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Research on the Production of Black Garlic Juice

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ABSTRACT*:* The aim of this research is contribute to diversification products from black garlic, create a new product line up which is high quality, usability and the price is affordable for daily using. The results showed that fresh garlic Phan Rang after 38 days of aging period at 60-70^oC, the content of reducing sugar increased 22-fold to 42.1 g/100g, the pH decreased to 3.9 and the moisture decreased to 34.74%, polyphenol increased 7-fold and reached 1.82%, SAC content increased 46-fold to 159.81 mg/kg, DPPH-antioxidant activity increased 22-fold and IC_{50} values were 3262 µg/ml. The study provided the suitable condition for the hydrolysis of black garlic at a pectinase enzyme concentration 0.2% (w/v), hydrolysis temperature was 45^oC, pH 3.5 and hydrolysis period was 120 minutes, reducing sugar content was 43.12 g/100g, polyphenol was 2.19%, IC_{50} value was 2440 µg/ml. At the same time, the experiment to produce black garlic juice with dilution ratio of black garlic extract and sweet grass herb was 4:4 g/g in 200 ml, the highest sensory score, characteristic color, harmonious taste. The product has high biological activity with polyphenol was 3.05% and IC_{50} value was 1730 µg/ml, SAC content was less than MLOQ = 10.

KEYWORDS: antioxidant, black garlic juice, pectinase enzyme, SAC content, the aging period. Date of Submission: 03-07-2018 Date of acceptance:

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I. INTRODUCTION

Black garlic was originated in Japan and has been on the market for over 10 years. Black garlic has a lot of advantages with a light sweet and light sour of fruit, no smell of spicy like fresh garlic, hypoallergenic and can be used daily for a long time without any side effect. On the other hand, black garlic has clinical evidences show that it has a lot of benefit to the health such as antioxidant activity, anti-obesity activity, free radicals scavenging, reduce cholesterol level, liver protection, inhibit the growth of cancer cell lines. Despite of having a lot of benefits, the consuming of black garlic has not been very popular due to 2 main reasons. Firstly, the black garlic price is not affordable for every objects. Secondly, product line-ups originated from black garlic have not diversified yet. At the present, they only include peel black garlic; black garlic wine; soft capsule with ginseng or Ophiocordyceps militaris. They require cold storation for the best effect or not suitable for some subjects such as pregnant, people who is on diet or diabetic. So that, the aim of this research is contribute to diversification products from black garlic, create a new product line up which is high quality, usability and the price is affordable for daily using.

II. MATERIALS AND METHODS

1.1. Materials and reagents

Fresh garlic Phan Rang,enzyme pectinase Ultra SPL (9500 PGU/mL)were supplied by Novozymes,dry sweet grass herb.

DNS reagent, Folin–Ciocalteu reagent (Merck), gallic acid (Merck), DPPH (2.2-Diphenyl-1-picrylhydrazyl) Sigma, methanol, ascorbic acid (Sigma) and the other reagents were of analytical grade.

1.2. Methods

Moisture content: was determined by measuring the weight loss using a moisture analyzer (105^oC, MX-50, A&D Company, Japan).

pH: 1 g of heated garlic samples was blended in 100 mL distilled water, the pH of heated garlic sample was then measured using a pH meter.

Reducing sugar content: was determined according described by Miller (1959). This method is based on the reaction of reducing sugar with 3.5-dinitrosalicylic reagent (DNS). The color intensity of the reaction mixture was proportional to the reduction sugar concentration. Based on the standard graph of pure glucose with dinitrosalicylic acid reagent, the reduction sugar content of the sample was calculated [2].

Total polyphenols content (% dry weight):was determined according described by Wolfe et al, 2003 and Xiaoming Lu et al, 2016, with minor modifications. In brief, an aliquot of diluted black garlic (0.5 mL) was mixed with Folin-Ciocalteu reagent (2.5 mL, 10%), the reaction was then allowed to proceed for 3 min, followed by the addition of Na_2CO_3 solution (2 mL, 7.5%). Subsequently, the mixtures were incubated in the dark for 30 minutes. After incubation, the absorbance was recorded at 765 nm. Gallic acid was used as a standard for the calibration curve [6, 12].

