International Journal of Multidisciplinary Research and Publications
ISSN (Online): 2581-6187

Improving the Recovery of Dry Matter and Bioactive Compounds from the Fibrous Strands By-Products of Pumpkin (*Cucurbita Moschata*) by Enzyme Method

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Abstract— *Pumpkin (Cucurbita moschata) is a popular vegetable grown in many parts of the world, including Vietnam. Most of food processing technology uses only the fresh and omits by-products. In particular, the fibrous strands of pumpkin have been proven to have many beneficial nutritional values for human health. The purpose of the study was to improve the recovery of dry matter and beneficial compounds from the fibrous strands by combining two enzymes Cellulase (C) and Pectinase (P). Investigate the influence of extraction conditions including C/P enzyme ratio (0/0, 0/1, 1/0, 1/2, 2/1); hydrolysis temperature (35 – 55ºC); enzyme concentration (0.05 – 0.2%) and hydrolysis time (30 – 150 minutes) were studied. Besides, the ratio of dilution of raw materials/water is 1/2; The natural pH of raw materials. The obtained results show that, with the condition that the C/P enzyme ratio is 1/2; hydrolysis temperature 45ºC; 0.15% enzyme concentration and 90 min hydrolysis time gave the highest extraction efficiency. With this condition, the recovery efficiency of dry matter, total polyphenols, ABTS antioxidant activity and β-carotene, respectively, were significantly higher than those of the control sample without using enzymes. This research result opens the possibility of effective application of pumpkin by-products, avoiding waste and improving the value of this vegetable.*

Keywords— *Fibrous strands of pumpkin, hydrolysis, β-carotene.*

I. INTRODUCTION

Pumpkin (Cucurbita moschata) is widely grown around the world, including Vietnam [1]. In Vietnam, pumpkin is gradually asserting an important position in agricultural production with an area of 42 thousand hectares in 2020, the average yield of 15.35 tons/ha, the total output of 644.6 thousand tons [2]. Pumpkin is packed with nutrients and beneficial compounds that provide many health benefits. Pumpkin is a rich source of carotenoids, a provitamin A, an antioxidant and a good natural coloring compound present mainly in the fibrous strands, along with other bioactive compounds such as polyphenol compounds, minerals, vitamin C, ... [3] help prevent cancer, blood pressure, support the development of intestinal microflora, neurological disorders or related diseases eyes [4]. Pumpkin contains 3 - 4% of the intestine, and 4 - 6% of the seeds, 10 - 12% of the skin and 79 - 82% of the fresh. In the food processing process, most of us mainly use fruit pulp, which can create 17 - 20% of waste from by-products [5]. This not only causes environmental pollution but also wastes a valuable resource. The above loss and waste not only represent a waste of food goods but also indirectly leads to the waste of important resources such as land, water, fertilizers, chemicals, energy and labor [6]. Although the pumpkin by-products are rarely used, they have been shown to contain about 10 - 40% of carotenoids and provitamin A such as α - carotene, β - carotene, β - cryptoxanthin, lutein, lycopene, etc. applications in many fields [9]. Therefore, pumpkin by-products have attracted the great attention of researchers.

The biological treatment of this by-product source is the first choice because of the safety and effectiveness of the process. In particular, the combination of two enzymes, cellulase and pectinase, affects the cellulase of cells and the pectin adhesion layer between cells in the plant tissue structure, making the extraction process easier and at the same time obtaining more cell fluid and extract more efficient [11]. Therefore, the study used enzymatic methods, namely the combined use of two enzyme preparations, pectinase and cellulase, in the stage of extracting the fibrous stands from pumpkin to maximize the yield of extracts and compounds. Precious biology from this raw material is made. This study not only demonstrates the effectiveness of enzymatic hydrolysis but also helps provide important information about the nutritional composition and beneficial compounds from the intestines of pumpkin, creating a premise to diversify more food products from by-products, helping to avoid waste and contribute to environmental protection.

II. MATERIALS AND RESEARCH METHODS

A. Material

F1 hybrid pumpkin variety is used for research, grown at Hanh My Cooperative in My Long Bac commune, Cau Ngang district - Tra Vinh province.

Enzyme: Cellulase enzyme (from *Trichoderma* mold, Novozymes brand, Denmark), the active pH ranges from 3 ÷ 7, temperature about 40 ÷ 50ºC, specific activity according to analytical results is 5021 U/ml. Pectinase enzyme (from Aspergillus mold strain, Novozymes company, Denmark), active pH range 3 ÷ 5, operating temperature 40 ÷ 50ºC, specific activity according to analytical results is 4751 U/ml.

