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Study on extraction Anthocyanin from red Artichokes (*Hibiscus Sabdariffa Linn)* and apply to produce fermented beverage

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Abstract: Anthocyanin is a compound which dissolves in water and creates multicolor fruit. Anthocyanin has many precious biological activities such as: high antioxidant capacity, reduce the decline of resistance, anti-inflammation, restrict the growth of cancer cells. Anthocyanin can be extracted in red artichokes with high concentrations.In this study, anthocyanin extracted and used to produce the biological active beverage that would enhance the value of red artichokes. Anthocyanin is extracted from red artichokes by water in experimental conditions as follows 80^0C of temperature, 100 minutes of extraction time, 1/160, $g.ml^{-1}$ for the rate of dry materials and solvent, pH = 3.5. The results of fermentation study are as follows 2% of yeast extraction rate; pH = 4; 5 days of fermentation time for the highest quality beverage.

Key words: anthocyanin; red artichokes; fermented beverage; extraction; ferment.

I. Introduction

Red artichoke is a type of flower with a sour taste; has the effect of heat clearing, refreshment, diuretic; very beneficial for health. According to Herrear et al. (2004) [3] as well as Mc Kay et al. (2010) [5] demonstrated that anthocyanins in red artichoke flowers have antibacterial, antifungal, anti-inflammatory, and antioxidant properties, prevent cardiovascular disease, enhance digestive function, laxative, lower blood pressure, lower blood cholesterol, prevent arteriosclerosis and protect liver cells... Therefore, the study of extracting this biologically active natural ingredient is great interest.

Fermented beverage is a drink that has existed for a very long time in Eastern European countries, especially in Russia, fermented apple juice has existed for more than 1000 years. As the food industry develops, this drink is more popular and diverse in quality and type. Vietnam is a country with a hot and dry tropical climate, so the demand for soft drinks is huge. The source of fruit and vegetable materials in Vietnam is extremely rich and diverse, which can be fully met for stable and long-term production. On the other hand, in recent years, the demand for alcoholic beverages in our country is increasing day by day. Low-alcohol fermented fruit juice is a new product with high nutritional value and health benefits. This product is currently a very popular beverage in the world market, especially in the UK, France, and the US. Consumers love this product not only because of its nutritional value but also because it is a cheap drink, suitable for all ages. The alcohol content is not high in the product, which stimulates digestion, making the meal delicious without causing intoxication like some other alcoholic beverages.

Therefore, the study of anthocyanin extraction to produce fermented beverage from red artichoke flowers is very necessary and completely consistent with the general development trend of the world as well as Vietnam.

II. Material and Methods

2.1. Material

This study was conducted on the raw material source of dried red artichoke flower (*Hibiscus Sabdariffa Linn*) purchased at Roselle Vietnam Trading and Service Joint Stock Company, Danang city, Viet Nam.

2.2. Methods

2.2.1. Determination of moisture

The moisture content of red artichoke flowers (%) was determined based on the principle of drying the sample at 105^oC to evaporate all the water vapor [2]. Weigh the ingredients before and after drying, then calculate the percentage of water in the material. Humidity (X%) is determined by the following formula:

$$X\ (\%) = \frac{(m_1 - m_2) * 100\%}{m_1} \quad \text{(Equation 1)}$$

Including:

m_1: Weight of sample before drying, g

m_2: Weight of sample after drying at 105^{o}C for 3 hours, g.

2.2.2. Determination of total acids

Based on the neutralization reaction of the acids present in the sample with NaOH alkaline solution (0.1N) with bromothymol blue as indicator. From the amount of alkaline consumed, calculate the total amount of acid present in the sample [1].

The amount of total acid (Ax, g/l) is calculated by the formula:

$$\text{Ax (g/l)} = \frac{n * K * 1000}{V} \text{ (Equation 2)}$$

Including:

Ax: Amount of acid in 1 liter of product, g/l

n: Volume of NaOH (0,1N) consumed in the determination of the real sample, ml.

