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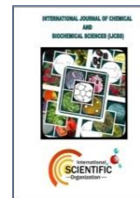

The Efficiency of Temperature and Time Pasteurization on the Quality of a Canned Tea from Dried Asparagus Root (*Asparagus Officinalis* L.)

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Abstract

The root of *Asparagus officinalis* L. shows potential as a valuable by-product for developing new food products. Known for its rich nutritional profile and unique flavor and aroma, asparagus root is being explored for use in creating a new herbal tea. Pasteurization plays a critical role in ensuring the final product's quality, necessitating careful research to determine the optimal pasteurization parameters for preserving the quality of the fermented asparagus beverage. This investigation mainly focused on the efficiency of different pasteurization's temperatures (80, 85, 90 and 95°C) and durations (15, 30, 45 and 60 minutes) on the pasteurization value, physicochemical properties as well as bioactive compounds of the pasteurized product. The study selected a temperature of 90°C for 20 minutes for the pasteurization of the extracted canned tea from dried asparagus root to ensure microbiological safety, maintain high levels of bioactive compounds, and be economically advantageous. With this condition, the levels of saccharose, vitamin C, bioactive compounds (phenolic, flavonoid, and saponin), and antioxidant capacity of the tea (per liter of product) were 153.16 g, 2.43 g, 0.52 g TAE, 0.15 g QE, 1.41 g SE, 66.74%, and 1.04 M $FeSO_4$, respectively. The optimized pasteurization technique could pave the way for developing nutrient-rich beverages in future research.

Keywords: Asparagus roots, antioxidant, beverages, bioactive compounds, herbal tea

Full length article **Corresponding Author*, e-mail: *ntngiang@agu.edu.vn* https://doi.org/10.62877/30-IJCBS-24-26-20-30

1. Introduction

Asparagus (*Asparagus officinalis L.*) is a highly potential vegetable known for its rich nutritional content and distinctive flavor, attributed to volatile compounds like pyrazines and sulfur compounds [1]. It is also a rich source of bioactive compounds, including saponins, flavonoids, vitamins, polysaccharides, and dietary fiber [2], offering a range of health benefits such as anti-cancer, antioxidant, and anti-hypertensive effects [1-3]. Notably, fructans, one of the bioactive compounds, are concentrated in the roots and lower sections of the spears [3-5]. Despite these benefits, the asparagus roots are often discarded as by-products, presenting an opportunity for the creation of value-added products [6]. Herbal infusions have been a staple in the traditional medicine and are widely enjoyed as beverages globally [7]. The growing popularity of herbal beverages is driven by their high content of the natural bioactive compounds, such as alkaloids, flavonoids, phenolic, carotenoids, saponin, coumarins, and the terpenoids [8]. Evidence suggests that the bioactive compounds in the herbal infusions may offer various health benefits, including the anti-bacterial, antioxidant, anti-inflammatory, anti-allergic, and the vasodilatory effects, as well as the anti-carcinogenic and anti-aging properties [8]. A crucial element in completing research on the development of herbal beverage products is the pasteurization process. The pasteurization is a heat treatment technique used to eliminate harmful microorganisms in food and beverages, ensuring safety and extending shelf life. The primary objective is to deactivate the non-spore-forming pathogenic bacteria and most spoilage organisms while also inhibiting microbial and enzymatic activity. Two widely used pasteurization methods are low-temperature long-time (LTLT) and high-temperature short-time (HTST). This study aimed to identify the optimal pasteurization conditions to maintain the quality of fermented asparagus beverages, considering factors such as the pasteurization value, physicochemical properties, and the bioactive compounds. The optimized process could contribute to the development of nourishing drinks in the future research.

