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Effect of Drying Temperatures and Aids on Polyphenol Content, Antioxidant Activity, and β Glucosidase Enzyme Inhibition Activity of Powder *Stylissa flexibilis*

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Abstract: Sponge *Stylissa flexibilis* is Porifera belong to a medicine animal group in the Vietnam sea, contains numerous valuable bioactive substances. The study focused on the impact of spray-drying input temperatures (90°C, 100°C, 110°C, and 120°C) and aids (Maltodextrin, saccharose: maltodextrin mixture (1:1, w/w and 1:2, w/w)) on the physical-chemical properties (moisture content, solubility, and polyphenol content), antioxidant activities (mechanism Mo^{6+} and Fe^{3+}), and inhibition enzyme β Glucosidase of sponge extract powder. The sponge was collected from the sponge in May 2017 in the Ninh Thuan sea. The results showed the polyphenol content, total antioxidant activity, reducing power activity, and β Glucosidase enzyme inhibition activity was affected by the temperature and aids of the drying process and got the highest value of 77.692 ± 2.025 mg phloroglucinol equivalent/100g DW, 98.526 ± 1.997 mg ascorbic acid equivalent/100g DW, 28.945 ± 0.754 mg $FeSO_4$ equivalent/100g DW, and 75.38 ± 2.516/100 µg/ml was at 90°C, respectively, compared to the others. The moisture of powder was a negative-correlation to the increase of drying temperature. All the powder dissolved fully in the water. Polyphenol content and bioactivities (antioxidant activity, β Glucosidase enzyme inhibition activity) was a strong correlation with each other (R^2>0.8) and a positive-correlation to drying temperature. The suitable condition for drying of active polyphenol powder was at 90°C and 10% of maltodextrin. Active polyphenol powder can be useful for applicating into functional foods and pharmaceutics.

Keywords: Antioxidant Activity, Marine Sponge, Polyphenol, Solubility, Sponge Powder, Spray-Drying, Glucosidase Enzyme

1. Introduction

Marine sponge, a porifera animal, is mostly grown in numerous regions in the world from the deep sea (8,500 m) to the coast [1, 2] with over 9,000 marine species [3], for example, Mediterranean, America, Cuba, the Caribbean [4], Vietnam, Thailand, Cambodia, and Singapore [5]. Every year hundreds of new compounds are detected in a marine sponge, and nowadays, more than 5,300 different metabolites were noticed [6] with their activity, such as anti-inflammatory, anticancer, immune, anti-HIV, antimalarial, antibiotic, antifouling [3, 7-9], antioxidant [10-13], acetylcholinesterase enzyme inhibition, and glucosidase enzyme inhibition [11, 13]. The metabolites from sponge are diverse, for example, polyphenol [10, 11], lipid groups, quinone, terpenoid, alkaloid, halogen derivatives, peptide groups [3]. Almost previous studies on sponges are the cultivation, the metabolites isolation, and the bioactive evaluation from the sponge. Some studies noticed on the extraction [14] and the purification [15] of active polyphenol from the sponge, but no notices on spray drying of active polyphenol from marine sponge extract.

Spray drying is useful in the industry of the pharmaceutics, food, and chemistry, help moisture decrease of products, bacterial degradation and extend of storage time for the product, and fit for different types of materials.

Stylissa flexibilis distributes numerous regions in the world, for example, Vietnam, and possesses value bioactive ingredients with different activities [10, 11, 14, 16]. At the same time, the active polyphenol of marine sponge *Stylissa flexibilis* is temperature sensitivity.

Therefore, the study focused on the effect of the input temperature and aids (kind and ratio) of spray drying process on the polyphenol content, antioxidant activity, and β glucosidase enzyme inhibition, and the evaluation of physical characterization of powder (moisture and water solubility).