V: Amount of sample to be analyzed, ml

K: Number of grams of acid corresponding to 1ml of NaOH (0,1N).

2.2.3. Determination of total proteins

Total nitrogen content in artichoke flower was determined by Kjeldal method [2] and determined by the formula:

$$\text{X(\%)} = \frac{(a-b) * 0{,}0014 * 100 * 6{,}25}{n} \text{ (Equation 3)}$$

Including:

a: Volume of H_2SO_4 0,1N put into the conical flask, ml.

b: Volume of NaOH solution (0,1N) for quantitative determination of excess acid, ml.

0.0014: The number of gram nitrogen corresponds to 1 ml of H_2SO_4 0,1N.

n: The number of grams of material contained in the sample to be analyzed,

($n = \frac{10}{100} * 40$).

6.25: Nitrogen to protein conversion factor.

2.2.4. Determination of anthocyanin content

a. Preparation of anthocyanin extract from red artichoke flower

Take 1g of dried artichoke flowers and crush them, then put them in a 250 ml flask, add 160 ml of water, and steam them in an automatic thermostatic bath. Depending on the experimental conditions, anthocyanin extraction was carried out at specific time intervals, temperatures and pH.

The solution after extraction was filtered with filter paper and used for titration to determine anthocyanin content.

b. Determination of total anthocyanin content

Anthocyanin content in red artichoke flower extract was determined by colorimetric method [1].

Total anthocyanin content (X, mg/l) was calculated by the formula:

$$\text{X} = 20 * \left[A_{520nm}^{HCl} - \frac{5}{3} A_{520nm}^{SO2} \right] \text{ (Equation 4)}$$

Including:

X: Total anthocyanin content, mg/l.

A_{520nm}^{SO2} : Absorbance of the sample in metabisulfite solution at 520 nm.

A_{520nm}^{HCl} : Absorbance of the sample in HCl solution at 520 nm.

2.2.5. Determination of total sugar

The amount of total sugar in the extract of red artichoke flower was determined by Bx meter [1].

Using a glass cylinder rinse 3 times with the solution to be measured. Fill the foam tube with the solution, then gently insert the Bx meter, after the Bx meter is kept in equilibrium, read on the Bx line.

2.2.6. Determination of ancohol content

The ethylic alcohol content (%v) of the fermented beverage is determined by distillation of the product, using an alcohol meter to measure the alcohol content of the distillate remaining after reconstitution original volume [1].

III. Results and Discussion

3.1. The chemical composition of red artichoke flowers

The chemical components in red artichoke flowers can affect the extraction of anthocyanin. Hence, this study determined the chemical components of this flowers and the results are shown in Table 1.

Table 1. Some basic chemical components of red artichoke flower

No	Composition	Unit	Content
1	Humidity	% mass	11.8
2	Protein	% mass	0.9
3	Anthocyanins	% dry matter	1.4
4	Acid total	% dry matter	28.5

From Table 1, the result shown that the total protein content is not significant (0.9%). Acid total were obtained (28.5%). According to E. Nicole Bridgers et al. (2010) [4], the anthocyanin structure is stable when pH ranges from 1 to 3. However, pH is > 4, the anthocyanin structure is unstable and can be denatured. Therefore, the presence of acid in the extract will create a suitable pH (<4), contributing to stabilizing the state and structure of anthocyanin. Besides, the anthocyanin content is quite large (1.4%). According to Nguyen Di Khanh (2015) [9] anthocyanin in vegetables and fruits are compounds rich in antioxidant activity, preventing diabetes so they can be applied to the production of drugs to treat diabetes, heart disease and cancer.

3.2. Studyon extraction conditions of anthocyanins from red artichoke flowers

3.2.1. The effect of pH on anthocyanin extraction efficiency

Anthocyanin has polar polyphenol functional groups that dissolve well in polar solvents.In this study, the water was chosen as the solvent to extract anthocyanin. The choice of solvent depends on many factors, for example, organic solvents often have low evaporation temperatures, thus saving energy for later product collection. However, because of its good permeability, when extracting with organic solvents, in addition to the necessary components, there are many undesirable components such as proteins, chlorophyll... entering the extract, causing difficulties in the extraction process refined products and some organic solvents that are generally not safe for use in food. Water is an inexpensive, food-safe solvent, so it was suitable for use in this study.