Chemicals: Foline-Ciocalteu, Na_2CO_3, C_2H_5OH, standard solution of NaOH and 0.1 N H_2SO_4 provided by Merck,

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Germany; Gallic acid, trolox and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) were supplied by Sigma-aldrich, USA; n-Hexan was provided by Fisher, USA. Other chemicals are supplied from China.

B. Research Methods

Prepare materials: After purchasing, pumpkins are washed, peeled, halved and the intestines are collected. Next, the ingredients are added to water according to the ratio of pulp: pure water is 1: 2. After that, the mixture is pureed for 2 minutes. The mixture was then homogenized for 2 min by a homogenizer to obtain a homogeneous mixture prepared for hydrolysis. The mass of each sample is 100 grams with the natural pH of the material.

Analytical methods:

Moisture determination method: The moisture content of the extract will be determined by drying to constant weight at 105^{o}C [11].

Determination of total polyphenols (TPC): TPC content was determined by the Foline-Ciocalteu method [12].

Determination of free radical scavenging capacity by ABTS radical cationic decolorization: The free radical scavenging activity was determined by the method described by Re et al. [13].

Determination of β-carotene content: The content of beta-carotene was determined according to the description of Suo et al. [14].

The recovery (Y) of the components (Dry matter, TPC, β-carotene, ABTS with trolox equivalent) from hydrolysis was calculated as:

$$Y\ (\%) = \frac{Content\ of\ ingredients\ in\ extract}{Content\ of\ ingredients\ in\ raw\ materials} x100$$

Methods of statistics analysis:

Using the ANOVA analysis method to test the reliability at a 5% significance level to evaluate the difference of the experimental results, using statistical software STATGRAPHICS® Centurion XV (USA).

III. Results and Discussion

A. Results of determination of the nutritional components the fibrous strands of pumpkin.

The content of dry matter and some bioactive compounds in the fibrous strands of pumpkin is used both to evaluate the quality of the raw materials to ensure the homogeneity of the starting material source and to serve as the basis for determining the effectiveness of the raw materials recovery rate of the experiments. The results of the analysis are shown in Table 2

TABLE 1. The nutritional components in the fibrous strands of pumpkin.

Compositions	Unit	Content*
Dry matter	g/100g	14.3 ± 0.02
TPC	mg GAE/100g	454.8 ± 16.1
ABTS antioxidant activity	µmol TEAC/100g	155.7 ± 18.2
β-carotene	µg/100g	733.3 ± 24.4

*Note: *Mean ± standard deviation of three analyses.*

The results from Table 1 show that the fibrous strands of pumpkin contain high dry matter content (14.3 ± 0.02 g/100g), β-carotene (733,3 ± 24,4 µg/100g), TPC (454,8 ± 16,1 mg GAE/100g) and ABTS antioxidant activity (155,7 ± 18,2 µmol TEAC/100g). The results shown in Table 2 show that the fibrous strands contain high dry matter content, high content of β-carotene and total polyphenols. These are important antioxidant compounds in human nutritional transformation such as immune enhancement, and cancer prevention. Similar to Sharma's previous study on dry matter and polyphenol content, but less than carotenoid content [15]. In addition, the content of carotenoids in these materials was reported to be higher than that of the flesh and peel. The report also showed that the polyphenol content in the flesh was higher than that of the flesh and lower than that of the peel [16]. This promises high applicability of this material in enhancing the value of pumpkin by-products.

B. Effect of the enzyme Cellulase/Pectinase (C/P) ratio on the recovery of dry matter and biological compounds in fibrous strands of pumpkin.

The combined use of enzymes in different ratios or alone have shown significant effects on the efficiency of the extraction process. The results are shown in Table 2.

TABLE 2. Effect of enzyme C/P ratio on the recovery (Y%) of the hydrolysis.

C/P ratio	Y (%)*			
	Dry matter (%)	TPC (mg GAE/ 100g)	ABTS (µM TEAC/ 100g)	β-carotene (µg/100g)
0/0	33.9^{e} ± 0.5	29.9^{e} ± 0.5	34.1^{d} ± 0.9	24.8^{e} ± 0.4
0/1	40.7^{c} ± 0.4	43.1^{d} ± 0.1	42.5^{bc} ±0.1	31.3^{d} ± 0.7
1/0	38.3^{d} ± 0.1	44.2^{c} ± 0.8	41.4^{c} ± 0.4	31.9^{d} ± 0.5
1/1	46.4^{b} ± 0.9	46.1^{b} ± 0.3	44.1^{ab} ± 1.1	34.2^{c} ± 0.4
1/2	47.9^{a} ± 0.7	48.7^{a} ± 0.5	45.1^{a} ± 1.4	37.3^{a} ± 0.4
2/1	45.5^{b} ± 0.8	46.8^{b} ± 0.1	42.9^{bc} ± 0.9	35.5^{b} ± 0.4

