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Research Article

Effects of enzymatic treatment on seed mucilage degradation and air-drying temperature on quality attributes of dragon fruit seeds (*Hylocereus* spp.)

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ABSTRACT

Dragon fruit is a typical crop being grown commercially in tropical areas, especially in Viet Nam. Dragon fruit seeds are the waste type disposed of in beverage processing due to difficulties in seed separation and the presence of seed mucilage. The objective of this study was to investigate the effects of enzymatic treatment on seed mucilage degradation as well as the impacts of air-drying temperature on quality attributes of red-flesh (*Hylocereus polyrhizus*) dragon fruit seeds. The maximum mucilaginous layer degradation by commercial pectinase preparation (Pectinex® Ultra SP-L) was 84.9% when the seed:water ratio, enzyme concentration, and treatment time were 1:0 (w/w), 10 U/g seed, and 75 min, respectively. The increase in the drying temperature from 50°C to 70°C decreased the drying time by 61.9% but increased the total phenolic content of dehydrated seeds by 20.7%. Further increase in drying temperature from 70°C to 90°C reduced drying time and total phenolic content by 75% and 9.5%, respectively. Seeds dried at 70°C for 8 h indicated the highest retention of the total phenolic content (588±11 mg GAE/100g dry matter) and antioxidant activity (DPPH free radical scavenging activity: 9000±59 μmol TE/100g dry matter). Dragon fruit dried seeds were considered a valuable source of nutrients and antioxidants that can be further used in formulation of different food products.

Key words: Antioxidant, dragon fruit seed, drying, enzyme, mucilage

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INTRODUCTION

Due to an increase in population, the development of food industry is essential to provide a broader choice of food products for consumers[1]. However, huge amounts of by-products from fruit and vegetable processing are regarded as waste and easy disposal, which cause ecosystem problems as they are disposed to microbial degradation and environmental pollution. In addition, these by-products are potential source of bioactive compounds and valuable nutrients for health benefits. The manufacturing of value-added products of fruit and vegetable by-products makes a huge contribution to solve problems in food waste management[2].

Dragon fruit, also known as pitaya or pitahaya is a species of the *Cactaceae* family and its most common cultivated varieties are from the *Hylocereus* genus. Vietnam is one of the potential dragon fruit producers due to its large cultivated area and high productivity. *Hylocereus polyrhizus*, *Hylocereus undatus* and *Selenicereus megalanthus* are three commercially grown varieties. There are many processed products prepared from dragon fruit and its pulp is mostly the main part used in the food industry[3].

The seeds of dragon fruit are small black seeds scattered closely throughout its pulp which occupy about 2.7 – 14.7% by fresh fruit weight. The phytoconstituents of dragon fruit seed including fatty acids, carbohydrates, protein, and phenolic compounds are essential for human diet[4]. Seeds are properly discarded as waste in beverage processing due to difficulties in seed separation and the presence of seed mucilage[3,5]. According to Bellec and Vaillant (2011), the mucilage of *Hylocereus* species has similar characteristics of other cactus species. In this case, mucilage is defined as a complex combination of at least five types of polysaccharides, less than half of which corresponds to a pectin-like polymer[6]. Human body can not absorb nutrients from dragon fruit seeds if they are covered with mucilaginous layer[7]. There are two main steps to collect dragon fruit seeds that include seed mucilage decomposition and seed separation. To our knowledge, little study was carried out on seed mucilage decomposition by enzymatic treatment. Nutritional and functional properties of dragon fruit seeds have attracted great attention of

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many research groups[8]. Rui et al. (2009) reported that microwave-assisted extraction was the most efficient method to obtain the highest dragon fruit seed oil yield (7.78% w/w)[9]. Research of Ariffin et al. (2009) showed that dragon fruit seeds consisted of nearly 50% essential fatty acid, including 48% linoleic acid and 1.5% linolenic acid[10]. Liaotrakoon et al. (2013) observed that dragon fruit seed oil containing remarkably high amount of tocopherols and low oxidation rate[11].

Food drying is one of the fundamental industrial operations that reduces moisture content of material to extend the shelf-life of most products. Hot air is widely used to provide heat to material for the reduction of moisture content to the level at which deterioration reactions and microbial spoilage are minimized[12].

In this study, degradation of dragon fruit seeds was performed by enzymatic treatment. The obtained seeds were then dried to achieve the appropriate moisture content for their preservation. This study aimed to examine conditions for degrading seed mucilage by enzymatic treatment and evaluate the influence of temperature during air-drying process on quality attributes of dragon fruit seeds.