Anthocyanin is stable in pH < 4 [8], the extraction process was carried out with acidified aqueous solvents with pH from 1; 1.5; 2; 2.5; 3; 3.5 and 4 in this study. The results are shown in Figure 1.

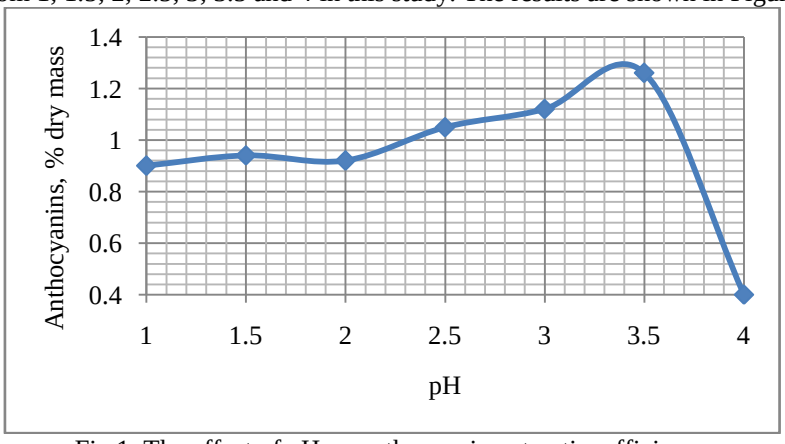

Fig 1. The effect of pH on anthocyanin extractionefficiency

The results obtained in Fig 1 presented that the anthocyanin recovery efficiency increases gradually and reaches the maximum at pH = 3.5. This is completely consistent with the research results of E. Nicole Bridgers et al. (2010) [4]. When the pH range from 1 to 3, the anthocyanin structure is stable and the recovery efficiency is also increased when pH >4. Therefore, the pH for anthocyanin extraction will be chosen as 3.5 in this study.

3.2.2. The effect of temperature on anthocyanin extraction efficiency

Temperature is also an important factor, a decisive influence on the efficiency of the extraction process. Normally, when increasing the temperature, the extraction efficiency will be high, but this rule is a limiting factor. When the temperature is too high, other unnecessary reactions may occur, making it difficult for the technological process, changing the properties of the components to be extracted and consuming energy. Therefore, it is necessary to choose the appropriate temperature level so that the extraction efficiency is the highest while limiting the adverse factors. This study conducted anthocyanin extraction at temperatures of 60^{o}C, 70^{o}C, 80^{o}C, 90^{o}C, and 100^{o}C and obtained the results as shown in Figure 2.

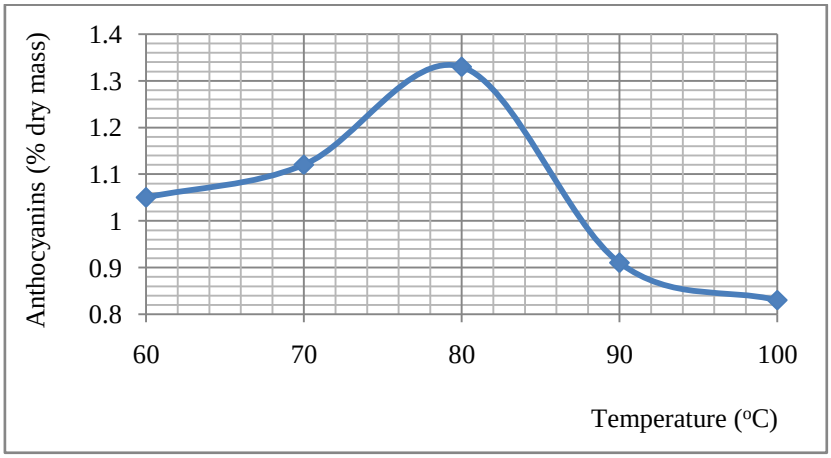

Fig 2. The effect of temperature on anthocyanin extraction efficiency.