*Note: * Mean ± standard deviation of three analysis results. Values in the same column with different uppercase letters are significantly different ($p<0.05$) according to Duncan's multi-interval test.*

For the control, the recoveries were all below 30% (Table 3). When using enzymes at different C/P ratios, the recovery efficiency of the components increased significantly. The combination of both enzymes improved the recovery efficiency of the reaction variables, which could explain that pectinase cleaved the pectins that bind the components in the cell structure, helping to reduce the viscosity, and making it easier to recover nutrients and bioactive ingredients [17]. Besides, the cellulase enzyme hydrolyzes cellulose molecules in the cell wall structure, helping to release nutrients in the extract better [18]. The combination of the two enzymes is effective and necessary to aid in the release of intracellular biomolecules and to increase the soluble dry matter content of the extract [19]. The combined use of both enzymes resulted in significantly higher extraction efficiency than using each enzyme alone, which is also seen in our experimental results [19]. Besides, increasing the enzyme ratio of pectinase to be greater than cellulase can help improve extraction efficiency better than doing the opposite at the same concentration of C/P combination. With a C/P ratio of 1/2, the highest recoveries

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were obtained, for dry matter (47.9% ± 0.7), TPC (48.7% ± 0.5), active antioxidant activity ABTS (45.1% ± 0.5), β-carotene (37.3% ± 0.4). With this ratio, the recovery efficiency was higher than the other rates and increased by 14%, 18.8%, 11%, 12.5% respectively compared to the control. This may be related to the influence of the type of substrate involved in the reaction. In addition, the use of a higher ratio of pectinase may help to better break down the cell wall-binding pectins. At the same time, together with the effect of cellulase, the cell wall of the raw material can be disrupted leading to better nutrient release and increased recovery efficiency [19]. Our results are consistent with previous studies by Sharma and Bahramian both showing that the combination of both enzymes increases the extraction yield higher than hydrolysis using only one enzyme or without using enzymes [22]. From the above results, we decided to choose the enzyme C/P ratio of 1/2 as the basis for the next experiments.

C. Effect of hydrolysis temperature on the recovery of dry matter and biological compounds in fibrous strands of pumpkin.

The selection of the appropriate hydrolysis temperature is very important, which directly affects the efficiency of the hydrolysis process. The results showed that the extraction yield of the extract was significantly different between the control sample and the enzyme-treated sample at different processing temperatures. The experimental results are shown in Table 3.

TABLE 3. Effect of hydrolysis temperature on the recovery (Y%) of the hydrolysis

Temperature (ºC)	Y (%)*			
	Dry matter (%)	TPC (mg GAE/ 100g)	ABTS (µM TEAC/ 100g)	β-carotene (µg/100g)
0	34.73^{e} ± 0.7	31.52^{e} ± 0.6	28.49^{f} ± 1.07	27.58^{e} ± 0.1
35	39.57^{d} ± 0.6	45.81^{d} ± 0.5	32.38^{e} ± 0.5	37.21^{d} ± 0.8
40	44.77^{c} ± 0.4	47.71^{c} ± 0.4	39.06^{d} ±0.1	39.69^{c} ± 0.7
45	48.22^{a} ± 0.4	50.42^{a} ± 0.4	48.74^{a} ± 0.2	41.88^{a} ± 0.3
50	47.45^{a} ± 0.3	49.40^{b} ± 0.1	45.96^{b} ± 0.8	40.71^{b} ± 0.4
55	45.81^{b} ± 0.5	48.08^{c} ± 0.1	40.61^{c} ± 0.1	39.15^{c} ± 0.3

*Note: * Mean ± standard deviation of three analysis results. Values in the same column with different uppercase letters are significantly different (p<0.05) according to Duncan's multi-interval test.*