MATERIALS AND METHODS

Materials

Red-flesh (*Hylocereus polyrhizus*) dragon fruits were obtained from a local fruit supplier, Tan Nghia Town, Ham Tan District, Binh Thuan Province, Vietnam. Upon arrival at the laboratory, fruits were cut in quarts and hand peeled after being washed under running tap water. The pulps were crushed into puree, mixed with tap water and then rubbed against 35-mesh sieve (The pore size was approximately 0.5 mm) to separate mucilaginous seeds from the fruit pulp. The collected mucilaginous seeds were wrapped in polyethylene bags, sealed, and preserved at –20°C until further analysis.

Commercial pectinase preparation used to degrade dragon fruit seed mucilage was Pectinex® Ultra SP-L (Novozymes, Denmark) obtained from *Aspergillus aculeatus* with enzyme activity of 3300 pectolytic units per gram (U/g). The optimum pH and temperature of the preparation are 4.5–5.0 and 50–55°C, respectively.

Chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (USA). Other enzymes for quantitative analysis of fiber (alpha-amylase Termamyl® SC, glucoamylase Dextrozyme® DX, and protease Alcalase® 2.5L) were purchased from Novozymes (Denmark).

Methods

Effects of enzymatic treatment on dragon fruit seed mucilage degradation

Certain amounts of mucilaginous seeds were added to 0–300 mL distilled water the seed:water ratio was changed from 1:0 to 1:15 (w/w). The pH of mixture was adjusted at 5 using 1% (w/v) citric acid solution. The samples were incubated with different concentrations of pectinase preparation (from 0 to 25 U/g seed). The enzymatic treatment was done in amble glass bottles which were put in a thermostat shaker at 50±5°C and the mixing rate was 50 rpm. The time of the enzymatic treatment was varied from 0 to 90 min. Heating the mixture to 90°C for 1 minute was applied to stop the reaction. The mixture was cooled to room temperature and strained through a 35-mesh sieve (The pore size was 0.5 mm) to collect fresh dragon fruit seeds and remove the seed mucilage.

The yield of seed mucilage removal was estimated using the following calculation formula:

$$\text{Yield of seed mucilage removal (\%)} = \left(1 - \frac{Mass\ of\ fresh\ seeds}{Mass\ of\ mucilaginous\ seeds}\right) \times 100\% \quad (1)$$

Effects of air-drying temperature on quality attributes of dragon fruit seeds

Fresh dragon fruit seeds were evenly spread on drying trays. The drying was performed at different temperatures (50, 60, 70, 80, and 90°C) using a laboratory convective dryer (UM400, Memmert, Germany). The samples were dried until they reached approximately 10% moisture content to limit growth of spoilage microorganisms and prevent lipid oxidation. During the drying, sampling was taken for measurement of moisture content. At the end of the drying, the dehydrated seeds were sampled to determine total phenolic content and antioxidant activity. Proximate composition of the fresh seeds and the dehydrated seeds at the selected drying temperature was also analyzed and compared.

Analytical methods

Proximate composition analysis

The moisture content was determined by using a moisture analyzer (ML-50, A&D, Japan) drying at 105°C. The lipid content was analyzed following Soxhlet extraction (AOAC 960.39, 2000). Acid value and peroxide value were determined by titration method according to the TCVN 6127:2010 and TCVN 6121:2010, respectively. The crude protein content was evaluated using Kjeldahl method with a conversion factor of 6.25 (AOAC 984.13, 2000).

The crude ash content was gravimetrically estimated (AOAC method 930.30, 2000). The total carbohydrate was calculated as the arithmetical difference between 100% and the sum of the percentage of the analyzed components (protein, lipid, ash). The total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) content were determined using AOAC 985.29, AOAC 993.19, and AOAC 991.42 methods, respectively (AOAC, 2000).

Determination of total phenolic content and antioxidant activity

Extraction of phenolic compounds was conducted by using ground seed samples mixed with 60% acetone following sample:solvent ratio (1:10, w/v) at room temperature for 60 min. Total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteau reagent according to Agbor et al. (2014)[13]. The antioxidant activity of the extract was evaluated using 2,2-diphenyl-2-picryl-hydrazyl (DPPH) according to Brand-Williams et al. (1995)[14].

Pectinase assay

Pectinase activity was determined by colorimetric method using 3,5-dinitrosalicylic acid (DNS) reagent according to Oyeleke et al. (2012). One unit (U) of pectinolytic activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol galacturonic acid under the assay condition (50–55°C, pH = 4.5–5)[15].