Antioxidant capacity: DPPH radical scavenging activity was determined according to Zesheng Zhang et al (2014) and Brand-William et al (1995), with slight modification. Briefly, 2 mL of 0.2 mM DPPH radical solution prepared in methanol was mixed with 1 mL of the test sample, the mixtures were then shaken up vigorously for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm. DPPH solution without garlic samples (instead of distilled water) was used as control sample. Ascorbic acid was used as a standard. The results reported by the IC_{50} value are the concentration of the eluant extracted at 50% DPPH radical under conditions of determination. The lower the IC_{50}, the higher the DPPH radical elimination [13, 11].

Determination ofS-allyl-cystein (SAC) by high performance liquid chromatography (HPLC):the sample was sent to Institute of Hygience and Public Health, district 8, Ho Chi Minh city.

Sensory evaluation: evaluated consumer acceptance testing.

Statistical analysis: the experiment was repeated three times. The means and standard deviations were also caculated and plotted using Microsoft Excel and Statistics 20 software. The data were analyzed by one-way ANOVA with LSD was used to determine significant differences ($p < 0.05$) between mean values

II. RESULTS AND DISCUSSION

2.1. Changes in during the aging period

2.1.1. Changes in the color of black garlic during the aging period

Black garlic, produced by aging whole bulbs of garlic for 42 days at 60-70°C in 80-90% relative humidity. Changes in the color of black garlic during the aging period are presented as Fig. 1.

0 7 14 21 28 35ngà 38 42

Fig. 1. Changes in the color of black garlic during the aging period

After 7 days of aging period, the white color in garlic samples decreased gradually, while the brown color increased gradually. The inside colour of the garlic sample showed an uneven distribution of white flecks. From 14 to 28 days, the colour development of the garlic sample was from pale brown to dark brown. The inside of garlic sample had some white flecks. From 35 to 38 days, the colour of the garlic sample was from dark brown to black, the texture of black garlic products become sticky and jelly like, the taste is more sweet and sour compared with fresh garlic. After 42 days, aged black garlic had a fruity taste. During the black garlic processing, development of the black colour is usually connected with the non-enzymatic browning reaction and greatly depends on the heating temperature. There were melanoidins forming in Maillard reaction. The colour of melanoidins might be connected with the enolisation of sugars and racemisation of amino acids.

2.1.2. Physicochemical properties of black garlic

The physicochemical characteristics of black garlic during the aging period are presented in Fig. 2 and Fig. 3.