2. Material and Methods

2.1. Sample Preparation

2.1.1. Extract Preparation for Spray Drying

Sponge *Stylissa flexibilis* collected from Hon Mot island in Nha Trang Bay, Khanh Hoa province, Vietnam, were transported to the laboratory in the day at the temperature under 4°C. Dirt and any impurities on the sponge were washed under running tap. They were ground into small pieces by nitrogen liquid and stored at -20°C for further study. To produce an extract by the Soxhlet technique of extraction at the temperature of 60°C for 80 minutes with the ratio of solvent and material (30/1 (v/w)) and using methanol as extraction solvent. After extraction, filtering the mixture was with Whatman no. 1 paper, and the extract volume decrease was to one part three volume by the concentration at 40°C. The concentrated extract storage was at 4°C until further study.

2.1.2. Solution Preparation of Aid for Spray Drying

Various aid solutions vortexed with sponge extracts using Wisd Homogenizer (Wisd, Frankfurt, Germany) were prepared in turn by dissolving maltodextrin (MD); maltodextrin: saccharose (1:1) or maltodextrin and saccharose (2:1) powder mixture into 50°C distilled water at 10:3 (v/w) ratio. Before spray drying, the sponge extract and aid solutions were vortexed according to the ratio of 1:1 (v/v) at 3.000 rpm. Collecting the antioxidant polyphenol powder was by the spray drying of the mixture on a Lab Plant SD06 spray dryer (Keison, Chelmsford, UK), and the powders storage at 4°C for further analysis.

2.1.3. Extract Preparation from Spray Drying Powder

The sponge powder was vortexed to 96% methanol according to the ratio of solvent to powder (50/1, v/w) (Merck, Darmstadt, Germany) at room temperature for 30 min in the dark. The mixture centrifugation was then at 5000 rpm at 4°C for 15 min (Z326 K, Hermle Labortechnik GmbH, Wehingen, Germany) for collecting supernatant which stored at 4°C for the analysis of polyphenol content and antioxidant activities.

2.2. Study on the Effect of Spray Drying Temperature and Aids on the Powder Properties

2.2.1. Study on the Effects of Aid Kind on the Powder Properties

To find the effects of aids on physical-chemical properties and antioxidant activities of the sponge powder by spray drying a mixture of sponge extract and aids was at the temperatures of 110°C with the feed flow rate of 10 mL/minutes, pump pressure of 0.8 bar and output temperature of 45°C. Maltodextrin content was 15% of the mixture. Various aids included maltodextrin (MA), the mixture of maltodextrin: saccharose at ratio 2:1 (w/w) (MAS21) and 1:1 (w/w) (MAS11).

2.2.2. Study on the Effects of Aid Ratio on the Powder Properties

To evaluate effects of spray-drying temperature on physical-chemical properties and antioxidant activities of the sponge powder by spray drying a mixture of sponge extract and maltodextrin was at temperatures of 110°C with the feed flow rate of 10 mL/minutes, pump pressure of 0.8 bar and output temperature of 45°C. Maltodextrin content was varied from 5%, 10%, 15% and 20% of the mixture.

2.2.3. Study on the Effects of Temperature on the Powder Properties

Evaluating effects of spray-drying temperature on physical-chemical properties and antioxidant activities of the sponge powder by spray drying a mixture of sponge extract and maltodextrin was at different temperatures of 90°C, 100°C, 110°C, and 120°C with the feed flow rate of 10 mL/minutes, pump pressure of 0.8 bar and output temperature of 45°C. Maltodextrin content was 10% of the mixture.

2.3. Determination of Physical Chemistry Characterization

2.3.1. Determination of Moisture Content

Determining moisture contents of the powders were according to the AOAC method 976.05.