Optical microscopy

Microscopic observation of dragon fruit seed was performed before and after the enzymatic treatment of seed mucilage. The seed samples were examined under a microscope (Olympus CX23, Japan) at 40× magnification.

Statistical analysis

All experiments were carried out in triplicate. The obtained experimental results were expressed as mean±standard deviation (SD). Mean values were considered significantly different when $p<0.05$. Analysis of variance (ANOVA) and Tukey's comparison test were performed using the software Statgraphics Centurion 18.

RESULTS AND DISCUSSION

Effects of enzymatic treatment on dragon fruit seed mucilage degradation

Change in yield of seed mucilage removal at different seed:water ratios is indicated in Figure 1. In this experiment, the pectinase concentration and enzymatic treatment time were set at 5 U/g seed and 60 min, respectively.

Decrease in seed:water ratio reduced the yield of seed mucilage removal. Dragon fruit seed mucilage is formed by polymer chains the main component of which is pectin[6]. Hydrolysis of pectin resulted in degradation of the mucilage layer. Our results showed that the moisture content of dragon fruit seed mucilage was varied from 89–92%. Due to high moisture content, pectinase enzyme effectively interacts with pectin in mucilaginous layers for the pectolysis. Enzyme molecules would exist in water phase more than mucilaginous seed coats if there was an increase in amounts of distilled water. In this case, sample without water addition achieved the highest yield of seed mucilage removal.

Figure 2 demonstrates the effect of pectinase concentration on the yield of seed mucilage removal. In this experiment, the seed:water ratio and enzymatic treatment time were 1:0 (w/w) and 60 min, respectively.

The yield of seed mucilage removal of the sample with 5 U/g seed was approximately 6.1 times higher than that of the control sample. Commercial pectinase preparation was found efficiently degrading mucilaginous layer embedded seed by disrupting the long chain pectin molecules of mucilage into shorter chain molecules[16,17]. When the enzyme concentration increased from 5 to 10 U/g seed, the yield of seed mucilage removal was enhanced by 2%. Further increase in pectinase concentration from 10 to 15 U/g seed improved the yield by 1.6%. The yield of seed mucilage removal was insignificantly different when the enzyme concentration increased from 15 to 25 U/g seed. In conclusion, the pectinase concentration of 10 U/g seed was effectively performed in degrading dragon fruit seed mucilage.

The impacts of enzymatic treatment time on the yield of seed mucilage removal are illustrated in Figure 3. In this experiment, the seed:water ratio of 1:0 (w/w) and pectinase concentration of 10 U/g seed were used.

At the beginning of the enzymatic treatment, the yield of dragon fruit seed mucilage removal was nearly 0%. Pectin degradation of seed mucilage samples achieved 79.3% after 30 min treatment. Moreover, an increase in yield of seed mucilage removal was approximately 7.6% when the enzymatic treatment time increased from 30 min to 90 min. Similar observation was reported by Wu et al. (2010) when Pectinex® Ultra SP-L was used to degrade the mucilage of flaxseed[18]. Nevertheless, insignificant difference was recorded between the seed samples treated for 75 min and 90 min. The appropriate time of the enzymatic treatment was

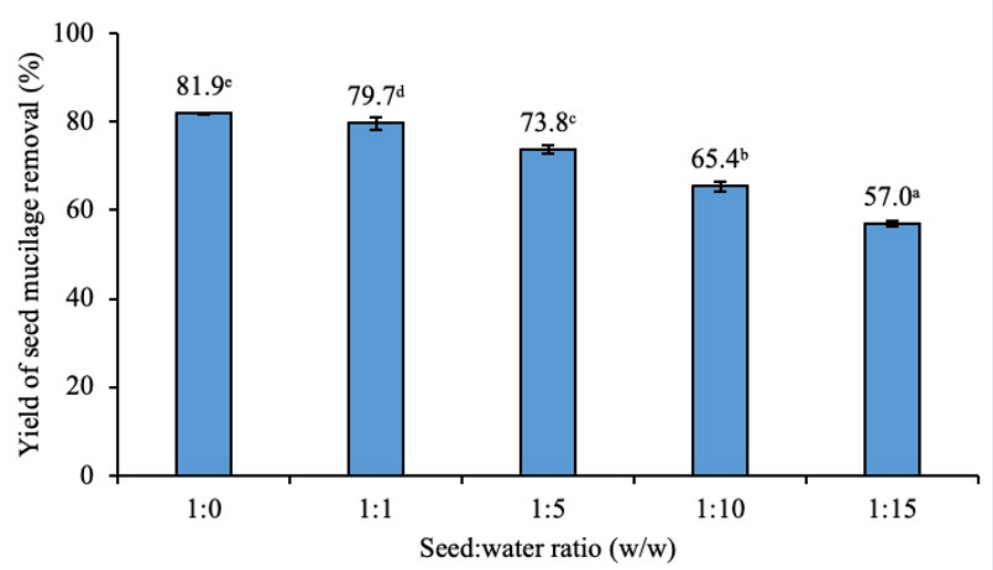

Figure 1: Yield of seed mucilage removal with different seed:water ratios (Insignificant difference is shown by same letters above the bars, $p<0.05$)