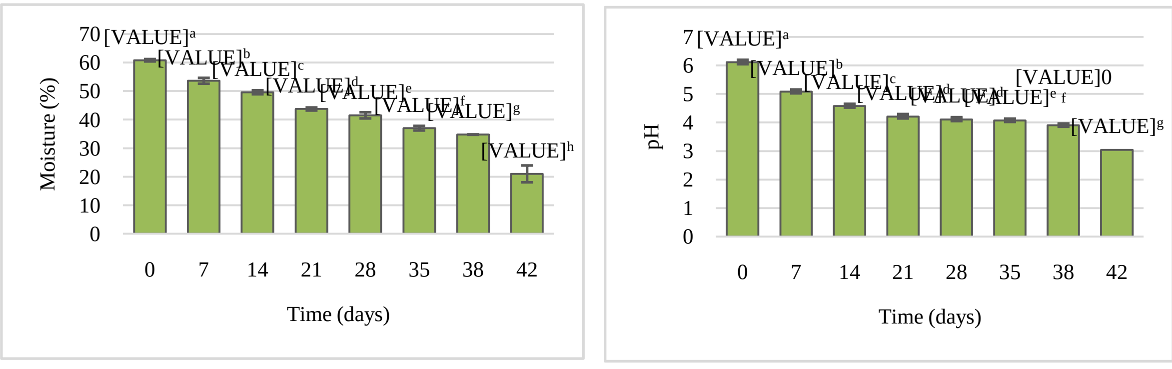

Fig. 2. Changes in moisture and pH of black garlic during the aging period

The moisture and pH value decreased as a function of processing time. The moisture content of fresh garlic was 60.77 g/100g. During the black garlic manufacturing process, the moisture content decreased continuously over time. The moisture content of the heated garlic samples decreased to 20.99 g/100g over a period of 42 days. The decreased rate of moisture content was faster at longer time. The moisture and time required for producing black garlic in the present study might have created a situation in which the moisture of the heated garlic sample reached a state of equilibrium with the moisture in the chamber in which the black garlic was produced. This moisture condition is thought to facilitate the browning reaction in heated garlic samples. The browning reaction is also affected by the moisture content of the garlic sample [3]. The water content appeared to affect the activation energy of non-enzymatic.

A significant decrease ($p < 0.05$) in pH was also observed with the progression of the heat treatment process. The pH of fresh garlic was 6.11. Thereafter, the pH decreased gradually with time, reaching 3.04 after 42 days. The pH decrease in the heated garlic sample was, in part, associated with the production of browning materials upon heat treatment during the black garlic manufacturing process. The formation of carboxylic acids (which are produced by the oxidation of the aldehyde group in aldohexose, acidic compounds and decreases in basic amino acids by combining with sugar) has been reported to be responsible for the decrease in pH in the browning reactions [5,7]. Besides, the decrease of pH value not only contributes to acidic preservative action of black garlic but also produces a sour taste mouthfeel.

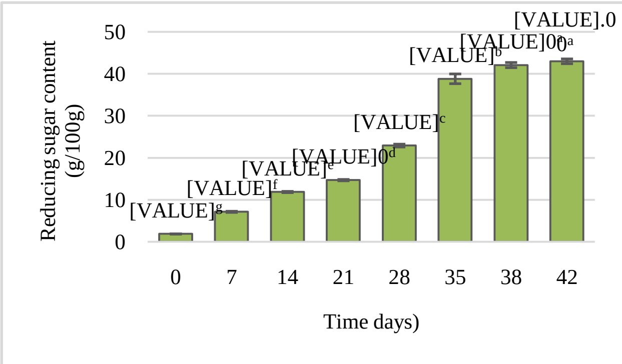

Fig. 3. Changes in reducing sugar content of black garlic during the aging period

During the black garlic processing, the reducing sugar content in black garlic depended on two factors. On the one hand, polysaccharide in garlic was degraded to reducing sugar. On the other hand, reducing sugar was consumed during the Maillard reaction. The reducing sugar content showed a rising trend during the whole process, indicating that under these temperatures the rate of formation of reducing sugar was faster than its rate of consumption. After 42 days, black garlic did not have the appropriate sweet flavour because of the large amount consumption of reducing sugar.

2.1.3. Changes in polyphenol content and DPPH radical scavenging activity

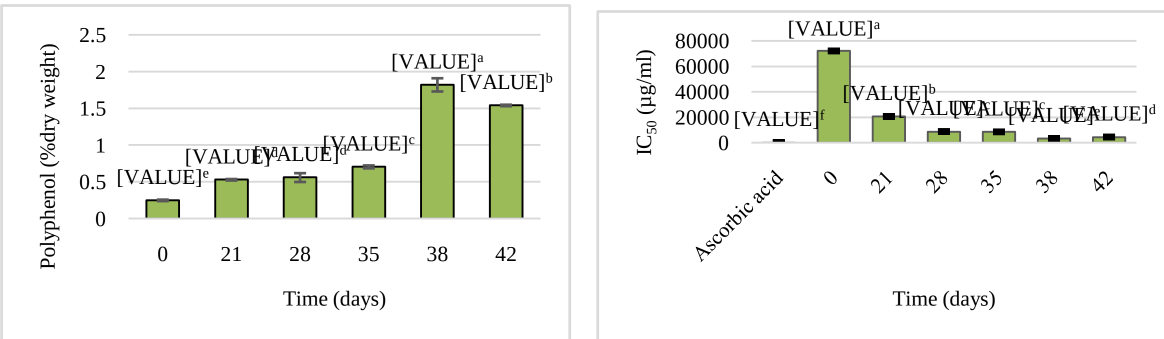

Fig. 4. Changes in polyphenol and DPPH radical scavenging activity of black garlic during the aging period