In detail, drying samples were at 105°C until constant weight, named final weight and calculating the moisture contents were as follows.

$$Moisture\ content\ (\%) = \frac{Initial\ weight - final\ weight}{Initial\ weight}\ x100\%$$

2.3.2. Determination of Water Solubility of Powder

Determining the solubility of the powders was according to the description of Takasi et al. [20] with some slight modifications. The first, dissolving 0.1 g of sample was into 1 mL of distilled water at 30°C and centrifuged at 9500 rpm for 10 min. Drying the supernatant was then to reach a moisture content of the initial sample at 105°C, named the dissolve weight. The final, using the dissolve weight for the calculation of the solubility of the samples, shown in according to the following equation.

$$The\ water\ solubility\ of powder\ (\%) = \frac{Dissolve\ weight}{Initial\ weight} x100\%$$

2.3.3. Determination of Polyphenol Content of Powder

The quantification of polyphenol content was according to the description of Swanson et al. In brief, adding 0.5 mL of 10% Folin-Ciocalteau (Sigma-Aldrich, Germany) was into 300 mL extract, vortexed and kept for 5 min. After that, adding 2 mL of 5% sodium carbonate solution (Merck, Germany) into the mixture, and kept the mixture for 90 min at room temperature. The absorbance measurement of the mixture was at a wavelength 750 nm on a UV-visible spectrophotometer machine and using gallic acid (Merck, Germany) as the standard.

2.4. Evaluation of Biological Activity

2.4.1. Evaluation of Total Antioxidant Activity

Total antioxidant activity was determined basing on the absorbance measurement of the metabolism reaction of Mo^{6+} to Mo^{5+} at the wavelength of 695 nm with ascorbic acid standard [17].

2.4.2. Evaluation of Reducing Power Activity

Reducing power activity was determined to base on the absorbance measurement of Fe^{2+} at 655 nm that was formed from the metabolism of Fe^{3+} to Fe^{2+}, with $FeSO_4$ standard. [18].

2.4.3. Evaluation of β Glucosidase Inhibition Activity

The extract dissolved into phosphate buffer, and then added 0.1 mL enzyme, vortex, and kept for 5 minutes at 37°C. Then add 0.1 ml of the substrate solution, mix well, incubate at 37°C for 25 minutes, finally add 1.5 ml of 0.1 M Na_2CO_3 solution and measure the optical density at 405 nm. Determination of the ability of enzyme inhibition based on the optical density, compared to the control sample (the control sample similar the sample but replacing the extract by the dissolving solvent of the extract) [19].

2.5. Data Analysis

All the experiments were triplication and data presentation under mean ± standard deviations. Analysis of ANOVA, regression, and descriptive statistics were by using MS. Excel 2010. To remove unnormal value was by the method of Duncan.

3. Results and Discussion

3.1. Effects of Different Spray-Drying Aids on Sponge Powder Characterization

3.1.1. Polyphenol Content, Moisture, and Water Solubility

The dry aids affected the polyphenol content of powder ($p < 0.05$). The highest polyphenol content of powder was with the maltodextrin aids. The polyphenol content of powder was arranged in the decreasing order as follows: 15% of MA, 15% of MAS21, and 15% of MAS11. The polyphenol content of powder using MAS11 was 0.93 and 0.97 times of MAS21 and MA, respectively. The saccharose ratio in drying aids increased by 33.33% (Figure 1), polyphenol content of powder was decreased by about 3%. The moisture of polyphenol powder was in the range of 6.7 and 7.8% DW, corresponding to MA and MAS11, respectively, and all various powder dissolved fully in the water for 5 minutes.

3.1.2. Mo^{6+} Metabolism Activity

The Mo^{6+} metabolism activity of different powders were significant differences ($p \leq 0.05$) and possessed the highest value of 96.523 ± 2.317 (mg ascorbic acid equivalent/g DW) with MA. In contrast, the Mo^{6+} metabolism activity of MAS11 powder was the lowest with 90.517 ± 2.101 (mg ascorbic acid equivalent/g DW) (Figure 1). The Mo^{6+} metabolism activity of MAS21 powder was 1.03 times of MAS11 powder. However, an insignificant difference in the Mo^{6+} metabolism activity occurred between MA and MAS21, and between MAS21 and MAS11. The maltodextrin ratio in drying aids increased by double in comparison to the saccharose ratio, Mo^{6+} metabolism activity of powder was increased by 2.95%. A good correlation between polyphenol content and Mo^{6+} metabolism activity was showed through ANOVA analysis ($R^2 > 0.9$).