2. Materials and methods

2.1 Materials and Equipment

The roots of *Asparagus officinalis* L. with excellent quality and no signs of physical damage or infestation were harvested in My Thoi Ward, Long Xuyen City, An Giang Province, Vietnam. The heat-resistant variety UC 157 F2 supplied by Walker Brothers (USA) was used. The green asparagus shoots were harvested when they reach 25-30 cm [10]. The roots were cut from the shoots, with a length approximately one-third of the shoot's length. Every sample comprised 2 kg of green asparagus roots, cut into approximately 1 cm pieces. The roots underwent sorting and washing, followed by a preliminary treatment of blanching, involving immersion in hot water at 85°C for 2 minutes in order to ensure better quality and stability of the material [11]. After blanching, the samples were dried using a Forced Convection Oven (ESCO, OFA-110-8, Indonesia) at 70ºC with an airflow velocity of 1 m/s. The dried samples were crushed finely and sieved through a mesh with a diameter of 1 mm [12]. The finely crushed asparagus material was mixed with green tea powder at an optimal ratio of 3% [11] and extracted at a temperature of 70°C with the assistance of ultrasound (180 W for 20 minutes) [13]. After extraction, the mixture filtered and adjusted to a Brix of 10 (using refined sugar) and a pH of 4.0-4.5 (using ascorbic acid). Tea solution then canned, sealed and ready to perform pasteurization.

2.2 Experimental design

The pasteurization process was conducted at the investigated parameters, including temperatures (80, 85, 90 and 95°C) and times (15, 20, 25 and 30 minutes). After pasteurization, the product was collected and analyzed to evaluate the physicochemical and microbiological.

2.3 Determination of the pasteurization units (PU)

The determination of pasteurization units (PU) is a way to quantify the effectiveness of the pasteurization process in inactivating microorganisms, particularly those that are heat-sensitive, without compromising the product's quality [14]. Briefly, record the temperature of the product during the pasteurization process at regular intervals, PU is typically calculated using the equation (1).

$$PU = \int_{t_o}^{t} 10^{\left(\frac{T-T_{ref}}{z}\right)} \quad \text{(Eq.1)}$$

Where: T: Temperature at time t(ºC); T_{ref}: Reference temperature; z: The z-value, which represents the temperature increase required to achieve a tenfold reduction in microbial population, specific to the microorganism of interest (usually between 5-10°C for common pathogens).

2.4 Determination of color

Color assessment was conducted by analyzing L, a, b values using a colorimeter (Konica Minolta CR400, Japan) to evaluate the extracted solution's color.

2.5 Determination of vitamin C

Vitamin C content was assessed using a modified version of the 2, 4-dinitrophenyl hydrazine colorimetric method as outlined by Sharaa and Mussa [15]. About 1 gram of sample was mixed with 5 mL of a solution comprising 3% meta-phosphoric acid (w/v) and 8% glacial acetic acid (v/v) in a 15 mL centrifuge tube. This mixture was then placed on a Reciprocating shaker (Stuart, UK) for 1 hour. Following centrifugation, 1 mL of the supernatant was combined with 0.5 mL of 3% bromine, 0.25 mL of 10% thiourea, and 0.25 mL of 2, 4-dinitrophenyl hydrazine. The resulting mixture was incubated for 3 hours at 37°C. Subsequently, 10 mL of 85% H_2SO_4 was added to the tube, resulting in the formation of a red complex. After cooling the solution to room temperature, its absorbance was measured at 520 nm. The concentration of vitamin C was determined using a standard ascorbic acid graph, where the absorbance (y) was calculated using the equation y = 0.2253x + 0.0024 (R^2 = 0.9999), with x representing the concentration of the solution in the tube

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2.6 Determination of total phenolic

The phenolic content (g TAE/ 100 g of dry matter) was determined using the Folin-Ciocalteu method as described by Sumaiyah, Masfria and Dalimunthe [16]. In brief, 0.15 mL of the sample was combined with 1.2 mL of distilled water and 0.45 mL of 5% (w/v) Na_2CO_3 in a test tube. This mixture was then mixed with 0.1 mL of Folin-Ciocalteu reagent and allowed to react at room temperature for 90 minutes. Phenolic compounds in the extract reacted with the Folin-Ciocalteu reagent, resulting in the formation of a phosphomolybdenum complex with a blue color in an alkaline environment. The absorbance of the solution was measured at 750 nm, using a UV-visible spectrophotometer (V730, Jasco, Japan). The concentration of total phenolics was determined using a standard tannic acid graph (TAE), where the absorbance (y) was calculated using the equation y = 0.0021x + 0.0064 (R^2 = 0.9999), with x representing the concentration of the solution in the tube.

