RESEARCH ON THE EFFECT OF THERMAL PASTEURIZATION ON AEROBIC MICROORGANISMS COUNT, BETANIN CONTENT, AND PHENOLIC CONTENT OF 200ML BOTTLED BEETROOT EXTRACT

NGHIÊN CỨU ẢNH HƯỞNG CHẾ ĐỘ THANH TRÙNG NHIỆT NÓNG TỚI CHỈ TIÊU VI SINH VẬT HIẾU KHÍ, HÀM LƯỢNG BETANIN, PHENOLIC CỦA DỊCH CỦ DỀN ĐỎ ĐÓNG CHAI 200ML

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ABSTRACT

The study focused on investigating the high-temperature pasteurization process on aerobic microorganisms, betanin content and phenolic content of 200ml bottled beetroot extract to select the pasteurization mode $\frac{15-15-15}{85^oC}$ and the betanin content (mg/100ml) obtained was 4.096 ± 0.075[a], 3.939 ± 0.057[b], 3.863 ± 0.049[c], 3.789 ± 0.065[d], respectively; and phenolic content (mg galic/100ml) was 19.257 ± 0.407[a], 17.452 ± 0.374[b], 16.919 ± 0.298[c], and 15.883±0.289[d], respectively, during the stabilized time of 0 days, 7 days, 10 days, and 15 days.

Keywords: *Betanin, phenolic, beetroot, pasteurization, stabilization.*

TÓM TẮT

Nghiên cứu tập trung vào khảo sát chế độ thanh trùng nhiệt nóng ảnh hưởng tới chỉ tiêu vi sinh vật hiếu khí, hàm lượng betanin và hàm lượng phenolic của dịch củ dền đỏ đóng chai 200ml nhằm lựa chọn được chế độ thanh trùng $\frac{15-15-15}{85^oC}$ và thu nhận hàm lượng betanin (mg/100ml) lần lượt là 4,096 ± 0,075[a]; 3,939 ± 0,057[b]; 3,863 ± 0,049[c]; 3,789 ± 0,065[d]; hàm lượng phenolic (mg galic/100ml) lần lượt là 19,257 ± 0,407[a]; 17,452 ± 0,374[b]; 16,919 ± 0,298[c]; 15,883 ± 0,289[d] trong thời gian bảo ôn lần lượt 0 ngày, 7 ngày, 10 ngày, 15 ngày.

Từ khoá*: Betanin, phenolic, củ dền đỏ, thanh trùng, bảo ôn.*

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1. INTRODUCTION

Beetroot is an herbaceous plant with swollen, fleshy, sweet roots that are dark red in color. Its scientific name is *Beta vulgaris* L. The beetroot has been cultivated in North Africa for thousands of years, commonly found along the Mediterranean coast, in European countries,

and later spread to England, Western Asia, India, and Eastern China for medicinal purposes. It was first used as food around the 3rd century AD. In Vietnam, beetroot is rarely cultivated in the northern regions, and is mainly grown in Da Lat and some districts like Duc Trong and Don Duong in Lam Dong province. Beetroot is ranked among the top 10 vegetables rich in antioxidants, with a phenol content ranging from 50 to 60µmol/g of dry weight. Miller et al. confirmed that beetroot extract contains more antioxidants compared to extracts from other vegetable peels [15]. In beetroot, the majority of antioxidant compounds include phenolics, betanin, and others; these compounds are easily lost during heat processing, exposure to light, and oxygen over time. Processing methods have a significant impact on the phenolic content in beetroot treatment [11]. Thermal pasteurization is one of the safe methods for extending the shelf life of fruit and vegetable juice by inactivating microorganisms and enzymes that cause spoilage [13]. In general, heat treatment reduces the nutritional quality of food, but there are cases where it can enhance the food quality after thermal processing [11]. The factors that alter the quality of food are the time and temperature of food processing [4]. The nutrient content of food decreases significantly when processed at high temperatures [8]. Therefore, a study surveys the effect of thermal pasteurization on the aerobic microorganism count, betanin content, and phenolic content of 200ml bottled beetroot juice is necessary to determine the appropriate pasteurization process.

2. METERIALS AND METHODS

2.1. Materials and equipment

Materials: Concentrated beetroot peel extract, beetroot.