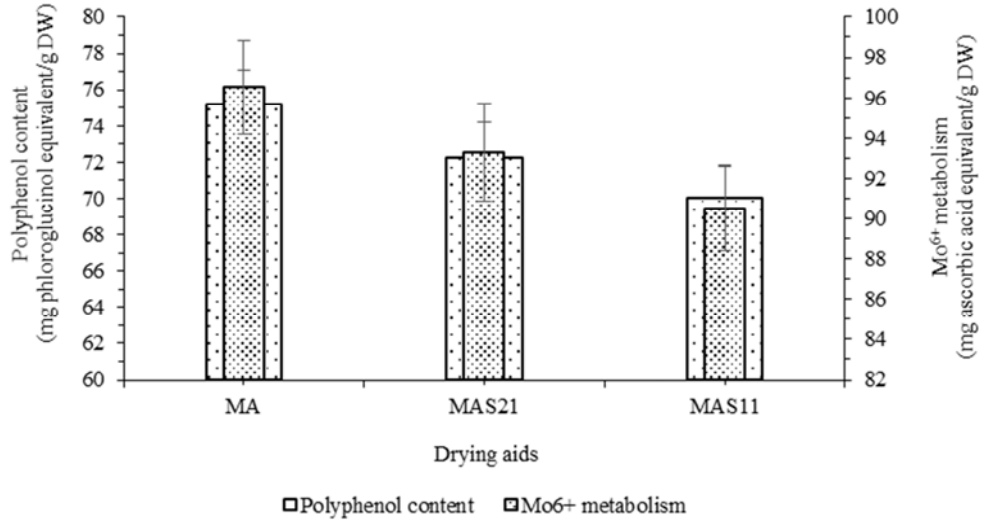

Figure 1. *Effect of spray-drying aids on polyphenol content and total antioxidant activity of powder.*

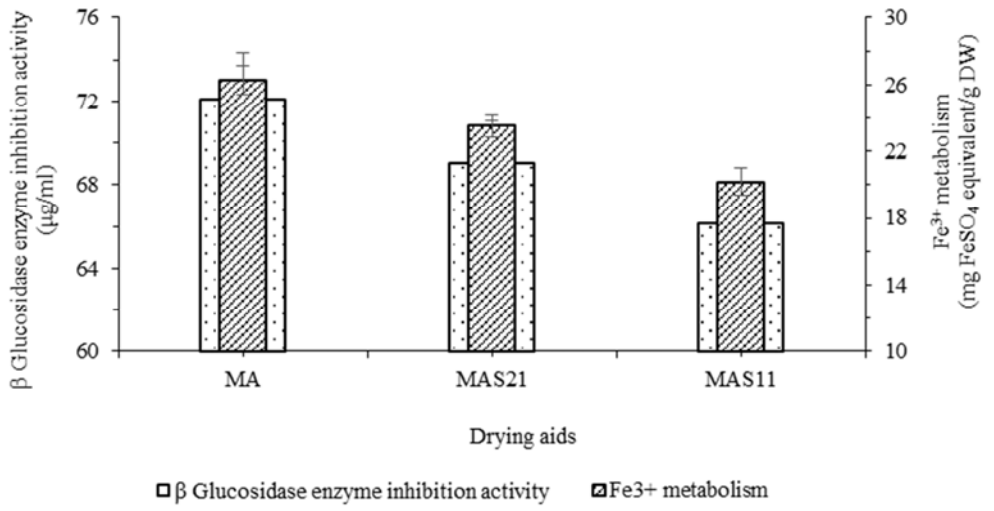

Figure 2. *Effect of spray-drying aids on reducing power activity and β glucosidase enzyme inhibition activity of powder.*