The increase of polyphenol improves the total antioxidant capacity of black garlic. Changes in polyphenol of garlic sample were shown in Fig. 4. The total polyphenol (0.53–1.82%dry weight) of black garlic were not only significantly higher than those of fresh garlic (0.25%dry weight) but also increased significantly until the 38th day of aging, before decreasing during the rest of the aging period ($p < 0.05$). According to Kim et al., hydroxycinnamic acid derivatives and other phenolic acid contents are increased over 5-fold in black garlic compared to fresh garlic. These increases of phenolic acids might also be related to an increase of the total acid contents of black garlic. According to Xu et al., heat treatment of the phenolic compounds increased the free fraction of phenolic acids, whereas it decreased the ester, glycoside, and ester-bound fractions, leading to an increase in free phenol forms [4]. Additionally, the total polyphenol contents decreased at the later stage of these conditions, which demonstrated that the accumulated rate of total phenols was less its consumed rate.

The DPPH radical scavenging activity of heated garlic samples is shown in Fig. 2. The DPPH radical scavenging activity indicates the ability of an antioxidant compound to donate electrons or hydrogen, thereby converting DPPH into a more stable molecule with a reduced absorbance [1]. The DPPH radical scavenging ability of the heated garlic samples was significantly higher than that of the fresh garlic samples until the 38th. The increase in antioxidant activity of black garlic during the aging periodis due to the major antioxidants of garlic such as polyphenols, flavonoids and sulfur compounds: SAC, DAS, DADS, DAT... increased. Additonally, the DPPH radical scavenging activity decreased at the later stage of these conditions, because the total polyphenol contents decreased. There is a close relationship between the total phenolic content and the DPPH radical scavenging rate. Based on the results of polyphenol with antioxidant activity, black garlic at the 38th day be used to testing SAC.

2.1.4. Changes in S-allyl cysteine content

Changes in the amount of SAC in the black garlic manufacturing process were shown in Table I.

Table I. Changes in the amount of SAC of black garlic during aging period.

Component	Fresh garlic	Black garlic
SAC (mg/kg)	Did not dectectLOD = 3.5	159.81

The amount of SAC increased significantly as the heat treatment process progressed. According to the research, the raw material was fresh garlic Phan Rang after 38 days of the heat treatment process with SAC content (159.81 mg/kg). The SAC content increased 46 times and significantly higher than Vietnam Military Medical Academy, with the materials used to Ly Son garlic after 32 days of the heat treatment process only increased by 5-6 times. On the other hand, the increase in SAC content depends on the temperature of the heat treatment process, and garlic samples heated at low temperatures have higher amounts of SAC than those heated at higher temperatures. According to a study by Bae et al., (2014), The SAC content of garlic samples heated at 40^{o}C for 45 days was 124.67 mg/g, whereas that of garlic samples heated at 85^{o}C was only 85.46 mg/g [8]. SAC was formed by γ-glutamyl-S-allyl cysteine (GSAC) hydrolysis due to the enzyme γ-glutamyl transferase. The formation of SAC is also affected by the water-facilitated reaction between GSAC and γ-glutamyl transferase. Water in garlic aids the γ-glutamyl transferase reaction, aiding hydrolysis, and an abundance of water supports the transformation of GSAC into SAC. The optimum temperature of the enzyme γ-glutamyl transferase was 40°C. In order to obtain a high level of SAC, it was necessary to control the temperature in the first stage at 40°C, then gradually increase and maintain at the temperature 70^{o}C. Thus, the appropriate time for high antioxidant activity was the 38th days.

2.2. Effect of factors to pectinase enzyme treatment process

2.2.1. Effect of enzyme concentration to pectinase treatment process

Pectin and hemicellulose compounds usually exists in cell wall of fruit. During grinding and extraction process, these compounds will be released together with fruit juice and turn fruit juice into a viscotous state. Thus, fruit juice can not escape during the extraction process and it has a high viscosity. Result of pectinase enzyme treatment showed that all black garlic samples treated with pectinase enzyme in all concentrations have polyphenol and antioxidant activity higher significantly than black garlic sample untreated with pectinase enzyme.