Chemicals: Gallic acid equivalent (GAE), phenolic, Folin - Ciocalteu, PEG5000 (polyethylene glycol), $(NH_4)_2SO_4$, $NaHCO_3$, chloroform, food additive vitamin C, potassium sorbate and erythorbate...

Sterile saline solution: Dissolve 9g of NaCl in 1 liter of distilled water. Draw 9ml into each test tube and sterilize by autoclave sterilization at 121°C for 15 minutes.

PCA environment (Plate Count Agar): The mixture consists of: casein pepton: 5g; yeast extract: 2.5g; pure glucose: 1g; agar: 15g, is dissolved in 1000ml of distilled water, with a pH of 7. Boil until fully dissolved and sterilize by autoclave sterilization at 121°C for 15 minutes.

Equipment: Incubator, spectrophotometer (Genesys 10S UV-VIS), moisture balance, analytical balance (3 decimal places), pasteurization equipment, centrifuge (15000rpm).

2.2. Experiments and measurements

2.2.1. Determination of phenolic content [6]

- *The phenolic content of beetroot juice* is determined by: Take 0.5ml of beetroot extract, add 2.5ml of 10% Folin-Ciocalteu reagent and 2.5ml of 7.5% $NaHCO_3$, then incubate at 45°C for 45 minutes. Measure the absorbance at 765nm. Gallic acid is used as the standard for calibration.

The phenolic acid content is calculated from the calibration curve equation of gallic acid (0 - 20µg/ml): $y = 110.58x - 0.1407$; $R^2 = 0.9986$. In which: x: absorbance at 765nm, y: the concentration of gallic acid), and the phenolic acid content is expressed as gallic acid equivalent (GAE) (mg per gram of dry weight). The formula to calculate:

$$\frac{C_x \times V_{solution}}{m \times a \times 1000}$$

In which:

C_x: The concentration of gallic acid derived from the calibration curve equation (µg/ml)

$V_{solution}$: The total volume of the solution obtained after centrifugation (ml)

m: The mass of the sample extracted (g)

a: The percentage of dry matter dissolved in the sample (%)

- *The phenolic content of the concentrated beetroot extract* is determined by: Dissolve 1 mg of the extract sample in 1ml of DMSO (Dimethyl sulfoxide) to prepare the test sample. Take 0.5ml of the test sample, add 2.5ml of 10% Folin-Ciocalteu reagent and 2.5 ml of 7.5% $NaHCO_3$, shake well, then incubate at 45°C for 45 minutes, avoiding light. Measure the absorbance at 765nm. Use gallic acid as the standard. The formula to caculate:

$$\frac{C \times V \times n \times \%_{dry}}{m \times 1000}$$

In which:

C: The sample concentration when substituted into the standard curve (µg/ml)

V: The volume of the sample dissolved in DMSO (ml)

n: The sample dilution factor

$\%_{dry}$: The dry matter content in the extract (%)

m: The mass of the extract used for determination

1000: Converting mg to g

2.2.2. Determination of betanin content [3]

- The betanin content of beetroot juice is determined by: 2ml of beetroot juice is mixed with 2g of PEG5000 and 2g of $(NH_4)_2SO_4$. Then, 10ml of distilled water is added, and the mixture is filtered through a membrane. The solution is stirred thoroughly for 1 hour and left to stand for 3 hours to allow phase separation. The upper phase is collected, followed by the addition of 2ml of chloroform. The mixture is shaken thoroughly and centrifuged at 15,000rpm for 10 minutes. Nitrogen gas is bubbled into the collected upper-phase sample to remove chloroform. The optical density (OD) is measured at a wavelength of 536nm.

The betanin concentration is determined using Lambert Beer's law: $OD_{536} = C \times l \times \varepsilon$.

In which:

OD_{536}: absorbance at 536nm

C: concentration (M)

l: Cuvette path length (cm)

$\varepsilon = 6{,}5 \times 10^4\ L \times mol^{-1} \times cm$

- The betanin content of the concentrated beetroot extract is determined by: 2ml of beetroot juice is mixed with 2g of PEG5000 and 2g of $(NH_4)_2SO_4$. Then, 10ml of distilled water is added, and the mixture is filtered through a membrane. The solution is stirred thoroughly for 1 hour and left to stand for 3 hours to allow phase separation. The upper phase is collected, followed by the addition of 2ml of chloroform. The mixture is shaken thoroughly and centrifuged at 15,000rpm for 10 minutes. Nitrogen gas is bubbled into the collected upper-phase sample to remove chloroform. The optical density (OD) is measured at a wavelength of 536nm.