3.1.3. Fe^{3+} Metabolism Activity

There was a significant difference in Fe^{3+} metabolism activity of sponge powders preparing from different carriers ($p < 0.05$). MA exhibited the highest Fe^{3+} metabolism activity (26.255 ± 0.873 mg $FeSO_4$ equivalent/g DW) (Figure 2). The followings were MAS21 (23.515 ± 0.689 mg $FeSO_4$ equivalent/g DW) and MAS11 (20.188 ± 0.837 mg $FeSO_4$ equivalent/g DW). The maltodextrin ratio in drying aids decreased by 33.33%, Fe^{3+} metabolism activity of powder was decreased by 14.15%. The Fe^{3+} metabolism activity was an insignificant difference in MA and MAS21, and MAS21 and MAS11. Polyphenol content and Fe^{3+} metabolism activity had a good correlation ($R^2 > 0.9$).

3.1.4. β glucosidase Enzyme Inhibition Activity

All polyphenol powders from sponge *Stylissa flexibilis* exhibited β glucosidase enzyme inhibition activity and was affected by the drying aids style ($p < 0.05$). β glucosidase enzyme inhibition activity had a similar trend for antioxidant activity and polyphenol content, was arranged in the increasing order as follows: MAS11, MAS21, and MA. β glucosidase enzyme inhibition activity of MA (72.116 ± 2.191 µg/ml) was 1.09 and 1.05 times of MAS11 and MAS21, respectively (Figure 2). The difference in β glucosidase enzyme inhibition activity of MA and MAS21, and MAS21 and MAS11 were not significant. Polyphenol content had a good correlation ($R^2 > 0.9$) for β glucosidase enzyme inhibition activity.

3.2. Effects of Drying Aids Ratio on Sponge Powder Characterization

3.2.1. Polyphenol Content, Moisture, and Water Solubility

The difference in the aids ratio caused the change of the polyphenol content in the powder that varied in the range of 72.262 ± 1.976 and 76,209 ± 1.734 mg phloroglucinol equivalent/g DW and exhibited the highest value at 10% of aids (Figure 3). The followings were 5%, 20%, and 15% of aids.

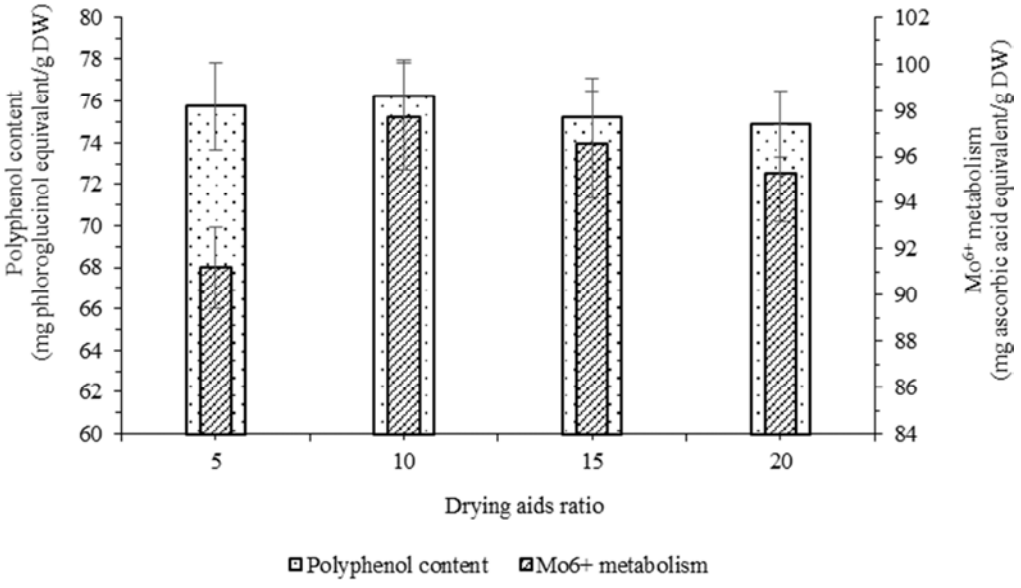

Figure 3. *Effect of drying aids ratio on polyphenol content and total antioxidant activity of powder.*