From the Fig 2, it can be seen that anthocyanin content have changed when the experiments carried out to different extraction temperatures.Temperature has the effect of increasing the diffusion rate and reducing the viscosity of the solution, making it easier for the solute particles to diffuse between the solvent particles, increasing the diffusion rate and leading to an increase in the ability to separate the cytosol.

The results obtained on the graph clearly show that when increasing the temperature, the anthocyanin content obtained also increases gradually and reaches the maximum at 80^{0}C. However, this does not mean that increasing the temperature will increase the extraction efficiency, because high temperature can change the properties of anthocyanin. In general, the obtained anthocyanin content is relatively high when the temperature fluctuates in the range of 60 - 80^{0}C. This is completely consistent with the research results of Gongjian Fan et al (2008) [6]. Therefore, according to the obtained experimental results, the extraction temperature was selected as 80^{0}C.

3.2.3. The effect of time on anthocyanin extraction efficiency

Zhang Hua et al. (2013) [7] confirmed that the extraction time of anthocyanin depends on the concentration of solvent used, extraction temperature and pressure, but normally when using solvent is water (pH = 3.5), the extraction time is about 60 - 120 minutes. In this study, the extraction times tested 60, 70, 80, 90, 100, 110, 120 minutes and the results are shown in Figure 3.

According to the Fig.3, when increasing the extraction time, the anthocyanin content obtained also increased. Specifically, when changing the time from 60 to 100 minutes, the extracted anthocyanin content was gradually increased and increased by about 1.37 times.Then, the extraction time ranged from 100 to 120 minutes, the obtained tannin content began to decrease gradually. It can be seen that time has a great influence on the efficiency of anthocyanin extraction. When the maximum value was reached at 100 min, the anthocyanin content obtained began to decrease, so prolonging the extraction time was ineffective. In addition, anthocyanin was easily oxidized in the presence of atmospheric oxygen. The extraction time is too long, it will cause anthocyanin denaturation. Therefore, the extraction time was selected as 100 minutes to ensure the highest anthocyanin extraction efficiency.

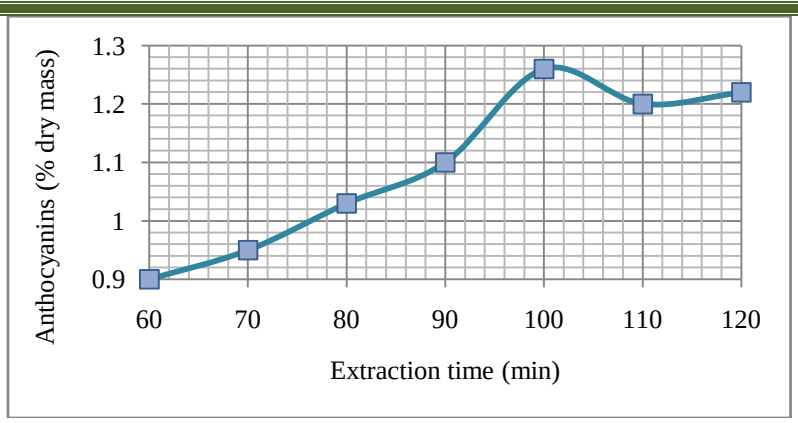

Fig 3. The effect of time on anthocyanin extraction efficiency

3.2.4. The effect of dry material/solvent ratio (g/ml) on anthocyanin extraction efficiency

In this study, the dry material/solvent ratio was investigated, respectively, 1/140; 1/160; 1/180; 1/200; 1/220 g/ml. The results are shown in Figure 4.