2.7 Determination of total flavonoid

The flavonoid content was assessed using a colorimetric reaction with aluminum chloride, with modifications to the method outlined by Sumaiyah, Masfria and Dalimunthe [16]. This reaction forms a stable acid complex between $AlCl_3$ and the C-4 keto groups, as well as the hydroxyl groups at C-3 or C-5 of the flavonoids and flavonols. In brief, 0.1 mL of the sample was mixed with 1.2 mL of distilled water and 30 µL of 5% (w/v) $NaNO_2$. After 5 minutes, the mixture was supplemented with 60 µL of 10% (w/v) $AlCl_3.H_2O$; 0.2 mL of 1 M NaOH, and 0.11 mL of distilled water. The resulting solution was then measured at 510 nm. The total flavonoid concentration was determined using a standard quercetin graph (QE), where the absorbance (y) was calculated using the equation y = 8.2634x + 0.0182 (R^2 = 0.9999), with x representing the concentration of the solution in the tube.

2.8 Determination of saponin

The determination of saponin content was conducted using the vanillin-sulfuric acid method, as described by Le, Parks, Nguyen and Roach [17]. This method relies on a red-violet color reaction resulting from the oxidation of triterpene saponins by sulfuric acid and vanillin. Approximately 0.25 mL of the sample was mixed with 0.25 mL of 8% (w/v) vanillin in 96% ethanol and 2.5 mL of 72% H_2SO_4. The mixture was then incubated for 30 minutes at 60°C and subsequently cooled to room temperature. The absorbance of the solution was measured at 560 nm. The concentration of saponin was determined using a standard saponin graph (SE), where the absorbance (y) was calculated using the equation y = 0.1348x + 0.0075 (R_2 = 0.9999), with x representing concentration of solution in tube.

2.9 Determination of DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity

The antioxidant activity of the sample was assessed by its ability to scavenge free radicals using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Following the protocol outlined by Molyneux [18], the DPPH assay was conducted with minor adjustments. Specifically, a mixture of the sample (1.5 mL) and DPPH solution in a 1:1 ratio (v/v) was prepared. This assay relies on electron transfer, resulting in the formation of a purple solution in ethanol, which was then analyzed the spectrophotometrically at 517 nm. The inhibition of DPPH free radicals was determined using Equation 2.

$$Inhibition\ of\ DPPH\ radical\ (\%) = 100 \times (Ac - As)/Ac \quad (2)$$

Where: A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.10 Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay (mM of $FeSO_4$/g dry matter) was conducted following the method outlined by Sudha, Vadivukkarasi, Shree and Lakshmanan [19] with some adjustments. This method relies on the reduction of the tripyridyltriazine complex Fe $(TPTZ)^{3+}$ to the blue-colored Fe $(TPTZ)^{2+}$ by antioxidants in an acidic medium. The FRAP reagent consisted of 100 mL of 200 mM acetate buffer (pH 3.6), 10 mL of 20 mM $FeCl_3.6H_2O$, and 10 mL of 10 mM TPTZ in 40 mM HCl. A volume of 0.05 mL of the sample was mixed with 1.5 mL of the FRAP reagent and 0.15 mL of distilled water. The mixture was then incubated at 37°C for 8 minutes and subsequently analyzed for absorbance at 593 nm.

2.11 Determination of saccharose

Saccharose content (g/100 g of dry matter) were measured by the DNS method with some modifications. This method is based on the oxidation of the C=O group by 3, 5-Dinitrosalicylic acid from yellow colour to orange-red in an alkaline medium [20]. An aliquot (1 mL) of sample was put in a test tube and then added 2 mL of reagent DNS. The tubes of blank, solution of standard glucose and samples were put in boiling water for 10 minutes. Next, 7 mL distilled water was added. The solution was analysed at an absorption of 575 nm, using a UV-visible spectrophotometer (V730, Jasco, Japan). The concentration of total sugar was based on a standard curve of glucose, y =23885x + 0.126 (R2=0.9999), where y is the absorbance and x is the concentration of the solution in the tube.

2.12 Data analysis

Data were collected and processed by STAGRAPHICS Centurion 16.1 software (Statistical Graphics Corp., USA) for analysis multifactor variance (ANOVA), LSD test to conclude the difference between the average of experiments at 5% confidence (p=0.05) and Microsoft Excel 2016 software for calculating. Results were presented using text, and figures, which illustrated the data based on statistical analyses, including p-values, confidence intervals, and other relevant metrics

3. Results and discussion

Before pasteurization, the tea water is adjusted to pH4.3-4.5 (3 g ascorbic acid/L) and 10°Brix.

