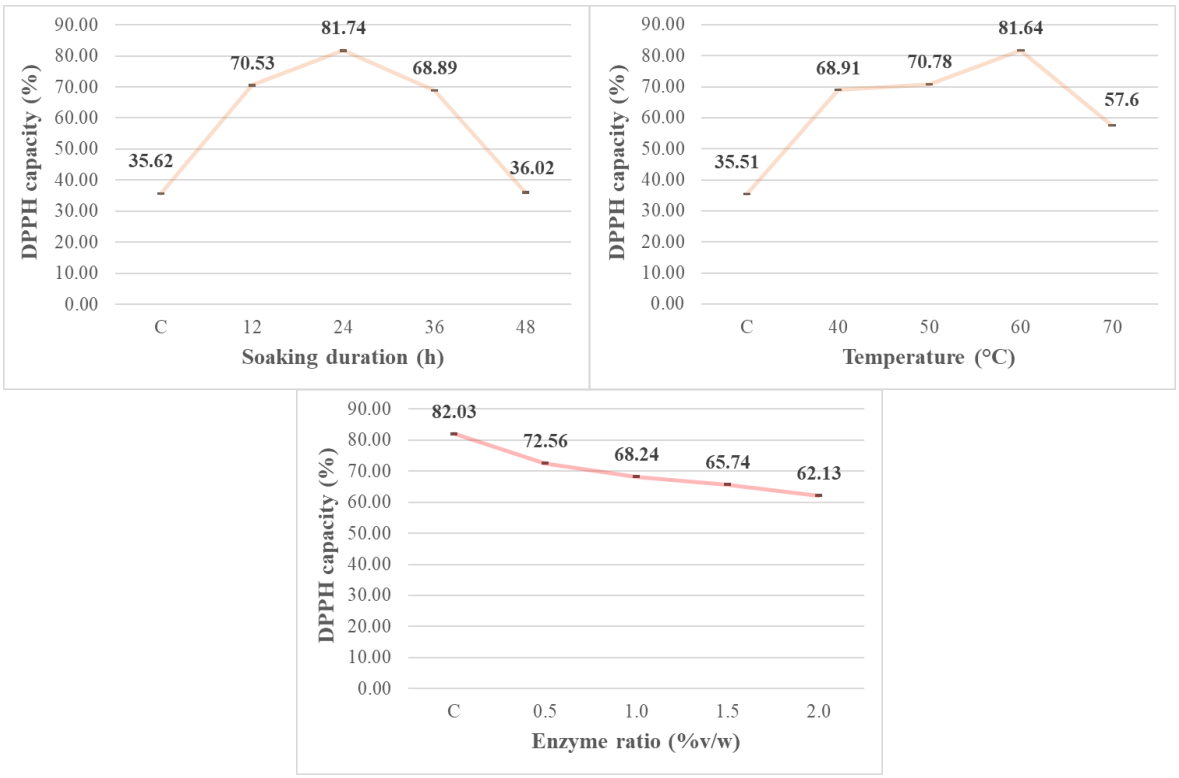


Figure 6: Antioxidant capacity of coffee beans over different (a) soaking durations; (b) soaking temperatures and (c) Viscozyme® L concentrations

3.6. Determination of soluble solids content (SSC)

Sucrose, fructose and glucose have the same values in refractometer. Thus, the result of SSC represented the amount of sugar in germinated coffee seeds. Sugar related to the sweetness and aroma of coffee has a significant relationship to the demand of many countries as well as the storage stability.

At an overall view from (a) and (b) of Figure 7, SSC extraction of coffee seeds increased slightly but declined after reached the highest value at a certain condition of soaking time (24h) and soaking temperature (60^oC). In the other case, soluble solids content decreased gradually with the increasing of enzyme concentration. Once again, the effect of studied factors on SSC was closely related to their effect on germination rate as could be observed in Figure 3.

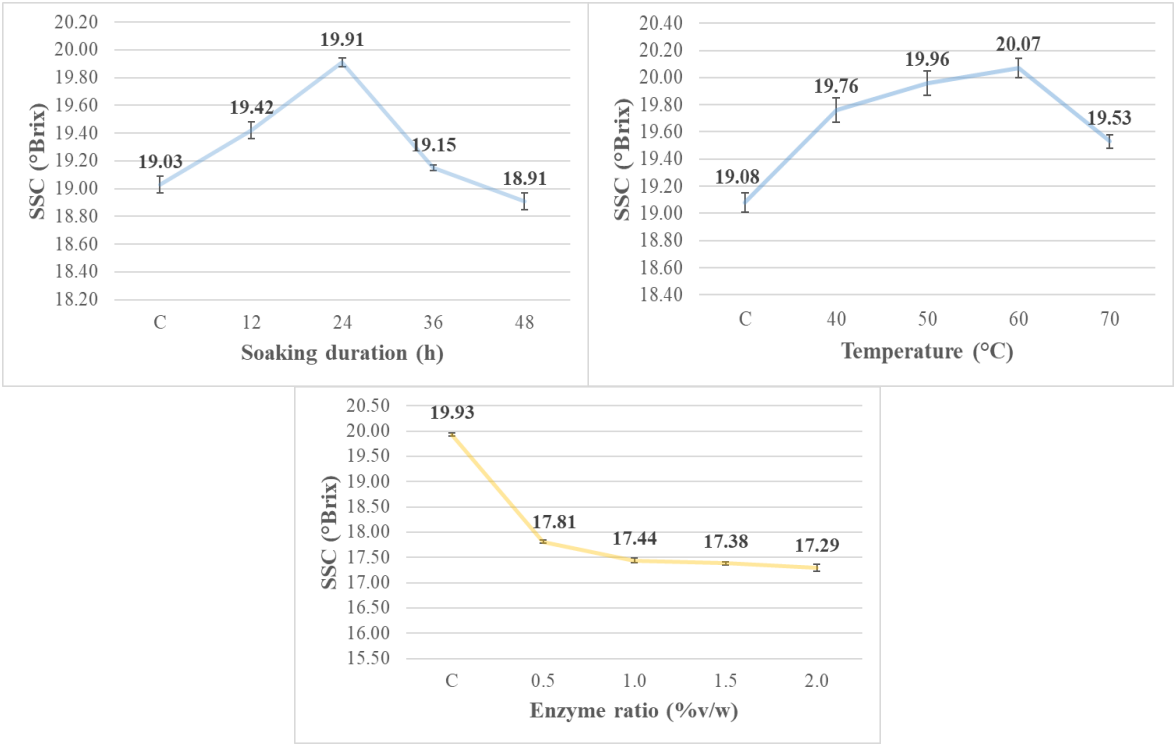


Figure 7: Soluble solids content (SSC) of coffee beans over different (a) soaking duration; (b) soaking temperatures and (c) Viscozyme® L concentrations.

IV. CONCLUSION

This study was carried out to find the best external condition of soaking duration, soaking temperature and Viscozyme® L concentration for coffee germination as well as chemical and biochemical characteristics related to cupping quality. The highest results of germination rate, lipase activity, antioxidant capacity and SSC were observed after green coffee beans had soaked for 24 hours in water at 60°C. On the contrary, α-amylase inhibitory activity of treated samples in these conditions had the lowest value. Besides, the application of enzyme performed negative effect on most of the characteristics studied, except α-amylase inhibitory activity. Therefore, 24 hours of soaking duration in 60°C should be selected as the optimal condition in the germination step of producing germinated coffee, while a further study on enzymatic factor need to be performed.

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