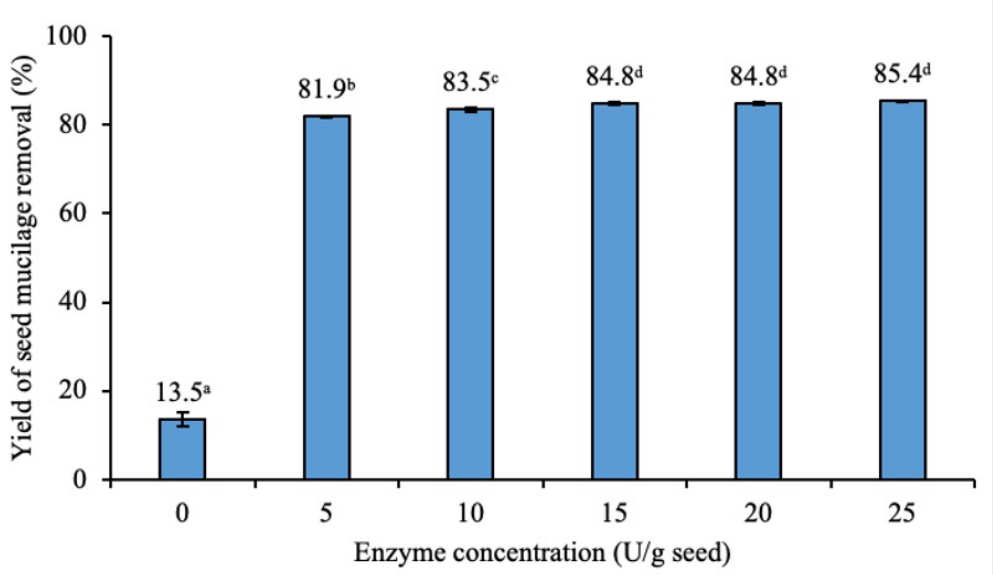

Figure 2: Yield of seed mucilage removal with different enzyme concentrations (Insignificant difference is shown by same letters above the bars, $p<0.05$)

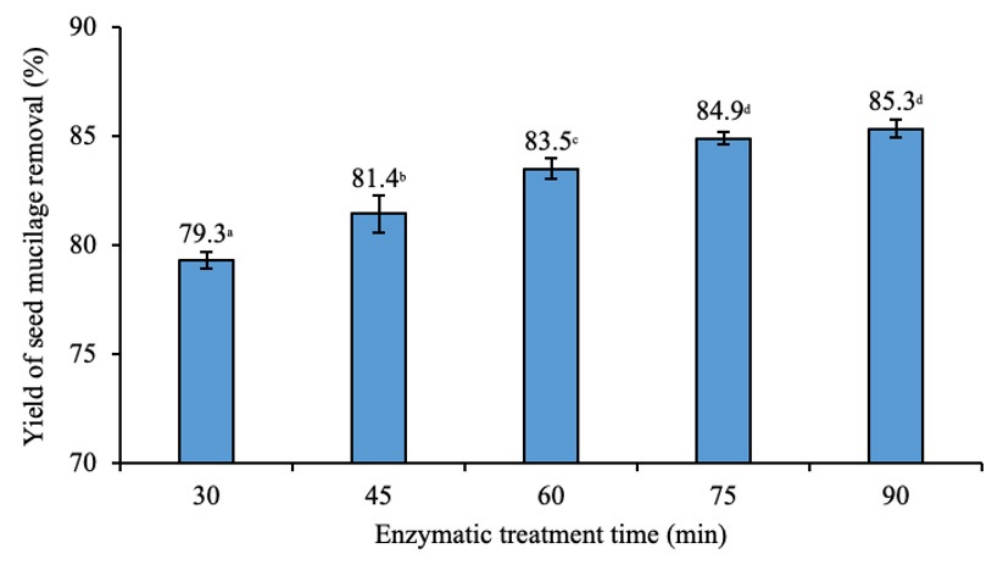

Figure 3: Yield of seed mucilage removal with different enzymatic treatment times (Insignificant difference is shown by same letters above the bars, $p<0.05$)