3.1 The impact of pasteurization temperature and time on the chemical and bioactive compounds of canned tea from dried asparagus roots

The results displayed in the Figure 1 indicate that the levels of saccharose and vitamin C in the tea decrease with increasing pasteurization temperature. Specifically, the levels of saccharose and vitamin C (per g/L) tend to decrease from 158.76 g and 2.63 g, respectively, when pasteurized at 80°C, to 147.34 g and 2.39 g, respectively, when pasteurized at 95°C. Similarly, the levels of saccharose and vitamin C decrease with increasing pasteurization time. During pasteurization, as temperature and pasteurization time increase, the level of saccharose decreases due to the involvement of non-enzymatic browning reactions (Maillard reaction). Additionally, vitamin C is highly sensitive to temperature, and heating or holding stages of pasteurization process also contribute to the loss of vitamin C content [21].

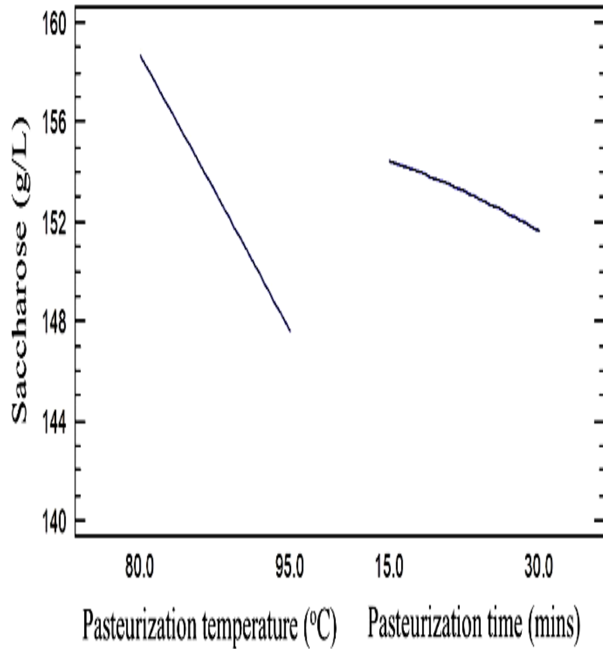
3.2 The impact of pasteurization temperature and time on bioactive compounds in canned tea from dried asparagus roots

The content of phenolics and flavonoids increase to an optimum value, then gradually decrease with the increasing pasteurization temperature (Figure 2a). Specifically, the phenolic content increases from 0.49 g TAE/L when pasteurized at 80°C to 0.52 g TAE/L at 90°C. The highest flavonoid content is achieved when pasteurized at 85°C (0.15 g QE/L), with no statistically significant difference compared to samples at 80°C and 90°C (p>0.05). Conversely, saponin content increases with the pasteurization temperature (Figure 2c), reaching its highest value at 95°C (1.43 g SE/L). Similarly, with increasing pasteurization time, phenolic and saponin contents increase to an optimum value, then gradually decrease. Specifically, phenolic content reaches its peak when pasteurized for 20 minutes (0.51 g TAE/L); however, there is no statistically significant difference between times of 15 and 25 minutes. The saponin content reaches its highest value when pasteurized for 25 minutes (1.41 g SE/L), with no statistically significant difference compared to pasteurization for 25 and 30 minutes.