The results on the graph show that depending on the different dry material/solvent ratio, the anthocyanin extraction efficiency will be different. This can be explained as follows: using a low dry material/solvent ratio which means a large amount of solvent is used, it will help to thoroughly dissolve the anthocyanin content in the raw material, leading to efficient extraction, get a raise. Besides, when using this large ratio, the solvent will not be enough to dissolve all anthocyanin in the raw materials, so the extraction efficiency is low. But when the highest extraction level is reached, continuing to increase the volume of solvent will not be effective because then some impurities will be extracted and waste solvent.

To ensure the highest extraction efficiency of anthocyanin from red artichoke flowers, the ratio of dry material/solvent was chosen to be 1/160, g/mlin this study.

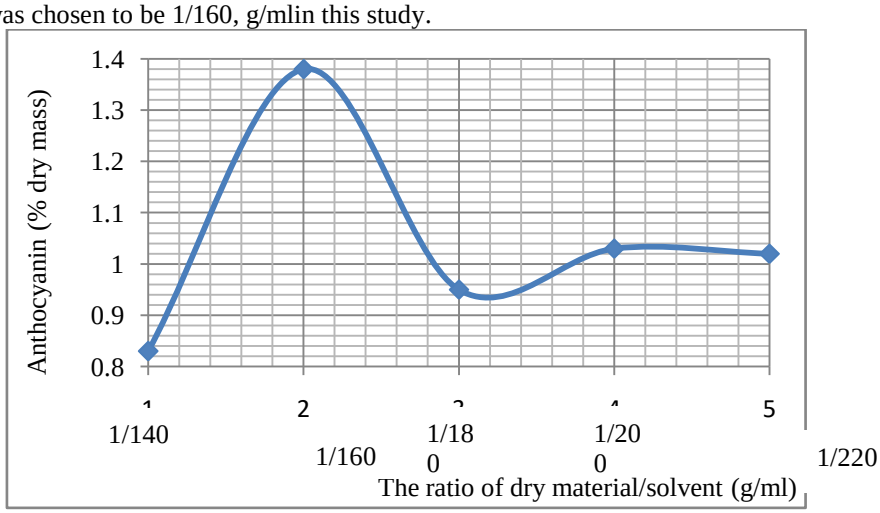

Fig 4. The effect of dry material/solvent ratio on anthocyanin extraction efficiency

3.3. Application to produce fermented beverage from red artichoke flower

Fermented beverage from red artichoke flower with low alcohol (3-6%) has both a refreshing effect and provides the body with a number of substances with high biological activity. Therefore, in this study, the conditions of fermentation were studied (the percentage of yeast added and the fermentation time) in order to produce the highest quality product.

3.3.1. The effect of the percentage of yeast

The amount of initial yeast culture is closely related to the fermentation process. If the initial amount of yeast culture is too small, the fermentation time is prolonged. On the contrary, a high density of cultured yeast can cause turbidity of the product and poor taste. Therefore, the selection of the appropriate yeast culture ratio is crucial to the product quality.

In this study, the submerged yeast *Saccharomyces carlsbergensis* was used. Surveying the percentages of yeast added as follows: 1%, 2%, 3%, 4% at pH 4, the sugar concentration of the fermentation solution was

adjusted to 20°Bx. Then measure the alcohol and residual sugar content. The results are shown in Figures 5 and 6.