The betanin concentration is determined using Lambert Beer's law: $OD_{536} = C \times l \times \varepsilon$.

In which:

OD_{536}: absorbance at 536nm

C: concentration (M)

l: Cuvette path length (cm)

$\varepsilon = 6{,}5 \times 10^4\ L \times mol^{-1} \times cm$

Calculation result: The betanin content in the extract is calculated by formula:

$$100 \times \frac{C}{0{,}002}(\%)$$

2.2.3. Method for determining total aerobic microorganisms

Diluting the sample to concentrations of 10^{-4}, 10^{-5}, 10^{-6} to determine the total aerobic microorganisms and perform the inoculation and incubation according to TCVN 11039-1:2015.

The formula: $N = \frac{\sum C}{n_1 v d_1 + ... + n_n v d_n}$ (CFU/g or CFU/ml)

In which:

N: The number of bacterial cells in 1g or 1ml of the sample

C: The total number of colonies counted on the selected petri dishes (approximately 25 - 250 colonies/plate)

n_i: the number of petri dishes inoculated at the first dilution; d_i: the corresponding dilution factor; v: the volume of sample solution (ml) inoculated into each plate.

2.2.4. Pre-treatment of the red beetroot flesh to obtain the juice

The beetroot is cleaned, peeled; the flesh is separated. Then pre-treated by cutting into pieces with dimensions of 1x1cm² to prepare a sample weighing 200g which is blanched in water at 85°C for 1 minute and the process is continued by pressing to separate the pulp. The juice obtained after pressing is filtered to collect the beetroot extract. Betanin and phenolic acid levels are measured in the final extract.

+ Procedure for obtaining the extract from the flesh of the beetroot:

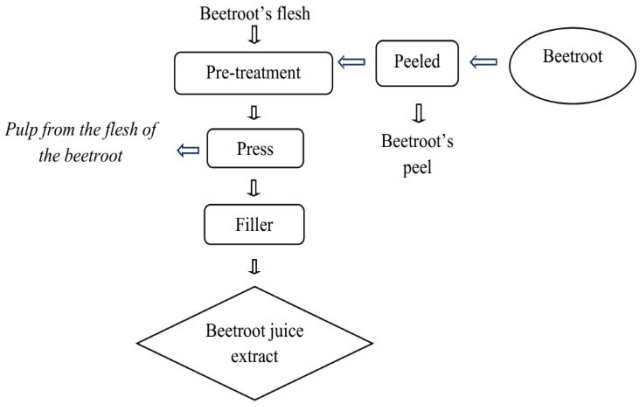

Figure 1. Diagram of the extraction process from the flesh of the beetroot

2.2.5. Standardization of beetroot extract

A 5% concentrated extract of beetroot peel is added to the beetroot juice obtained from Method 2.2.4. The mixture is then formulated with additives at the following concentrations: 0.015% vitamin C, 800mg/L potassium sorbate, and 1500mg/L erythorbate. After standardization, the betanin and phenolic content of the formulated beetroot juice is determined.

2.2.6. Evaluate and select the pasteurization formula

The standardized beetroot juice, prepared according to method 2.2.5, is bottled in 200ml containers and subjected to pasteurization based on the formulas presented in Table 1. The changes in phenolic and betanin content before and after pasteurization are evaluated. Additionally, the levels of aerobic microorganisms are assessed after 7 and 15 days of storage at 37°C, in order to select the most suitable pasteurization formula.

Table 1. Pasteurization formulas

CT1: $\frac{15-5-15}{85^{o}C}$	CT4: $\frac{15-5-15}{90^{o}C}$
CT2: $\frac{15-10-15}{85^{o}C}$	CT5: $\frac{15-10-15}{90^{o}C}$
CT3: $\frac{15-15-15}{85^{o}C}$	CT6: $\frac{15-15-15}{90^{o}C}$

Subsequently, pasteurization is carried out using the selected optimal formula. The phenolic and betanin contents are then evaluated at 0, 7, 10, and 15 days of storage under stabilization conditions.