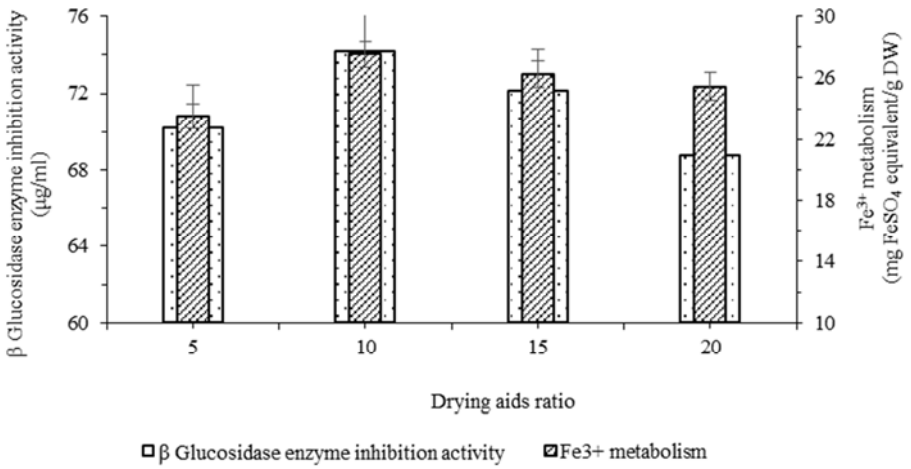

Figure 4. *Effect of drying aids ratio on reducing power activity and β glucosidase enzyme inhibition activity of powder.*

The aids ratio decreased from 20% to 5%, the polyphenol content of powder changed according to the model of level 2 with a maximum peak at 10% of aids. The significant difference in polyphenol content at 10% and 15% of aids was clear ($p < 0.05$). The difference in the moisture of various powders was insignificant ($p<0.05$). The full dissolving of all polyphenol powders into the water for 5 minutes was not different.

3.2.2. Mo^{6+} Metabolism Activity

The Mo^{6+} metabolism activity of active polyphenol powders was depended on the aids ratio ($p \leq 0.05$) and was from 91.186 ± 1.755 to 97.727 ± 2.318 mg ascorbic acid equivalent/g DW. The Mo^{6+} metabolism activity of active polyphenol powders got the highest and lowest value at 5% and 10% of drying aids, respectively. The model of level 2 with the maximum peak at 10% of drying aids was shown, as in Figure 3, and similar observation for the changing model of polyphenol content. Mo^{6+} metabolism activity and polyphenol content was a good correlation according to the change of drying aids ($R^2 > 0.9$).

3.2.3. Fe^{3+} Metabolism Activity

Fe^{3+} metabolism activity of polyphenol powder was from 91.186 ± 1.755 to 97.727 ± 2.318 mg $FeSO_4$ equivalent/g DW and its highest value at 10% of drying aids. The followings were at 20%, 15%, and 5% of drying aids. Fe^{3+} metabolism activity of polyphenol powder at 15% and 20% of drying aids corresponded to 95.44 and 97.5%, compared to 10% of drying aids (Figure 4). Fe^{3+} metabolism activity of polyphenol powder was a positive correlation to polyphenol content and impacted by the drying aids ratio with the changing trend according to the non-linear model of level 2 having the maximum peak.

3.2.4. β glucosidase Enzyme Inhibition Activity

β glucosidase enzyme inhibition activity is also not an exception when it was affected by the drying temperature similar to polyphenol content and antioxidant activity ($p < 0.05$). The changing trend of β glucosidase enzyme inhibition activity was like antioxidant activity and polyphenol content with the highest and the lowest value at 90°C (75.38 ± 2.516 μg/ml) and 120°C (73.116 ± 2.284 μg/ml), respectively. β glucosidase enzyme inhibition activity of powder at 110°C and 110°C was 98.45% and 99.7%, compared to 90°C (Figure 4). β glucosidase enzyme inhibition activity of powder drying from 90°C to 110°C was an insignificant difference ($p > 0.05$). Polyphenol content was positively proportional to β glucosidase enzyme inhibition activity ($R^2 > 0.9$).