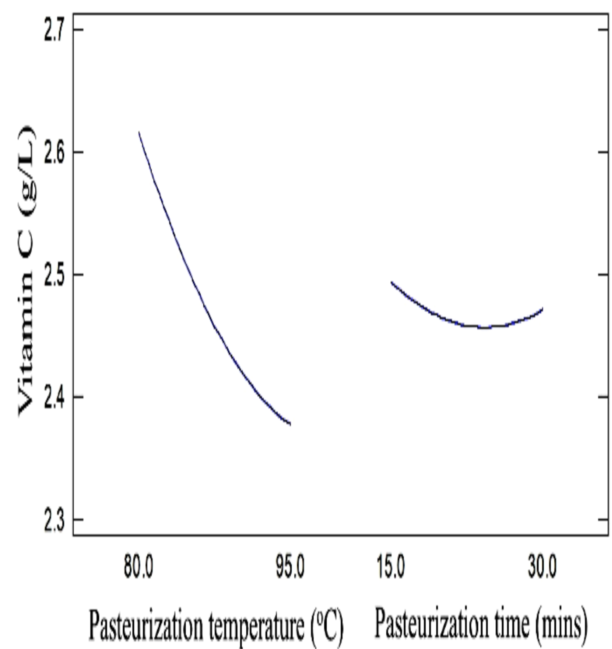
Results in Figure 2b show that flavonoid content decreases with increasing pasteurization time; however, decrease in flavonoids is not statistically significant among time periods of 10, 25, and 30 minutes. According to Jeong *et al*. [22], initial increase in phenolic content is due to release of phenolic compounds in bound form and transformation of insoluble phenolic compounds into soluble ones. Additionally, as suggested by Xiao [23], phenolic, flavonoid, and saponin contents will rapidly convert into various derivatives, and lower temperatures will help protect bioactive compounds during thermal processing. Moreover, it can be observed that prolonging pasteurization time leads to a loss of bioactive compound content. Increasing temperature and time significantly reduce microbial load but also decrease bioactive compounds [24]. This result is consistent with findings of Laslo [25] and Tan, Giang and Tuyen [26].

3.3 The impact of pasteurization temperature and time on antioxidant capacity (measured through DPPH and FRAP values) in tea canned extracted from dried asparagus root

Figure 3 indicated that as pasteurization temperature increases from 80 to 95°C, ability to scavenge free radicals (DPPH) and ferric reducing antioxidant power (FRAP).

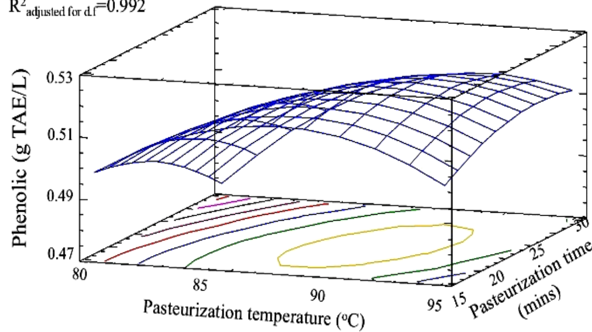


(a) Saccharose

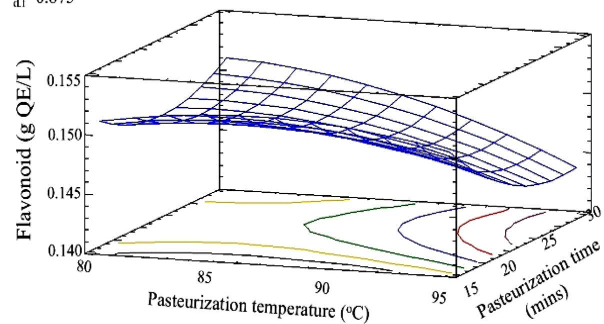


(b) Vitamin C

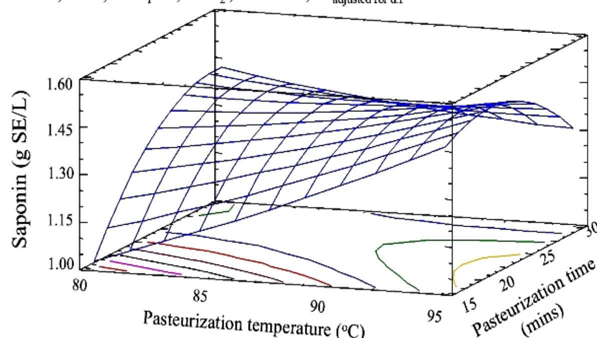
Figure 1. The correlation between pasteurization temperature and time on the chemical composition of extracted solution from dried asparagus roots (a) Saccharose and (b) Vitamin C