Description of pasteurization formula $\frac{a-b-c}{t^{o}C}$

In which:

a is the time to raise from ambient temperature to sterilization temperature (minute)

b is the holding time at sterilization temperature (minute)

c is the time it takes to lower the sterilization temperature to 40^{o}C (minute)

t^{o} is the sterilization temperature (^{o}C)

2.2.7. Statistical method for data processing

Data analysis using a one-way analysis of variance (ANOVA) model in Excel 2017 software.

2.2.8. Method of Determining Average Error

The experiments are repeated 3 times. The average error is calculated by standard deviation, excel 2017 software.

3. RESULTS AND DISCUSSION

3.1. Evaluate the betanin content, phenolic content of beetroot peel extract and beetroot juice

The beetroot juice obtained from method 2.2.4 and the concentrated beetroot peel extract were analyzed for betanin and phenolic content, as shown in Table 2.

Table 2. Betanin, phenolic content table

Subject	**Phenolic** (mg/g absolute dry mass)	**Betanin** (mg/g absolute dry mass)
Concentrated beetroot peel extract	14.703 ± 0.105	4.909 ± 0.085
Beetroot juice	7.215 ± 0.061	1.878 ± 0.021

3.2. Study on standardization of beetroot juice before bottling

The standardization of beetroot juice for bottling preparation, as described in method 2.2.5, involves homogenizing and evaluating the phenolic content and betanin of the juice. The results show a phenolic content of 23.794 ± 0.343mg gallic acid/100ml and a betanin content of 4.262 ± 0.068mg/100ml.

3.3. The effect of pasteurization method on the total aerobic microorganisms in 200ml bottled beetroot juice during stabilization time

The 200ml bottled beetroot juice is standardized as described in section 3.2. The samples are pasteurized according to the method in section 2.2.6, and the results are evaluated to compare the effect of pasteurization on the total aerobic microorganisms at the stabilization points of 0, 7, and 15 days, compared to the pre-pasteurization condition, as shown in Table 3.

The beetroot juice samples, before pasteurization, contained aerobic microorganisms at approximately 10^{6}CFU/ml. When they are pasteurized at temperatures ranging from 85°C to 90°C for 5 minutes (represented by pasteurization formulas CT1, CT4), and even when pasteurized at 85°C for 10 minutes (pasteurization formula CT2), the growth of aerobic microorganisms is not controlled. Specifically, the aerobic microorganism count is approximately 10^{3}CFU/ml to 10^{12}CFU/ml at the 7-day and 15-day stabilization points (as shown in CT1, CT4). At the 15-day stabilization point, the aerobic microorganism count is around 10^{3}CFU/ml (as shown in CT2). Pasteurization formulas CT3, CT5, and CT6 effectively inhibited the growth of aerobic microorganisms (no aerobic microorganisms are detected during the 15-day stabilization period).

Moreover, pasteurization formulas CT3, CT5, and CT6 is used to compare the effect of pasteurization on the bioactive compounds betanin and phenolic, with the results shown in Table 4.

Table 3. The effect of pasteurization method on the presence of aerobic microorganisms in 200ml bottled beetroot juice during stabilization time

Pasteurization formula	Total aerobic microorganisms (CFU/ml)			
	After pasteurization	Immediately after pasteurization (0 days of stabilization after pasteurization)	7 days of stabilization after pasteurization	15 days of stabilization after pasteurization
CT1: $\frac{15-5-15}{85^oC}$	$7.8x10^6$	0	$4,8.10^5$	$7.6x10^{12}$
CT2: $\frac{15-10-15}{85^oC}$	$3.6x10^6$	0	0	$2.8x10^3$
CT3: $\frac{15-15-15}{85^oC}$	$8.8x10^6$	0	0	0
CT4: $\frac{15-5-15}{90^oC}$	$8.6x10^6$	0	$2,9.10^5$	$5.3x10^{12}$
CT5: $\frac{15-10-15}{90^oC}$	$4.7x10^6$	0	0	0
CT6: $\frac{15-15-15}{90^oC}$	$7.3x10^6$	0	0	0