3.3. Effects of Spray-Drying Temperatures on Sponge Powder Characterization

3.3.1. Polyphenol Content, Moisture, and Water Solubility

The polyphenol content of powder was affected by the drying temperature ($p < 0.05$) and was from 70.035 ± 1.802 to 77.692 ± 2.025 mg phloroglucinol equivalent/g DW. When the temperature decreased from 120°C to 90°C, the polyphenol content of powder increased. The polyphenol content of powder at 120°C was 92%, 90.97%, and 90.22%, compared to at 110°C, 100°C, and 90°C, respectively (Figure 5). The insignificant difference in polyphenol content occurred at various temperatures of drying, except for 120°C. The water solubility of polyphenol powders drying at various temperatures was full. The moisture of polyphenol powders varied from 5.5 to 8.3% DW, corresponding to at 120°C to 90°C, respectively.

3.3.2. Mo^{6+} Metabolism Activity

The Mo^{6+} metabolism activity of different powders was affected by the drying temperature ($p \leq 0.05$) and got the highest value (98.526 ± 1.997 mg ascorbic acid equivalent/g DW) at 90°C. In contrast, the Mo^{6+} metabolism activity was the lowest (95.008 ± 1.926 mg ascorbic acid equivalent/g DW) at 120°C. The Mo^{6+} metabolism activity at 120°C was 0.97 and 0.96 times, compared to at 110°C and 100°C, respectively (Figure 5). Similar observation for polyphenol content, the significant difference in Mo^{6+} metabolism activity only occurred at 120°C in comparison to other drying temperature. Polyphenol content and Mo^{6+} metabolism activity was a good correlation under the effect of drying

temperature ($R^2 > 0.9$).

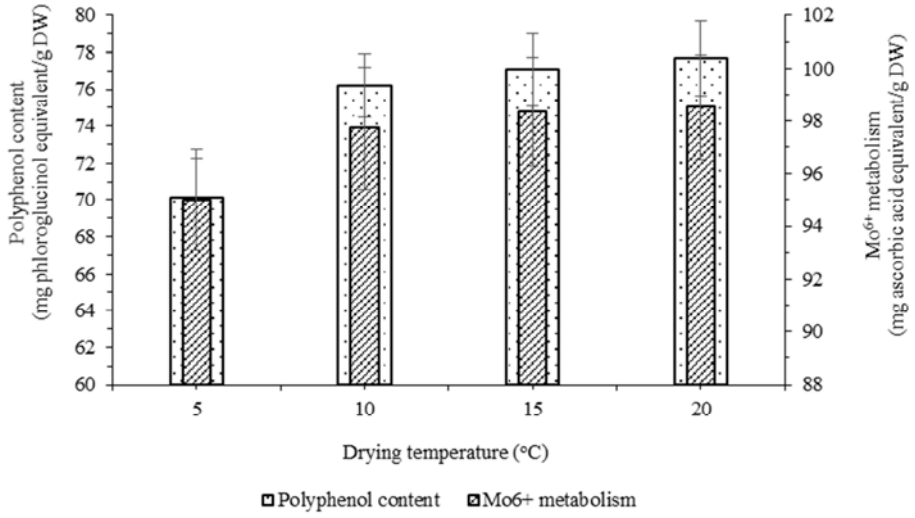

Figure 5. *Effect of drying temperature on polyphenol content and total antioxidant activity of powder.*