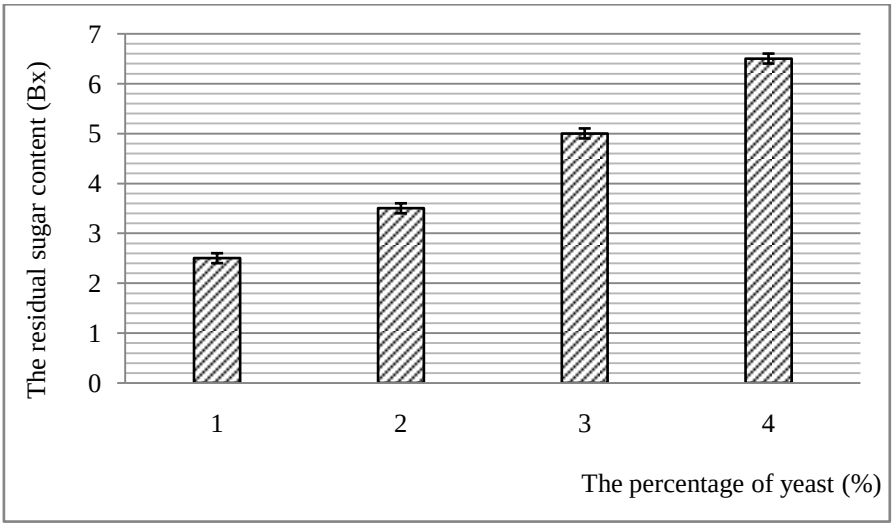

Fig 5. The effect of the percentage of yeast added and the alcohol content in the product.

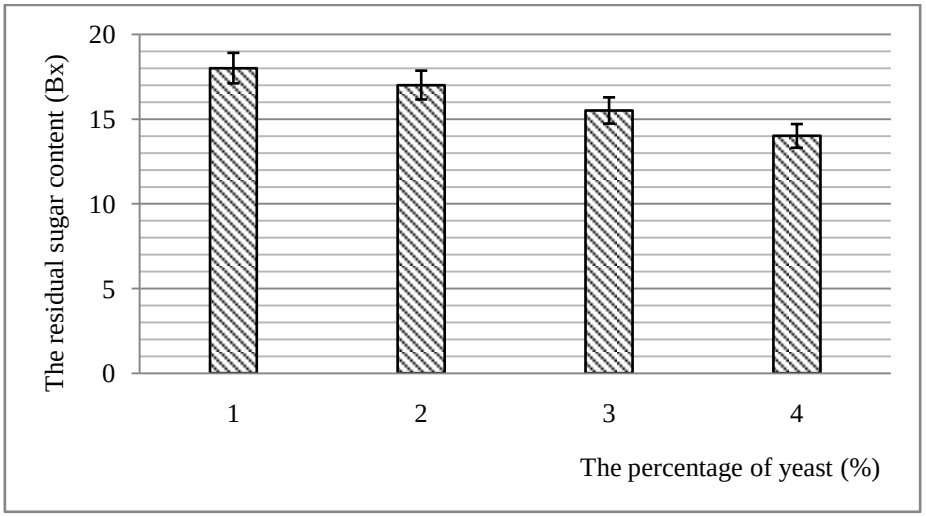

Fig 6. The effect of the percentage of yeast added and the amount of residual sugar in the product

In general, the more yeast is used, the more alcohol is formed and the smaller the residual sugar. However, according to the requirements of fermented beverage, the alcohol content is in the range of 3 - 6% and the sugar level is 15 - 18°Bx, so only 2% and 3% yeast rates are suitable. In two cultures with 2% and 3% yeast rates, 2% samples gave better sensory (smell and taste), so in this study, 2% yeast was selected.

3.3.2. The effect of fermentation time

Fermentation time has a decisive influence on the quality of low alcohol fermented beverage. If the fermentation time is short, the product has not achieved the characteristic properties (alcohol, color, smell, taste). If the fermentation time is long, the alcohol level is high and the taste is not good. Therefore, determining the appropriate fermentation time is necessary.

Conduct survey on fermentation time 4, 5, 6 and 7 days, respectively. The results are shown in Figures 7 and 8.