Table 4. Survey of phenolic and betanin content in standardized 200ml bottled beetroot juice before and immediately after pasteurization with different formulas

Content	Phenolic	Betanin
After pasteurization	23.794 ± 0.343	4.262 ± 0.068
Before pasteurization CT3: $\frac{15-15-15}{85^oC}$	19.257 ± 0.407	4.096 ± 0.075
Before pasteurization CT5: $\frac{15-10-15}{90^oC}$	18.093 ± 0.215	3.963 ± 0.068
Before pasteurization CT6: $\frac{15-15-15}{90^oC}$	14.990 ± 0.327	3.751 ± 0.088

From the results in Table 4, we have the results in Table 5.

Comparison of phenolic content in fresh and heat-treated beetroot juice samples reveals a clear reduction following heat pasteurization over time [10]. Heating at 70°C for 15 minutes results in a phenolic loss of approximately 0% - 20%. However, heating at 90°C for 15 minutes causes a phenolic loss of about 45% - 47% [10]. This loss is greater than that observed in our study: standardized beetroot juice heated at 85°C for 15 minutes (represented by pasteurization formula CT3) exhibited a phenolic loss of approximately 19%. Meanwhile, heating at 90°C for 15 minutes (pasteurization formula CT6) resulted in a phenolic loss of approximately 37%. In our study, phenolic content decreased by 37% and 24% when samples were pasteurized at 90°C for 15 minutes and 10 minutes, respectively (corresponding to pasteurization formulas CT6 and CT5). The reduction in total phenolic content during heat treatment is consistent with previous findings, which suggest that polyphenols are heat-sensitive and that prolonged exposure to high temperatures may lead to chemical transformations into other phenolic compounds [9].

Table 5. The effect of pasteurization on the remaining betanin and phenolic content in 200ml bottled beetroot juice

Pasteurization formula	% of the remaining betanin and penolic content in the product after pasteurization compared to before pasteurization	
	Phenolic	Betanin
CT3: $\frac{15-15-15}{85^{o}C}$	**81**	**96**
CT5: $\frac{15-10-15}{90^{o}C}$	76	93
CT6: $\frac{15-15-15}{90^{o}C}$	63	88

Betanin is an important pigment component of beetroot, known for its antioxidant properties and its ability to protect against free radicals. It is also considered an indicator of juice quality, with a higher reduction in betanin content leading to consumer dissatisfaction. One of the key factors affecting the stability of betanin in food processing and storage is pH and temperature. At pH = 7, betanin remains stable for less than 20 days at 4°C and stable for over 275 days at -30°C [14]. Betanin is heat-sensitive and decomposed at 50°C, which is a disadvantage when using betanin as a food pigment [2]. The red color of the betanin solution gradually fades and turns yellow-brown when heated at 100°C [1]. Another study suggests that boiling beetroot roots for 60 minutes reduces the betanin content by 51% [12].

A comparison of the betanin content in standardized beetroot juice before and after pasteurization shows that the betanin loss increases as the pasteurization time increases. Specifically, pasteurization at 90°C for 10 and 15 minutes (represented by pasteurization formulas CT5 and CT6) result in betanin losses of 7% and 12%, respectively. Besides, pasteurization at 85°C for 15 minutes (represented by pasteurization formula CT3) result in a betanin loss of 4%.

Pasteurization at 96°C for 540 to 900 seconds result in a decrease in betanin content by 40% to 42%, compared to before pasteurization [7]. During pasteurization at 85°C, the betanin pigment is decomposed and formed a yellow-red color [5].

Therefore, in our study, red beetroot juice is standardized with 0.015% vitamin C and 2000mg/L erythorbate (method 2.2.5) to stabilize the active ingredients penolic and betanin during hot pasteurization compared to other studies [5, 10].

The results of Table 2 and Table 4 show that: Pasteurization formula CT3 not only ensures the complete elimination of aerobic microorganisms during 15 days of storage but also causes the least loss of phenolic and betanin content. Therefore, pasteurization formula CT3: $\frac{15-15-15}{85^{o}C}$ is chosen for the study evaluating the quality of 200ml bottled beetroot juice during its stabilization time.