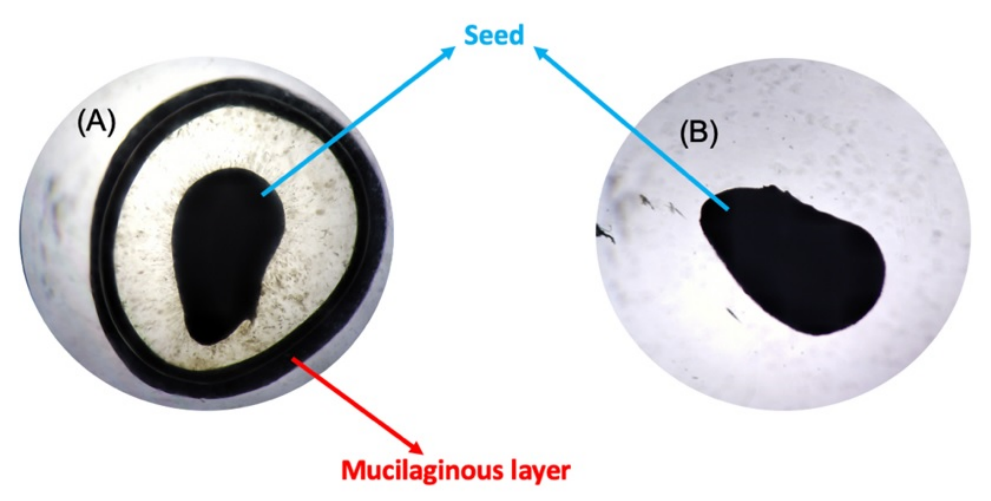

Figure 4: Pictures of dragon fruit seed under magnification (40×) of microscope before (A) and after the enzymatic treatment (B). (The seed:water ratio, pectinase concentration, and treatment time were 1:0 (w/w), 10 U/g seed, and 75 min, respectively).

75 min at which the yield of seed mucilage removal reached the maximum value.

Figure 4 displays the morphology of dragon fruit seed before and after the enzymatic treatment. It can be noted that the mucilaginous layers were completely removed from dragon fruit seeds by enzymatic treatment and the obtained fresh dragon fruit seeds were used for further analysis.

Effects of air-drying temperature on quality attributes of dragon fruit seeds

Change in moisture content of dragon fruit seeds during the drying is presented in Figure 5. Based on the obtained results, the drying time and final moisture content of all samples are displayed in Table 1.

The higher the drying temperature was, the shorter the drying time was. It can be explained by the improved heat and mass transfer at high temperature.

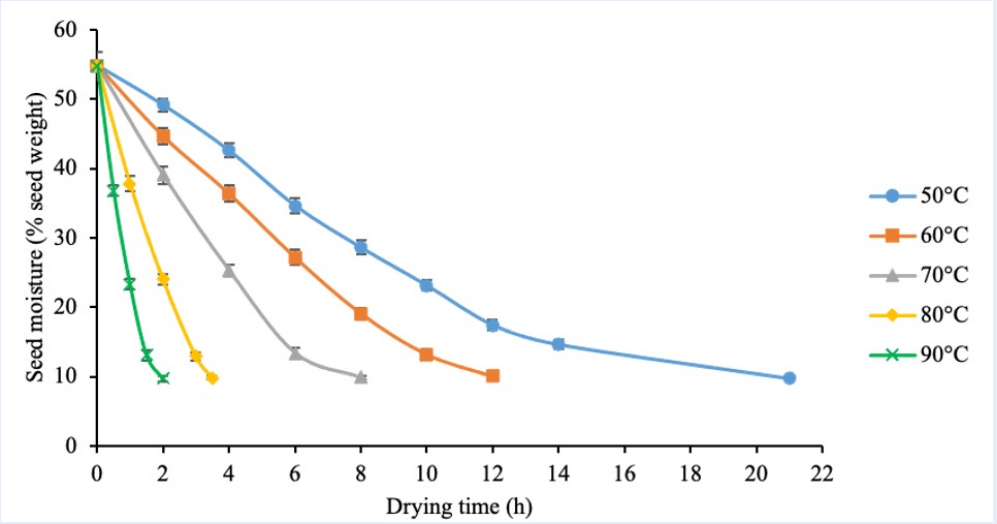

Figure 5: Change in seed moisture content during the drying at different air-drying temperatures

Table 1: Effect of drying temperature on the drying time and moisture content of the dried seed samples

Drying temperature (°C)	50	60	70	80	90
Drying time (h)	21	12	8	3.5	2
Moisture content of dried seed (% seed weight)	9.7±0.3[a]	10±0.3[a]	9.9±0.3[a]	9.8±0.3[a]	9.7±0.3[a]

(Insignificant difference is shown by identical letters within the same row, $p<0.05$)

The moisture diffusion from the internal to external surface of seed and the free water evaporation on the seed surface occurred rapidly due to increased drying temperature[19]. Similar trend was previously observed for orange by-products when the air-drying temperature increased from 30°C to 90°C[20].