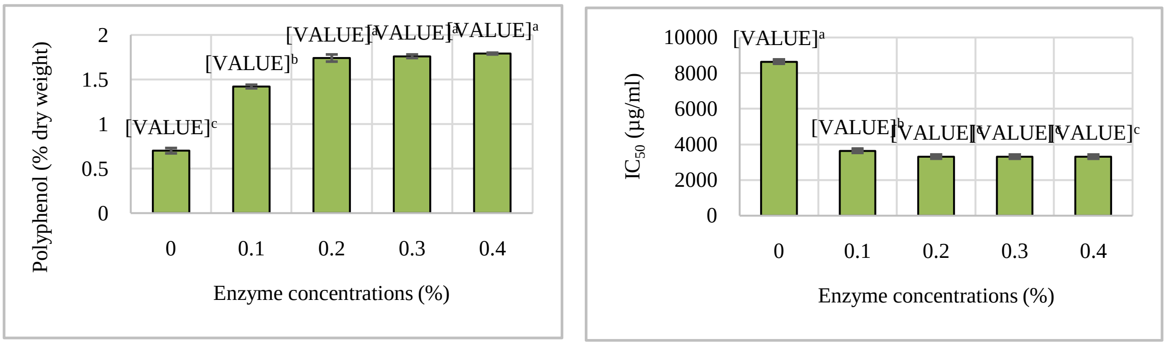

Figure 5. Effect of pectinase enzyme concentrations to polyphenol and antioxidant activity

Specifically, black garlic sample untreated with enzyme has polyphenol was 0.7% while polyphenol in black garlic samples treated with enzyme at different concentrations 0.1-0.4% increased significantly ($p<0.05$), 2-fold higher than control sample (untreated with enzyme). When enzyme concentrations increase, polyphenol and antioxidant activity also increase respectively. This is because, by increasing the enzyme concentrations, the pectin in the linkage between cells is broken, destroying the structure of the fruit tissue, making the polyphenol easier to extract into the juice. However, when increasing enzyme concentrations up to 0.3% and 0.4%, the polyphenol increased negligible. The reason is that the pectin substrate in black garlic is now linked to pectinase so the excess enzyme will not increase the efficiency of extracting polyphenol.

Along with the increase in polyphenol, the antioxidant activity of black garlic sample treated with pectinase enzyme also increased. At concentrations of 0.2 to 0.4%, the antioxidant activity of black garlic extract was not significantly different ($p<0.05$). When the enzyme concentration is high enough, the pectinase breaks off the linkage of the carbohydrate complex, thereby releasing proteins and short-chain pectins next to other proteins. Under acidic conditions and with phenolic compounds, the protein binds together to reduce solubility. At the same time, the polyphenol complex also forms in the less soluble state, thereby causing polyphenols to lose their ability to dissolve. Thus, reduces antioxidant activity when high enzyme concentrations. There, enzyme concentration 0.2% was chosen. However, the extraction of pectin substance depends on many factors such as: period of treatment, temperature, pH ....