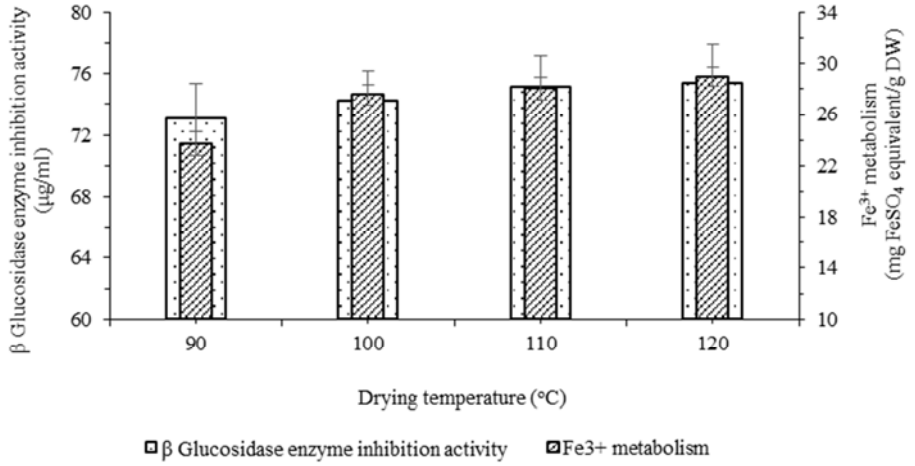

Figure 6. *Effect of drying temperature on reducing power activity and β glucosidase enzyme inhibition activity of powder.*

3.3.3. Fe^{3+} Metabolism Activity

The drying temperature impacted significantly on Fe^{3+} metabolism activity of sponge powders ($p < 0.05$) and had the highest Fe^{3+} metabolism activity (28.945 ± 0.754 mg $FeSO_4$ equivalent/g DW) at 90°C, compared to various drying temperatures. The followings were at 100°C (28.045 ± 0.851), 110°C (27.552 ± 0.832 mg $FeSO_4$ equivalent/g DW), and 120°C (23.756 ± 0.945 mg $FeSO_4$ equivalent/g DW) (Figure 6). Similar Mo^{6+} metabolism activity, Fe^{3+} metabolism activity of powder was a good correlation to polyphenol content ($R^2 > 0.9$) and insignificantly different at the drying temperature 90°C, 100°C, and 110°C, except for 120°C.

3.3.4. β glucosidase Enzyme Inhibition Activity

β glucosidase enzyme inhibition activity is also not an exception when it was affected by the drying temperature similar to polyphenol content and antioxidant activity ($p<0.05$). The changing trend of β glucosidase enzyme inhibition activity was like antioxidant activity and polyphenol content with the highest and the lowest value at 90°C (75.38 ± 2.516 µg/ml) and 120°C (73.116 ± 2.284 µg/ml), respectively (Figure 6). β glucosidase enzyme inhibition activity of powder at 110°C and 110°C was 98.45% and 99.7%, compared to 90°C. β glucosidase enzyme inhibition activity of powder drying from 90°C to 110°C was an insignificant difference ($p > 0.05$). Polyphenol content was positively proportional to β glucosidase enzyme inhibition activity ($R^2 > 0.9$).

4. Conclusion

In general, the inlet temperatures and aids (kinds and ratio) of the spray-drying process significantly affected the moisture content, polyphenol content, antioxidant activity (total antioxidant activity and reducing power), and β glucosidase enzyme inhibition activity of marine sponge extract powder *Stylissa flexibilis*, except for water solubility. The increase of spray-drying temperature caused by the decrease of moisture content, polyphenol content, antioxidant activity, and β glucosidase enzyme inhibition activity, and the suitable drying temperature of 90°C. 10% of maltodextrin was the best use for

the spray drying process, compared to the mixture of maltodextrin and saccharose. Polyphenol content had relatively high antioxidant activity and β glucosidase enzyme inhibition activity. It is suggested marine sponge extract powder *Stylissa flexibilis* containing high polyphenol content with high antioxidant activity, high β glucosidase enzyme inhibition activity, good water solubility, and low moisture content can be useful into applicating for functional foods and pharmaceutics.

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