Phenolics are representative bioactive compounds of dragon fruit seeds. Their content is demonstrated in Figure 6.

It can be observed that drying significantly decreased the phenolic content of dragon fruit seeds. When the drying temperature increased from 50°C to 70°C, the drying time reduced by 61.9% but the phenolic content of the dried dragon fruit seed increased by 20.7%. Further increase in drying temperature from 70°C to 90°C decreased both drying time and phenolic content by 75% and 9.5%, respectively. Some phenolic compounds are thermolabile and can be degraded during drying process[21]. However, the seed samples dried at 60, 70, 80, and 90°C had higher phenolic content than that dried at 50°C. This can be explained that high process temperature resulted in short drying time that limited oxidative damage of phenolic compounds caused by polyphenoloxidase[22,23]. Uribe et al. (2014) reported that total phenolic content of olive-waste cake dried at 50°C reduced by 11.3% as compared to that of dehydrated sample at 60°C[24]. The results of Figure 6 show that drying time had more influence on the total phenolic content of dried dragon fruit seeds than the use of high drying temperature.

The DPPH free radical scavenging activity of the fresh and dehydrated samples at different drying temperatures is showed in Figure 7.

The seed samples dried at 60, 70, 80, and 90°C showed higher antioxidant activity than that dried at 50°C. It can be noted that the variation of total phenolic content and DPPH scavenging activity at different drying temperatures had similar trend. A strong correlation was recorded between the total phenolic content and DPPH scavenging activity ($R^2 = 0.95$) which indicated that phenolic compounds were major antioxidant of dragon fruit seed; the correlation coefficient was 0.98. Adnan et al. (2011) identified catechin, quercetin, myricetin, epicatechin, and rutin which were main phenolics of dragon fruit seed and these compounds

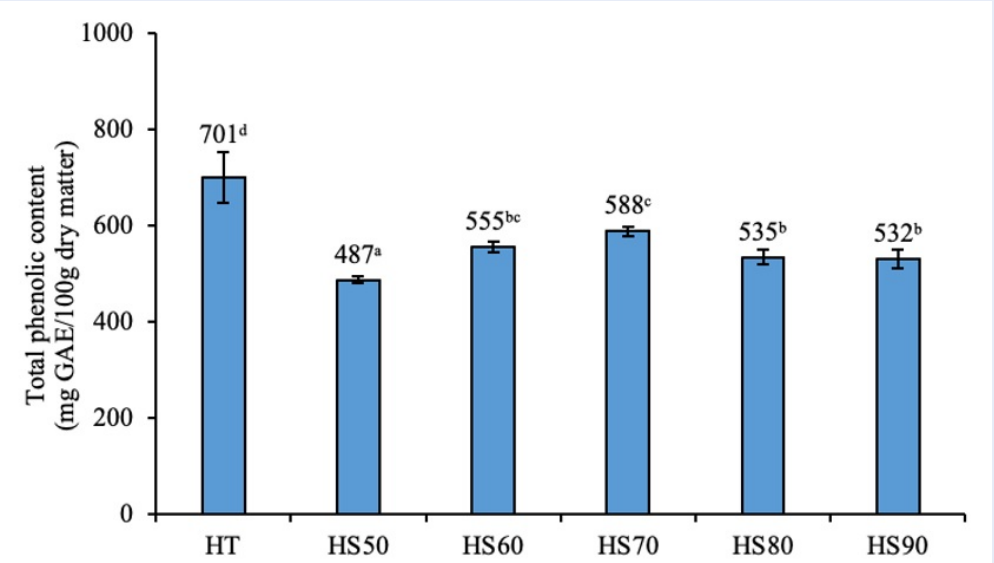

Figure 6: Total phenolic content of the fresh and dried seeds at different drying temperatures (HT-fresh, HS50-50°C, HS60-60°C, HS70-70°C, HS80-80°C, HS90-90°C) (Insignificant difference is shown by same letters above the bars, p<0.05)