(a) Phenolic

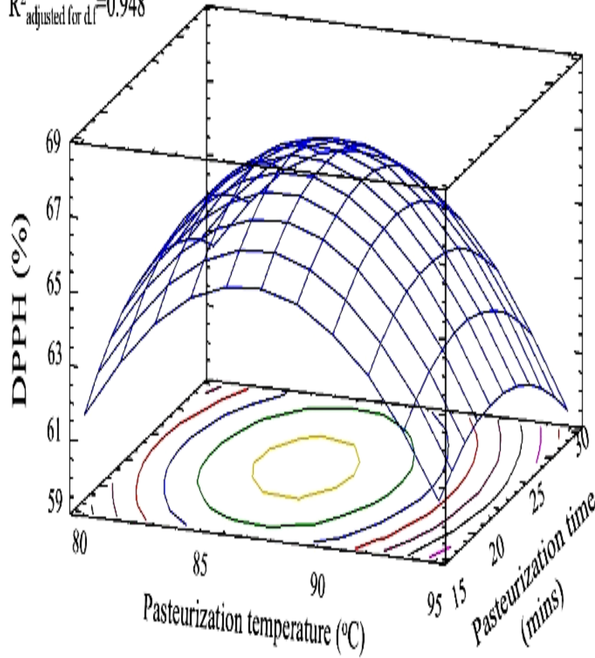


(b) Flavonoid

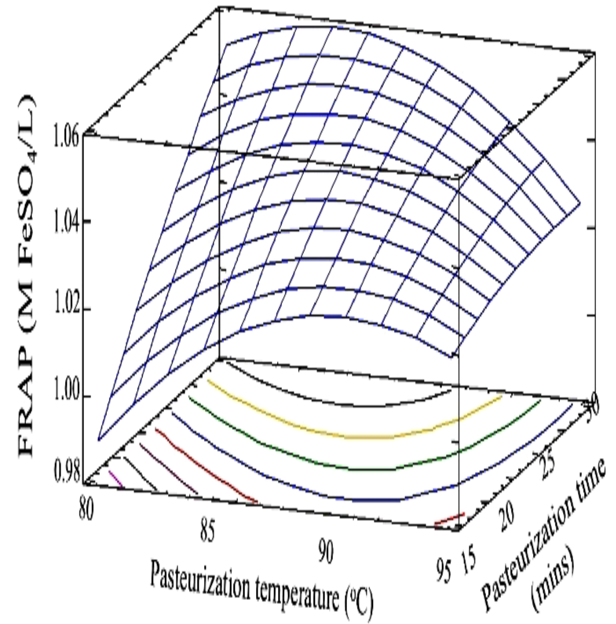


(c) Saponin

Figure 2. The correlation between pasteurization temperature and time on bioactive compounds of extracted solution from dried asparagus roots (a) Phenolic, (b) Flavonoid and (c) Saponin