At the hydrolysis temperature of 35ºC, the recovery efficiency was higher than that of the control sample, indicating that the effect of temperature on the extraction efficiency is significant. When continuing to raise the temperature, at 45ºC gave the highest Y values (%), specifically for dry matter, TPC, ABTS antioxidant activity, β - carotene, the recovery efficiency was 48.22 ± 0.4%, 50.42 ± 0.4%, 48.74 ± 0.2%, 41.88 ± 0.3% respectively. The reason is that when the temperature is low, the enzyme has not been activated, so increasing the temperature will increase the kinetic energy and the frequency of the enzyme-substrate complex per unit time. The increased temperature also helps to reduce the viscosity of the solution, making it easier for enzymes and solvents to contact the substrate, increasing the ability to hydrolyze. On the other hand, enzymes are proteins in nature, so reaching a certain critical temperature will denature them, causing inactivation and stopping the reaction [22]. High temperature also causes biologically active compounds such as phenolic compounds, carotenoids, vitamin C, etc. to decrease due to sensitivity to heat, oxidation, polymerization, etc. [23]. This rule is true with our research results, when continuing to raise the temperature to 50 and 55ºC, the recovery efficiency tends to decrease. Research results are similar to Liu Yi-don or Zhao Nen et al. Pectinase and Cellulase enzyme hydrolysis for juice yield gives the best hydrolysis temperature results in the range of 40 - 45ºC [24] [25]. To reduce the calorific cost and avoid the changes caused by chemical reactions when increasing the temperature, we choose the temperature of 45ºC as the basis for the next experiments.

D. Effect of enzyme concentration on the recovery of dry matter and biological compounds in fibrous strands of pumpkin.

The combination of two enzymes cellulase and pectinase is a reasonable and necessary job to help release intracellular biomolecules and increase the soluble dry matter content in the extract [19]. Different enzyme concentrations had a positive effect on the recovery yields. The results are presented in Table 4.

TABLE 4. Effect of enzyme concentration on the recovery (Y%) of the hydrolysis.

C/P ratio	Y (%)*			
	Dry matter (%)	TPC (mg GAE/ 100g)	ABTS (µM TEAC/ 100g)	β-carotene (µg/100g)
0	41.39^{d} ± 0.2	33.63^{d} ± 0.4	29.9^{d} ± 0.4	40.64^{d} ± 0.5
0.05	47.86^{c} ± 0.4	46.37^{c} ± 0.9	41.43^{c} ± 0.2	41.41^{cd} ± 0.5
0.1	49.98^{b} ± 0.3	50.05^{b} ± 0.8	51.87^{ab} ± 0.5	43.49^{c} ± 0.5
0.15	51.42^{a} ± 0.9	54.32^{a} ± 0.2	53.05^{a} ± 0.6	48.31^{a} ± 0.5
0.2	49.12^{b} ± 0.7	51.18^{b} ± 0.9	50.72^{b} ± 0.8	44.54^{b} ± 0.4

*Note: * Mean ± standard deviation of three analysis results. Values in the same column with different uppercase letters are significantly different (p<0.05) according to Duncan's multi-interval test.*

At the beginning of the hydrolysis process, the medium has an excess substrate, the reaction rate will be proportional to the concentration of added enzyme. In our study, when increasing the enzyme concentration, the recovery yield was significantly higher than that of the control sample. At the concentration of 0.15%, it showed good extraction ability, helping to obtain more dry matter and biologically active compounds, specifically the recovery efficiency of dry matter, TPC, and ABTS antioxidant activity, β - carotene reached 51.42 ± 0.9%, 54.32 ± 0.2%, 53.05 ± 0.6%, 48.31 ± 0.5%, respectively. As the enzyme concentration increases, the number of enzyme molecules per unit area increases, which allows them to cleave more glycoside bonds. However, up to a certain concentration sufficient for the enzyme to cleave all the bonds, continuing to increase the concentration of the enzyme will lead to excess, inefficiency, and does not make economic sense [26]. When the enzyme concentration is high enough, the hydrolyzed pectinase breaks the protein-carbohydrate complex (glycoprotein: in which protein

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accounts for 36% of the protein in the cell). This results in the release of proteins and negatively charged short-chain pectins in addition to other proteins, which, under acidic conditions, contain phenolic compounds that cause the protein to clump together and reduce its solubility. At the same time, the protein-polyphenol complex is also formed in a poorly soluble state, thereby reducing the loss of protein, phenolic compounds as well as antioxidant activity when the enzyme concentration is too high [27]. Besides, later on, the formed product both acts as a non-competitive inhibitor and reduces the amount of substrate in the medium, leading to the reaction rate at this time being unchanged or reduced [28]. This can be seen at the concentration of 0.2%, there was a tendency to decrease the ability to obtain recoverable values. This result coincides with the information from Khandare et al.'s study, which all showed that biologically active compounds tended to decrease when continuing to increase enzyme concentrations from 0.2 to 0.25% [29]. Therefore, we chose the enzyme C/P concentration of 0.15% (% v/w) as the basis for the next experiment to reduce economic costs while still bringing about the efficiency of the extraction process.