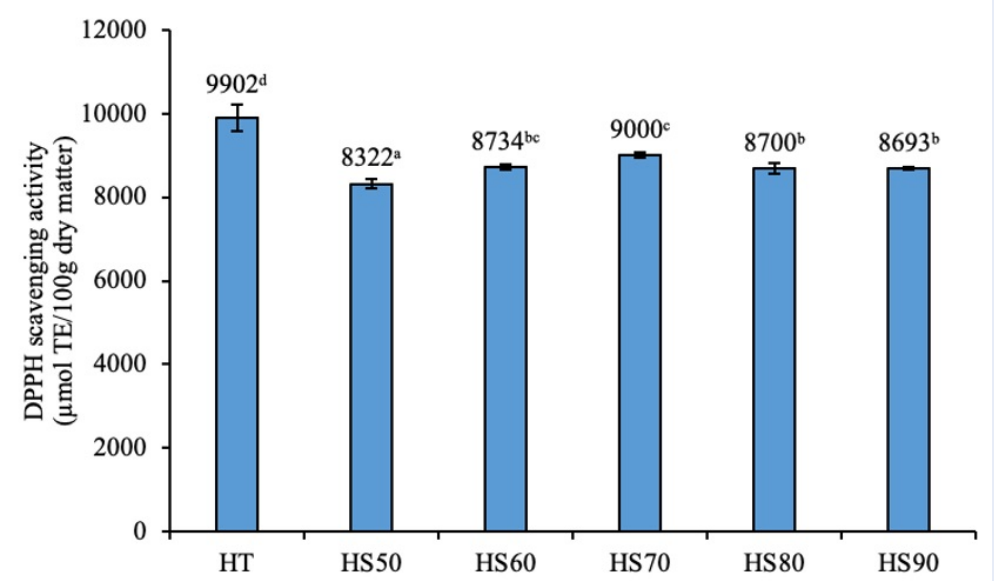

Figure 7: Effect of drying temperature on antioxidant activity of seed samples (HT-fresh, HS50-50°C, HS60-60°C, HS70-70°C, HS80-80°C, HS90-90°C) (Insignificant difference is shown by same letters above the bars, p<0.05)

had high antioxidant activity[25]. These results were in agreement with the findings of Li et al. (2019) who examined the impacts of air-drying temperatures on DPPH scavenging activity of okra; the antioxidant activity of okra samples dried at 60, 65, 70, 75, and 80°C was higher than that of samples dried at 55°C[26]. The results of Figure 7 reveal that the appropriate drying temperature for fresh dragon fruit seeds was 70°C at which the loss in phenolics and DPPH antiradical activity was the lowest.

Proximate composition of fresh and dried dragon fruit seed sample dried at 70°C for 8 h is showed in Table 2.

The dehydrated dragon fruit seeds had approximately 10% moisture content that permits to inhibit microbial growth as well as to prevent lipid oxidation during the preservation. The drying conditions used in this study did not change the carbohydrate, protein, lipid, and ash content of the dragon fruit seeds. Particularly, the oil quality of dehydrated dragon fruit seeds evaluated by acid value and peroxide value remained constant as compared to that of fresh seeds. However, drying process reduced the total phenolic content and antioxidant activity of dragon fruit seeds. Based on obtained results, it can be noted that dehydrated dragon fruit dried seed was considered a potential source of lipid, protein, and carbohydrates that can be further used in the formulation of different food products.

CONCLUSIONS

Appropriate enzymatic treatment conditions using enzyme preparation Pectinex® Ultra SP-L to degrade dragon fruit seed mucilage were as follows: seed:water ratio of 1:0 (w/w), pectinase concentration of 10 U/g seed, and enzymatic treatment time of 75 min. Under these conditions, the yield of seed mucilage removal was 84.9%. The drying time reduced by 61.9% and the phenolic content of the dried dragon fruit seed reduced by 16.1% when the air-drying temperature increased from 50°C to 70°C as compared to fresh sample. Further increase in air-drying temperature from 70°C to 90°C decreased the drying time and phenolic content by 75% and 24.1%, respectively. The appropriate drying temperature and time of dragon fruit seeds were 70°C and 8 h, respectively. Dragon fruit dried seeds were rich in nutrients and antioxidants. Future studies on use of dehydrated dragon fruit seeds in food processing should be investigated to develop new food products.

LIST OF ABBREVIATIONS

Total dietary fiber: TDF Soluble dietary fiber: SDF Insoluble dietary fiber: IDF; Total phenolic content: TPC; Standard deviation: SD; Analysis of variance: ANOVA.