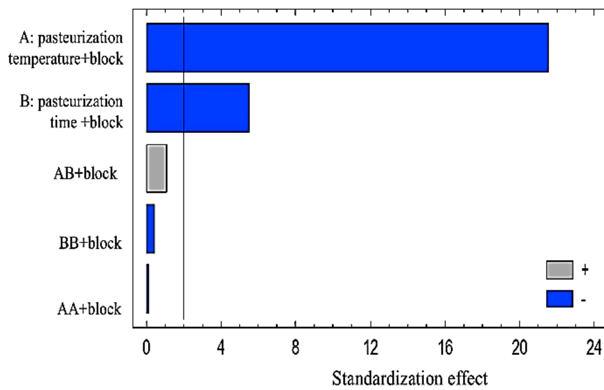


(a) DPPH

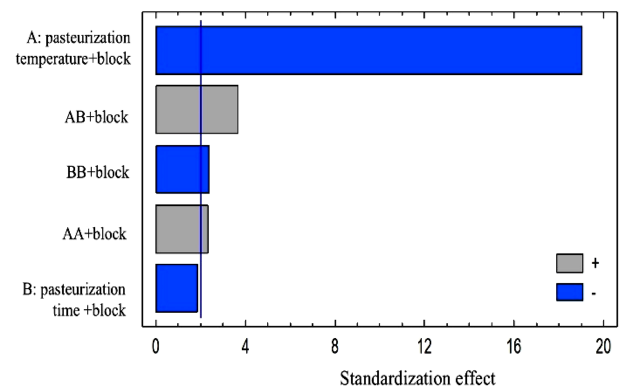


(b) FRAP

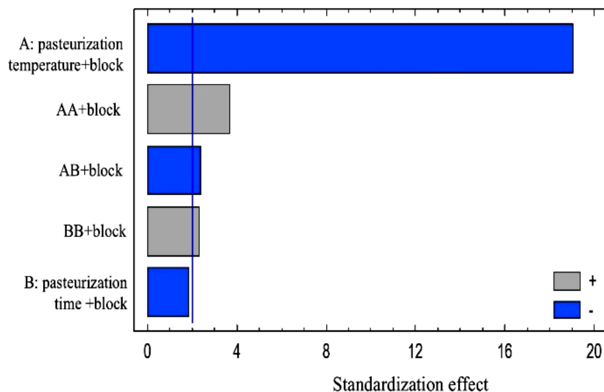
Figure 3. The effects of pasteurization temperature and time on (a) DPPH và (b) FRAP in the extracted solution from dried asparagus root