2.2.2. Effect of hydrolysis temperature to pectinase enzyme treatment process

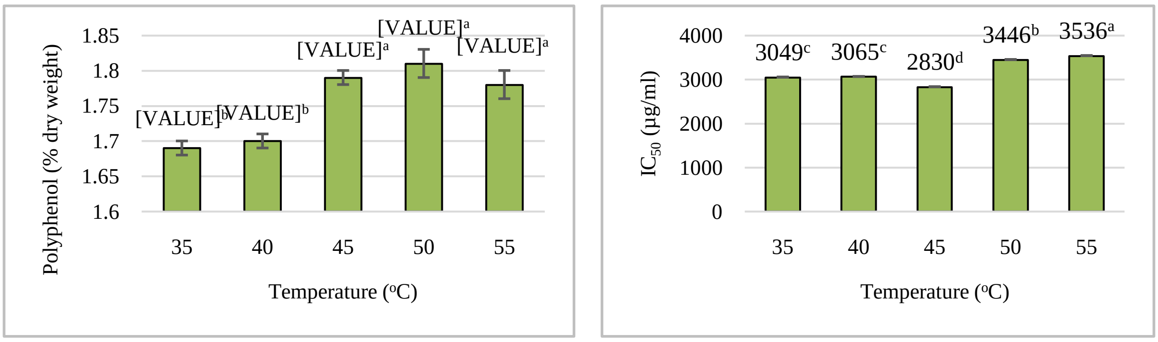

Fig.6. Effect of hydrolysis temperature to polyphenol content and antioxidant activity

Temperature has significant effect on enzyme activity. Proper temperature will increase the speed of enzymatic reaction. Based on the results obtained, the polyphenol increase significantly from 1.69% to 1.81% at 50°C and decrease slightly at 55°C. However, the results showed that there was no difference in the temperature range from 45 to 55°C ($p<0.05$). This can be explained by the fact that when the temperature increases, the enzyme's ability to catalyze increases. This diffuses the solutes out of the extraction medium, thus extracting more polyphenols. On the other hand, because the pectinase enzyme is optimally active at 45-55°C, it is more likely to be in the range of 45-55°C, with a stronger pectin degradation, which increases the polyphenol in black garlic juice.

The results also showed that, the free radicals DPPH scavenging ability is the lowest at 45°C corresponding to 2830 µg/ml. This means at the temperature 45°C, the ability to extract antioxidant compoundsis the highest. When the temperature rises to 50-55°C, the antioxidant activity decreases to 3536 µg/ml, which is different from the highest polyphenol extraction temperature at 50°C, which is due to the fact that apart from the polyphenols having antioxidant activity, other organic sulfur compounds such as flavonoids, ascorbic acid... in black garlic also have similar functions [9]. This finding is consistent with the study of Samantha Lemke Gonzalez et al (2011) that the optimum operating temperature of the pectinase enzyme is 45°C [10].

2.2.3. Effect of pH to pectinase enzyme treatment process

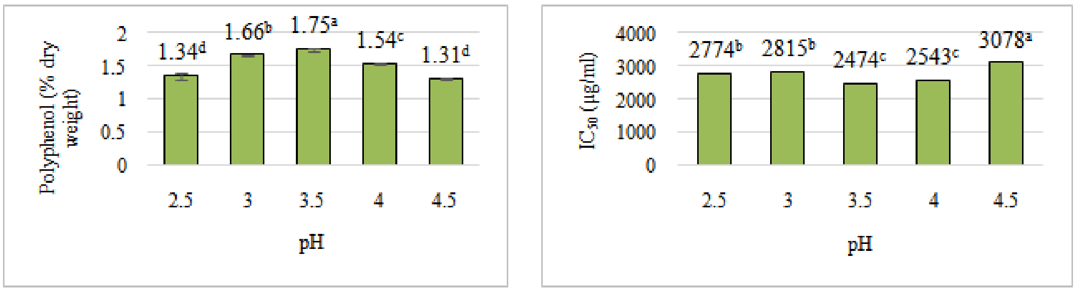

Fig.7. Effect of pH to polyphenol and antioxidant activity

The results showed that, the polyphenol increase respectively when the pH rises from 2.5 to 3.5 and after that it tended to decrease ($p<0.05$). At the range of pH from 3 to 4, the polyphenol content was higher than the polyphenol content of black garlic sample treated in pH value out of this range. The reason is that the pH of the medium usually affects the level of ionization of the substrate, enzyme and in particular affects the strength of the enzyme. In addition, the pH has a very strong influence on the rate of enzymatic reaction. The optimal pH of the covalent bonded enzyme with the positive charge carrier will shif to the acidic pH level relative to the soluble enzyme; otherwise, if the enzyme is covalently bonded to the negatively charged carrier, the optimal pH will shifted to alkaline pH. As a result of this study, the optimum pH of the pectinase enzyme is investigating the covalent bond with the positively charged substrate, which transfer to acid. Thus, depending on the source of enzyme production, they have different optimal pH, this study uses the enzyme produced from Aspergillus aculeatus. The results also showed that, at the range of pH from 3.5 to 4, the IC_{50} value was low and there was no difference ($p<0.05$), which means that at this pH range, antioxidant activity is high. At this pH level, the extraction of compounds have antioxidant activity is convenient. Therefore, when outside this pH range, the antioxidant activity will decrease.