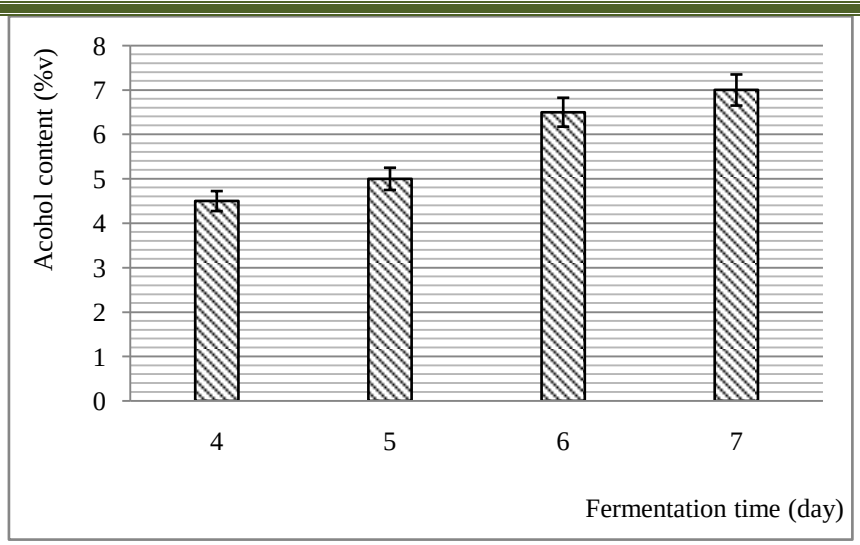

Fig 7. The effect of fermentation time and alcohol content in the product

Fermentation time has a direct effect on alcohol formation. The longer the fermentation time, the greater the alcohol produced, and vice versa, the shorter the fermentation time, the lower the alcohol content. At the fermentation time of 4 days and 5 days, the amount of substrate has not been broken down, so the alcohol level is satisfactory (in the range of 3 - 6%). In the remaining samples, the fermentation time is long, the amount of alcohol produced is very large, causing the product to have a strong odor. Based on the alcohol requirements of the fermented beverage, both samples with fermentation time of 4 and 5 days are suitable.

In the sample with a fermentation time of 4 days, due to the short fermentation time, the sugar content was not completely resolved, and the residual sugar content was high. Samples with fermentation time of 5 days, the sugar content gradually decreased to only 16^0Bx. The remaining samples due to the long fermentation time, the sugar metabolism is high, so the residual sugar content is only 14.5 and 13^oBx, the product starts to have a sour taste and strong alcohol smell. Based on the requirement of sugar content of fermented beverage, two samples of 4 and 5 days fermentation are appropriate.

However, in the two samples fermented for 4 and 5 days, the 5-day fermentation sample gave the product a harmonious taste and typical aroma of fermented beverage, so in this study, the fermentation time was selected as 5 days.

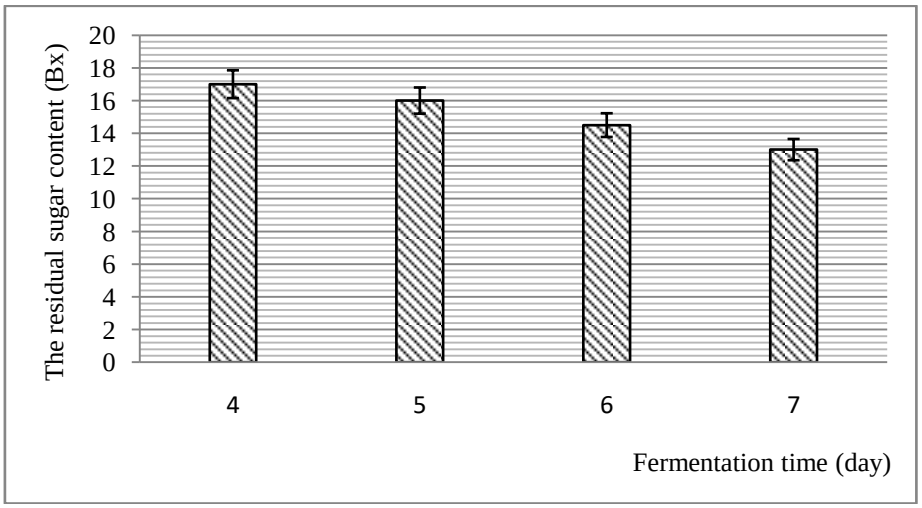

Fig 8. The effect of fermentation time and residual sugar in the product.

3.4. Technological process for anthocyanin extraction and fermented beverage

The extract obtained by extraction method has low dry matter concentration, so it is not convenient for storage and use. Therefore, in this study, the application of the extract is proposed by producing fermented beverage products that take advantage of the biological activity in the extract and create diversity for the product.