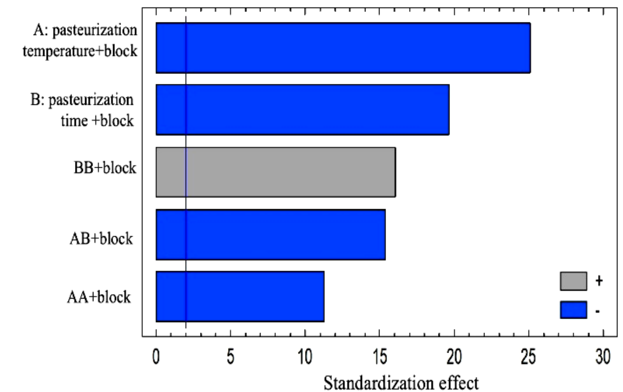
(a) Saccharose



(b) Vitamin C



(c) Phenolic



(d) Flavonoid

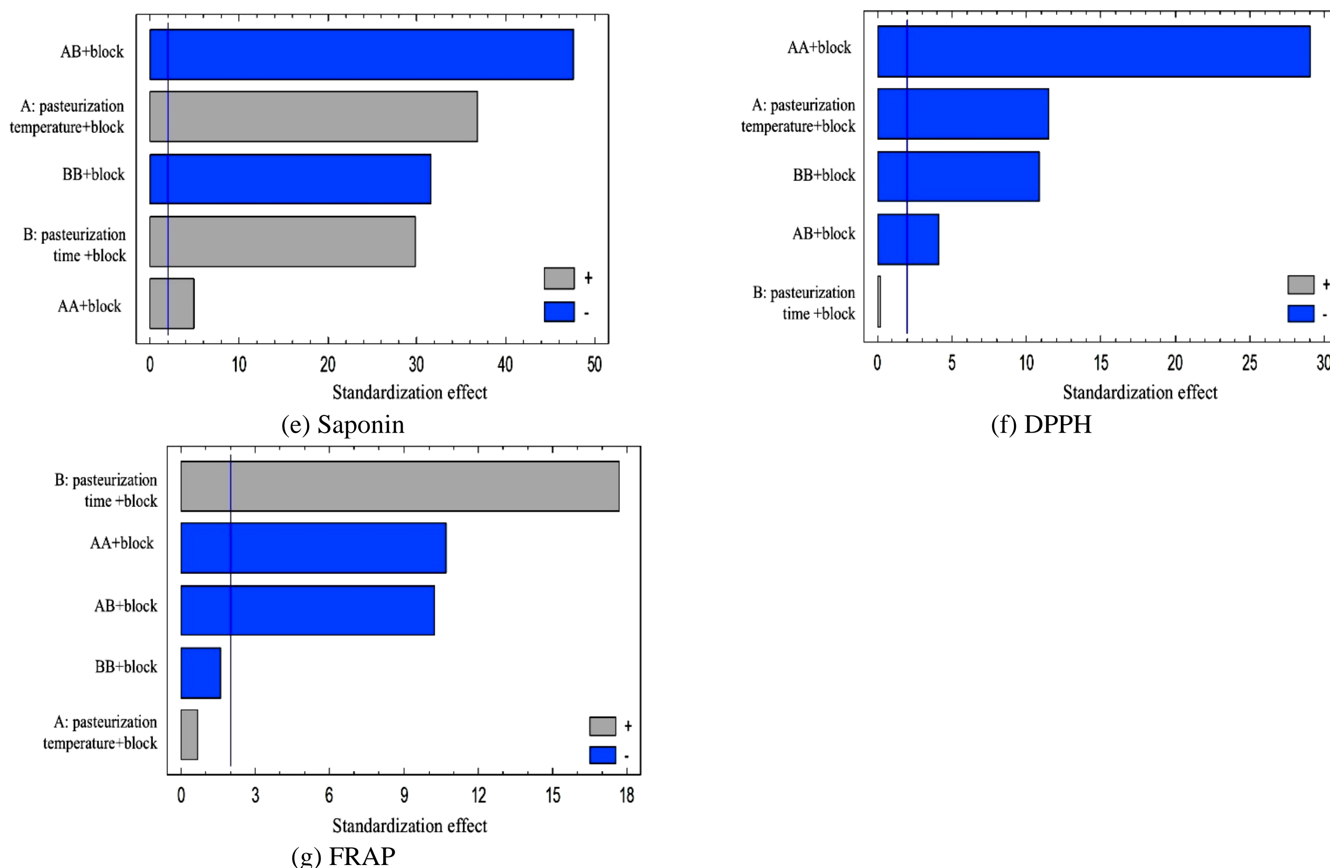


Figure 4. The level of influence of pasteurization temperature, time and the interaction between pasteurization temperature and time on (a) Saccharose, (b) Vitamin C, (c) Phenolic, (d) Flavonoid, (e) Saponin, (f) DPPH and (g) FRAP

It showed a tendency to increase to an optimal value, then decrease gradually. Specifically, DPPH increases from 62.99% to 66.01% when the pasteurization temperature rises from 80 to 85°C; however, there is no statistically significant difference b/w temperatures of 85 and 90°C. Continuing to increase pasteurization temperature to 95°C, DPPH decreases to 60.71%. Similar trends are observed for FRAP, reaching its highest value of 1.04 M $FeSO_4$/L when pasteurized at 85°C, with no statistically significant difference compared to sample at 90°C. Results also show that effect of pasteurization time on DPPH and FRAP is similar to the effect of pasteurization temperature. DPPH reaches its highest value when pasteurized for 25 minutes (64.62%), with no statistically significant difference compared to sample pasteurized for 25 minutes. FRAP reaches its highest value in sample pasteurized for 30 minutes (1.05 M FeSO4/L), with no statistically significant difference compared to sample pasteurized for 25 minutes. Heat treatment led to reduction in content of bioactive compounds in product, resulting in a decrease in antioxidant activity of product. Furthermore, increasing temperature and extraction time may either increase or decrease antioxidant capacity of a substance.

The decrease in bioactive compounds at high temperatures over a long period forms more stable compounds but with less bioactivity, thus affecting antioxidant activity [27]. The graphs in Figure 4 illustrate the influence of independent variables as well as interactions affecting the chemical composition and bioactive compounds of dried asparagus root solution. Results showed that the levels of variables A, B, AA, BB, and AB of phenolic, flavonoid, and saponin all exceeded reference line and were statistically significant at the 5% level. Similarly, variables A and B of saccharose, variables A, AB, BB, and AA of vitamin C and DPPH, and variables B, AA, and AB of FRAP surpassed reference line. From the Pareto chart, it is evident that the chemical composition and DPPH of tea significantly influenced by pasteurization temperature, while FRAP affected by pasteurization time. Additionally, regression equations were developed to predict levels of phenolic compounds, flavonoids, saponins, vitamin C, saccharose, and antioxidant capacity (DPPH and FRAP) of canned drink from dried asparagus root at different pasteurization temperatures & times (Figure 1-3). Insignificant factors or interactions removed from established model. The obtained equations had correlation coefficients R^2 and R^2_{adj}>0.87, thus allowing for prediction of changes in the levels of bioactive and chemical compounds as well as antioxidant capacity of tea based on pasteurization temperature and time investigated.

4. Conclusions

The study selected a temperature of 90°C for 20 minutes for the pasteurization of the extracted canned drink from dried asparagus root to ensure microbiological safety, maintain high levels of bioactive compounds, and be economically advantageous. This study not only aimed to provide insights into the optimal pasteurization for an extracted drink from dried asparagus roots but also underscores the broader implications for nutrition, health, economic development, and sustainability. This optimized process can lead to the development of nutritionally enriched, high-antioxidant products for future studies.

Conflict of interest

The authors declare no conflict of interest.

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