2.2.4. Effect of hydrolysis interval to pectinase enzyme treatment process

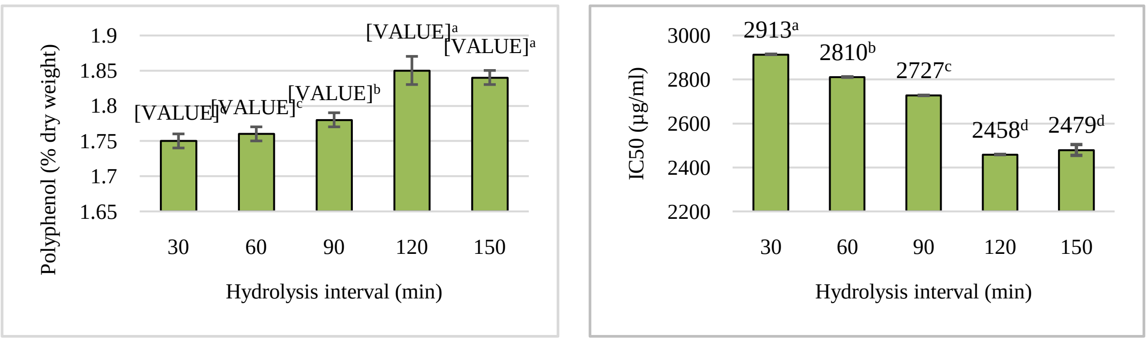

Fig.8. Effect of the hydrolysis interval to polyphenol and antioxidant activity

Based on the obtained results, there was a change in polyphenol in different hydrolysis period. The polyphenol increased with increasing hydrolysis interval and there was no difference ($p<0.05$) at 120-150 minutes. The increase in polyphenol over time was due to the increased reaction rate leading to the enzyme having time to react with the substrate, thus extracting more polyphenols. After 120 minutes the polyphenol increased negligible. The reason is that, in the early stages of enzyme treatment, most of the polyphenol compounds were extracted into the extract, as the amount of polyphenol in the black garlic was reduced so the extraction rate of these compounds began to decrease. In addition to the increase in polyphenol, the DPPH radical scavenging capacity of black garlic extract was also increased, and there was no significant difference ($p<0.05$) at 120 to 150 minutes. This indicates that the longer the hydrolysis interval, the greater the solubility of the antioxidant compounds, but the longer hydrolysis time will not be effective. This result is consistent with the theory that the enzyme affords both the product of the reaction and the substrate, the resulting products acting as a non-competitive inhibitor and inhibiting the activity of the enzyme.

2.2.5. Evaluation of black garlic extract treated with pectinase enzyme in optimized condition

Table II. The content of substances in black garlic extract at optimized condition

Component	Result
Reducing sugar (g/100g)	43.123 ± 0.36
Polyphenol content (% dry weight)	2.189 ± 0.704
IC_{50} (µg/ml)	2440 ± 0.006

The results showed that the reducing sugar content, polyphenol content and antioxidant activity after enzyme treatment were quite high in the black garlic extract juice. Therefore, the use of pectinase enzyme preparation to improve the quality of black garlic extract juice was shown by the increase in polyphenol compounds content, the antioxidant activity of black garlic extract was promising. Since then, our team conducted experiments to make black garlic juice.

3.3. Experiment to produce of black garlic juice

3.3.1. Effect of the dilution ratio on the state of the product

After diluting the black garlic with dilution ratio of black garlic extract and sweet grass herb was 3:4; 4:4; 5:4; 6:4. A survey of 30 people, mainly students, whether there was a distinct preference for the 4 samples to determine the appropriate dilution ratio.