3.4. Evaluate phenolic and betanin content in 200ml bottles of standardized beetroot juice, pasteurized according to the CT3 formula: $\frac{15\text{-}15\text{-}15}{85^{o}C}$ during stabilization time

The 200ml bottled standardized beetroot juice, which is prepared as described in section 3.2, is pasteurized according to the CT3 formula: $\frac{15-15-15}{85^{o}C}$ and then it is stabilized. The betanin and phenolic content are evaluated at stabilization times of 0, 7, 10, and 15 days, as show in Table 6.

Table 6. Survey of phenolic and betanin content in 200ml bottles of standardized beetroot juice pasteurized according to the CT3 formula: $\frac{15-15-15}{85^{o}C}$ during stabilization time

Stabilization time (days)	Phenolic content (mg/100ml)	Betanin content (mg/100ml)
0	19.257 ± 0.407^{a}	4.096 ± 0.075^{a}
7	17.452 ± 0.374^{b}	3.939 ± 0.057^{b}
10	16.919 ± 0.298^{c}	3.863 ± 0.049^{c}
15	15.883 ± 0.289^{d}	3.789 ± 0.065^{d}

Note: Data are presented as mean ± standard deviation (n = 3). Letters (a, b, c, d) that appear the same within a column indicate no significant statistical difference at the 95% confidence interval.

Compared to pre-pasteurization levels (betanin: 4.262 ± 0.068mg/100ml; phenolic: 23.794 ± 0.343mg/100ml), the betanin and phenolic contents decrease by approximately 4% and 19%, respectively, at the 0-day storage mark (i.e., immediately after pasteurization). Thus, the $\frac{15-15-15}{85^{o}C}$ pasteurization formula result in smaller reductions in betanin and phenolic contents (~3% and 5%, respectively) compared to the $\frac{15-15-15}{85^{o}C}$ formula.

Furthermore, the percentage reduction of both betanin and phenolic contents during stabilization time is presented in Figure 2.

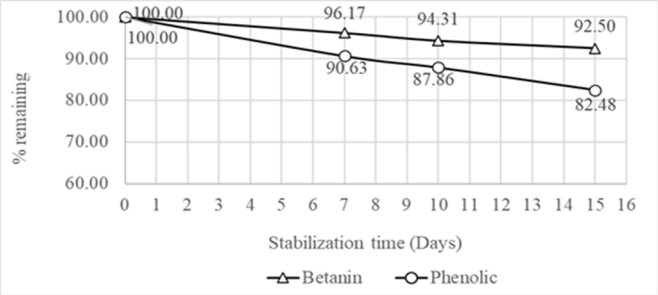

Figure 2. Investigation of remaining betanin and phenolic content percentages during stabilization time

When compared to the stabilization timepoint at day 0, the betanin and phenolic contents decreased by approximately 3.8% and 9.3% after 7 days of storage, by about 5.7% and 12.1% after 10 days, and by approximately 7.5% and 17.5% after 15 days, respectively. Accordingly, at the 15-day stabilization point, the remaining betanin and phenolic contents were 92.5% and 82.5%, respectively, relative to their levels immediately after pasteurization.

4. CONCLUSION

The study identifies the $\frac{15-15-15}{85^{o}C}$ pasteurization as optimal, as it effectively eliminates all aerobic microorganisms in the standardized 200ml bottled beetroot juice (composition: 0.015% vitamin C, 800mg/L potassium sorbate, 1500mg/L erythorbate; 23.794 ± 0.343mg GAE/100mL phenolics; 4.262 ± 0.068mg/100mL betanin) while retaining 81% phenolics and 96% betanin compared to pre-pasteurization. Using this thermal pasteurization method, the study obtains 200ml bottled beetroot juice with betanin content (mg/100ml) measuring 4.096 ± 0.075^{a}, 3.939 ± 0.057^{b}, 3.863 ± 0.049^{c}, and 3.789 ± 0.065^{d}, and phenolic content (mg galic/100ml) measuring 19.257 ± 0.407^{a}, 17.452 ± 0.374^{b}, 16.919 ± 0.298^{c}, and 15.883 ± 0.289^{d} at stabilization times of 0, 7, 10, and 15 days, respectively.

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