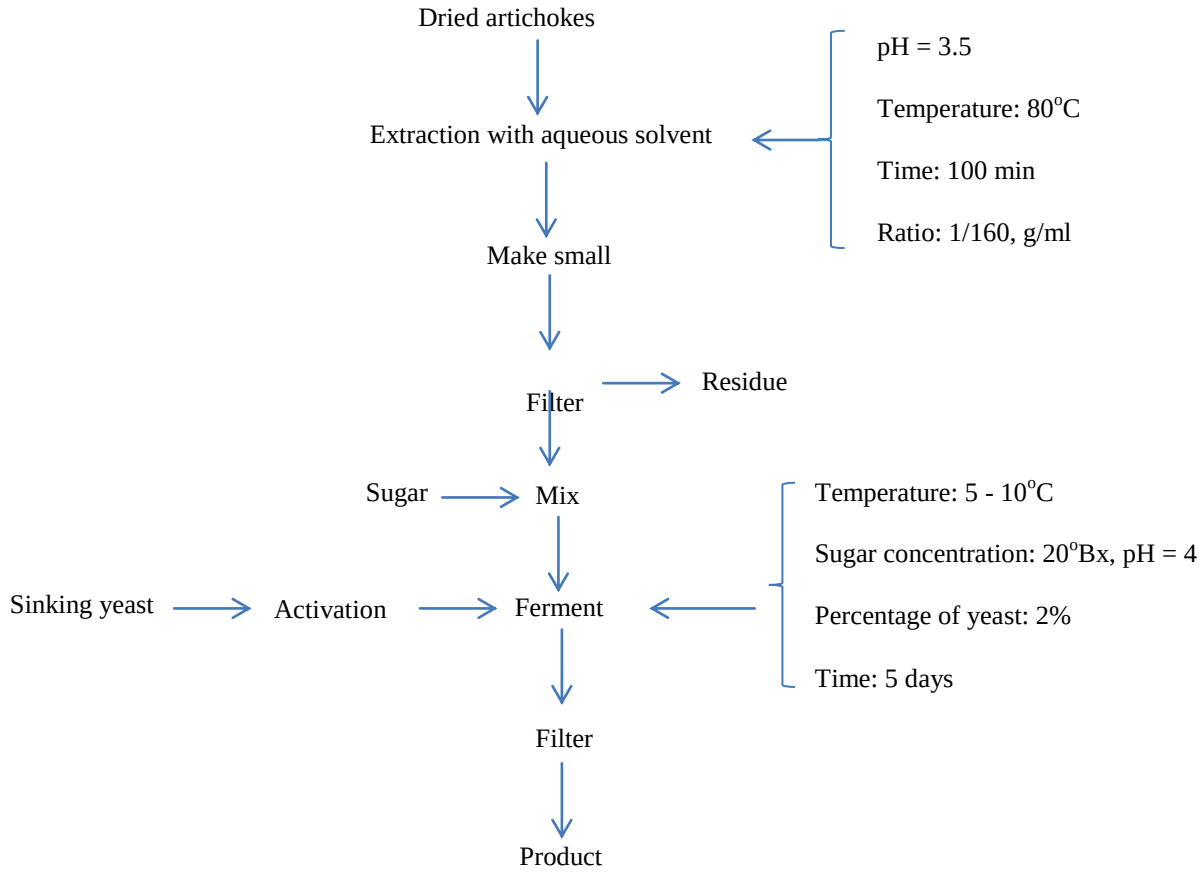

Fig 9. Technological process for anthocyanin extraction and fermented beverage

Fig 10. Beverage products fermented from red artichoke flowers.

IV. Conclusion

This study carried out the extraction of anthocyanin from red artichoke flowers with water solvent at 80^0C for 100 minutes and proposed a process to produce fermented beverage with low alcohol content. This is a product with very good antioxidant activity, which promises to contribute to improving consumer health and gradually replace current beverage products using unsafe colorants.

Acknowledgements

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