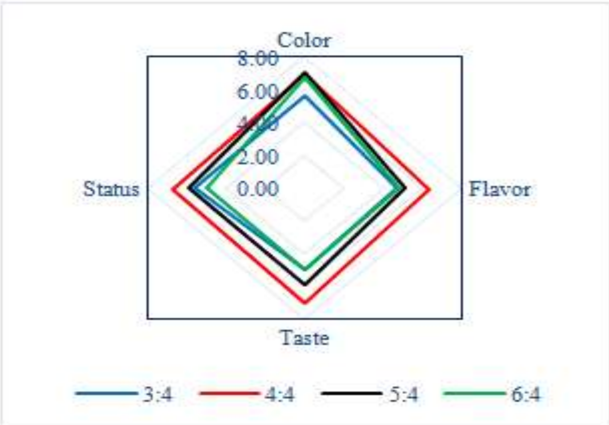

Fig. 9. Chart showing the sensory value of black garlic drink

Results showed that at the dilution ratio of black garlic extract and sweet grass herb was4: 4. The average score (color, flavor, taste) was higher than the others. At the same time, the 4: 4 ratio has a characteristic black color, harmonious taste. However, color did not differ between samples (4:4; 5:4; 6:4). The 3: 4 ratio was lighter than other ratios in color. At the same time, the 3:4 ratio was more acidic than rest. The 5: 4 ratio and 6: 4 ratio, the taste was so sweet and less preferred. Based on the results of the study, the 4: 4 ratio was selected to testing the biological activity shown in the table III.

Table III. Ingredients in the 4:4 ratio of garlic black extract and sweet grass herb

Components	Result
Reducing sugar (g/100g)	24.866 ± 0.019
Polyphenol (%)	3.11 ± 0.1
IC_{50} (µg/ml)	1727 ± 0.05

3.3.2.Product quality assessment

Black garlic juice is pasteurized at 95^{o}C for 15 minutes. Then, the product was cooled and stored for 3 weeks in the refrigerator with temperature 2-4^{o}C to consider changes in nutrient content. The results showed that polyphenol content and antioxidant activity of black garlic juice after 3 weeks of cold storage did not change the nutrient content. SAC content of the product was lower than SAC content of black garlic, which causes the product be diluted to suit the daily needs. In addition, product with low pH should inhibit microorganisms and increase product storage time. As a result, the finished black garlic drink produced by the team the appropriate procedure. The product be guaranteed food safety. The product has high biological activity at has the probe production step.

Tracking criteria	Result
pH	4.38 ± 0.01
Reducing sugar (g/100g)	24.741 ± 0.13
Polyphenol (%)	3.052 ± 0.01
IC_{50} (µg/ml)	1730 ± 0.01
SAC (mg/kg)	<MLOQ = 10
Total aerobic microorganisms, CFU/ml	<1(*)
Escherichia coli, CFU/ml	<1(*)
Staphylococcus aureus, CFU/ml	<1(*)
Clostridium perfringens, CFU/ml	<1(*)
Total yeast and bracket fungus, CFU/ml	<1(*)

Table IV. The quality criteria of black garlic juice

III. CONCLUSION

After 38 days of aging period, the content of reducing sugar was 42.10 g/100g, the pH decreased to 3.90 and the content of moisture decreased to 34.74%, polyphenol content reached 1.82%, SAC content was 159.81 mg/kg, IC_{50} values was 3262 µg/ml.

The study provided the suitable condition for the hydrolysis of black garlic at a pectinase enzyme concentration 0.2% (w/v), hydrolysis temperature was 45°C, pH 3.5 and hydrolysis period was 120 minutes, reducing sugar content was 43.12 g/100g, polyphenol was 2.19%, IC_{50} value was 2440 µg/ml.

The experiment to produce black garlic juice with dilution ratio of black garlic extract and sweet grass herb 4:4 g/g in 200 ml has the highest sensory score, characteristic color, harmonious taste. The product has high biological activity with the content of polyphenol is 3.05% and IC_{50} value was 1730 µg/ml.

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