E. Effect of hydrolysis time on the recovery of dry matter and biological compounds in fibrous strands of pumpkin.

Enzyme treatment time is also one of the important factors for enzyme processing. The investigation of the processing time both helps to improve the efficiency of the extraction process and saves time. The results of the survey are presented in Table 5.

TABLE 5. Effect of hydrolysis time on the recovery (Y%) of dry matter and biological compounds in fibrous strands of pumpkin.

Temperature (ºC)	Y (%)*			
	Dry matter (%)	TPC (mg GAE/ 100g)	ABTS (µM TEAC/ 100g)	β-carotene (µg/100g)
0	$41.32^{e} \pm 0.9$	$35.62^{e} \pm 0.4$	$30.35^{e} \pm 0.7$	$38.70^{d} \pm 0.5$
35	$46.55^{d} \pm 0.2$	$46.01^{d} \pm 0.3$	$38.69^{d} \pm 0.6$	$45.36^{c} \pm 0.5$
40	$49.83^{c} \pm 0.7$	$52.19^{c} \pm 0.5$	$52.59^{b} \pm 0.6$	$46.88^{bc} \pm 0.9$
45	$54.33^{a} \pm 0.6$	$55.42^{a} \pm 0.9$	$59.15^{a} \pm 1.0$	$52.29^{a} \pm 0.3$
50	$51.72^{b} \pm 0.4$	$53.59^{b} \pm 0.6$	$54.13^{b} \pm 0.6$	$47.24^{b} \pm 0.2$
55	$50.16^{c} \pm 0.2$	$51.51^{c} \pm 0.2$	$50.85^{c} \pm 0.5$	$46.19^{bc} \pm 0.8$

*Note: * Mean ± standard deviation of three analysis results. Values in the same column with different uppercase letters are significantly different ($p<0.05$) according to Duncan's multi-interval test.*

From the research results, we found that time is also a factor that greatly affects the efficiency of hydrolysis. At 30 minutes, Y (%) was low but also showed a difference compared to the control sample. As the hydrolysis time continues to increase, the efficiency of the process increases, it can be seen that the efficiency of the process is significantly influenced by the hydrolysis time. The short time is not enough for the enzyme to work and it does not completely hydrolyze all the substrates in the sample. With prolonged time, the more substrate and enzyme are in contact, breaking the cell wall will release phenolic compounds from the phenolic compounds in the conjugated form. In addition, there is a conversion of phenolic compounds in the insoluble to soluble form. The degradation of lignin leading to the release of phenolic acid derivatives or the generation of new phenolics also contributed to the increase in TPC in the recovered solution. With a longer time, the polyphenol content will decrease due to the degradation of phenolic compounds under atmospheric conditions in the presence of oxygen, thereby affecting the antioxidant capacity of the compounds [30]. In our study, when increasing the time to 90 minutes, the extraction efficiency was highest with the recovery efficiency reaching 54.33 ± 0.6%, 55.42 ± 0.9%, 59.15 ± 1.0%, 52.29 ± 0.3%, respectively. The content of carotenoids in the solution tends to decrease with the processing time because when extracted for a long time, they are easily destroyed by agents such as pectolytic reactions, light, temperature, oxidation, etc... [23] This can be seen when increasing the time to 120 and 150 minutes, both wasted time and did not achieve the desired extraction efficiency. On that basis, we chose a hydrolysis time of 90 min as the basis for hydrolysis.

IV. CONCLUSION

The results obtained in this study demonstrated the ability to enhance the efficiency of fibrous strands extraction by using the combination of Cellulase and Pectinase enzymes to degrade the cell wall. The best hydrolysis conditions were found: hydrolysis temperature 45ºC, enzyme concentration 0.15% (v/w) used in 90 minutes treatment time. At this condition, the recoveries in terms of dry matter, total polyphenol, ABTS and β-carotene antioxidant activities reached 554.33 ± 0.6%, 55.42 ± 0.9%, 59.15 ± 1.0%, 52.29 ± 0.3%, increased by 20.43%, 25.52%, 25.05%, 27.49% respectively compared to the control sample without using enzymes. Our findings help to better understand the applicability of both cellulase and pectinase to recover healthy nutritional components from the fibrous strands of pumpkin. Besides, it has also been proven that this is a potential source of by-products to develop into products to increase economic efficiency and improve the value of pumpkins.

ACKNOWLEDMENT

We are very grateful for the support of Le Thi Be Duy and Ngo Thi Hang, students of Tra Vinh University.

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