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AUTHORS' CONTRIBUTIONS

Luu Ngoc Bao Nguyen: Methodology, Investigation, Data curation, Writing-original draft.
Hoang Phong Nguyen: Investigation, Data curation.
Van Viet Man Le: Validation, Formal analysis.
Thi Thu Tra Tran: Validation, Formal analysis.
Nu Minh Nguyet Ton: Validation, Formal analysis, Writing-review and editing, Supervision.
Main author: Luu Ngoc Bao Nguyen
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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Table 2: Physico-chemical properties of fresh and dried dragon fruit seed samples

Parameters	Fresh dragon fruit seed	Dried dragon fruit seed
Moisture (% seed weight)	54.8 ± 2^{b}	9.9 ± 0.3^{a}
Lipid (% dry matter)	38.2 ± 0.9^{a}	37.4 ± 1^{a}
Acid value (mg KOH/g seed oil)	1.5 ± 0.1^{a}	1.7 ± 0.1^{a}
Peroxide value (meq/kg seed oil)	3.6 ± 0.3^{a}	4.1 ± 0.3^{a}
Protein (% dry matter)	27.3 ± 0.8^{a}	26.6 ± 0.7^{a}
Ash (% dry matter)	2.9 ± 0.1^{a}	2.8 ± 0.3^{a}
Carbohydrates (% dry matter)	31.6 ± 0.3^{a}	33.2 ± 1.3^{a}
TDF (% dry matter)	19.7 ± 0.2^{a}	19.4 ± 0.2^{a}
SDF (% dry matter)	2.1 ± 0.2^{a}	2 ± 0.2^{a}
IDF (% dry matter)	17.7 ± 0.2^{a}	17.5 ± 0.2^{a}
TPC (mg GAE/100g dry matter)	701 ± 53^{b}	588 ± 11^{a}
DPPH (μmol TE/100g dry matter)	9902 ± 325^{b}	9000 ± 59^{a}

(Insignificant difference is shown by identical letters within the same row, $p<0.05$)

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Bài nghiên cứu

Ảnh hưởng của quá trình xử lý enzyme đến màng nhầy hạt và nhiệt độ sấy đối lưu đến chất lượng hạt thanh long

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TÓM TẮT

Thanh long thuộc nhóm cây ăn trái được trồng chủ yếu tại các vùng nhiệt đới, điển hình tại Việt Nam. Hạt thanh long được xem là phụ phẩm trong quy trình sản xuất thức uống do khó khăn trong việc tách hạt và sự hiện diện của màng nhầy bao quanh hạt. Nghiên cứu tập trung vào đánh giá tác động của việc sử dụng chế phẩm enzyme thương mại đến hiệu quả làm sạch màng nhầy hạt và ảnh hưởng của nhiệt độ sấy đối lưu đến chất lượng của hạt thanh long ruột đỏ (*Hylocereus polyrhizus*). Đối với hạt thanh long, việc loại bỏ màng nhầy bao quanh hạt được thực hiện bằng quá trình xử lý với chế phẩm enzyme thương mại Pectinex® Ultra SP-L. Điều kiện làm sạch màng nhầy hạt phù hợp được chọn là: không bổ sung thêm nước vào hỗn hợp thuỷ phân, nồng độ enzyme pectinase sử dụng là 10 U/g hạt nhầy và thời gian xử lý enzyme trong 75 phút. Tỷ lệ màng nhầy bị loại bỏ chiếm 84.9% tại điều kiện xử lý này. Điều chỉnh nhiệt độ sấy tăng từ 50°C đến 70°C cho thấy thời gian sấy hạt giảm đi 61.9% và hàm lượng phenolic tổng của hạt sấy tăng 20.7%. Tuy nhiên nếu nhiệt độ sấy tiếp tục tăng từ 70°C đến 90°C thì thời gian sấy hạt và hàm lượng phenolic tổng của hạt sấy lần lượt giảm đi 75% và 9.5%. So với mẫu hạt tươi, điều kiện sấy đối lưu phù hợp nhằm thu nhận hạt thanh long sấy giữ hoạt tính kháng oxy hoá cao (hàm lượng phenolic tổng: 588±11 mg GAE/100g chất khô, hoạt tính kháng oxy hoá theo DPPH: 9000±59 μmol TE/100g chất khô) là tại nhiệt độ sấy 70°C và thời gian sấy 8 h. Hạt thanh long sấy là nguồn nguyên liệu giàu dưỡng chất thiết yếu và các hợp chất có hoạt tính kháng oxy hoá. Do đó cần có các nghiên cứu sâu hơn về việc ứng dụng loại nguyên liệu tiềm năng này trong lĩnh vực chế biến thực phẩm.

Từ khoá: Kháng oxy hoá, hạt thanh long, sấy, enzyme, màng nhầy

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