Mal J Nutr 31(2): 295-304, 2025

Optimising pasteurisation conditions to preserve the quality of fermented green asparagus roots (*Asparagus officinalis* L.) beverage

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ABSTRACT

Introduction: The root of *Asparagus officinalis* L. stands out as a potential by-product source for creating value-added food products. Characterised by its high nutritional content and distinctive flavour and fragrance, the green asparagus root was studied for development of a new herbal tea. Pasteurisation is the crucial stage to ensure the quality of the final product, which requires precise investigation to obtain optimal parameters for maintaining quality of fermented drinks made from green asparagus roots. **Methods:** This investigation mainly focused on efficiency of different pasteurisation temperatures (80, 85, 90, and 95°C) and durations (15, 30, 45, and 60 minutes) on pasteurisation value, physicochemical properties, and bioactive compounds of the pasteurised product. **Results:** Optimal pasteurisation parameters for canned fermented drinks from green asparagus root were identified at a temperature of 90°C for 20 minutes, which ensured that they were microbiologically safe, maintained high levels of bioactive compounds, and were economically beneficial. With this pasteurisation condition, levels of reducing sugar, saccharose, vitamin C, total acidity, and bioactive compounds (phenolic, flavonoid, and saponin) (per litre of product) were 19.84 g, 102.08 g, 2.38 g, 0.064 g, 0.97 g tannic acid graph, 0.35 g quercetin graph, and 2.39 g saponin graph, respectively. **Conclusion:** This study not only provided insights into optimal pasteurisation for fermented drinks made from green asparagus roots but also underscored the broader implications for nutrition, health, and sustainable economic developments. This optimised process can lead to development of nutritious beverages for future studies.

Keywords: fermentation, microbiological safety, pasteurisation unit

INTRODUCTION

Asparagus (*Asparagus officinalis L.*) is a highly valued vegetable, renowned for its rich nutritional profile and distinctive flavour, which comes from volatile compounds such as pyrazines and sulphur (Pegiou *et al.*, 2019). Moreover, this plant is rich in bioactive compounds like saponins, flavonoids, vitamins, polysaccharides, and dietary fibre, providing numerous health benefits, including anti-cancer, antioxidant, and

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doi: https://doi.org/10.31246/mjn-2024-0091

anti-hypertensive properties (Pegiou *et al.,* 2019; Guo *et al.*, 2020). Fructans, one of the bioactive compounds, are concentrated mainly in the roots and lower spear sections (Guo *et al.*, 2020). However, despite their nutritional benefits, green asparagus roots are often left unused as by-products, offering potential for the development of value-added products (Viera-Alcaide *et al.*, 2021).

Fermentation is one of the oldest methods of food preservation and enhances the nutritional value of foods. Beyond traditional beverages, there have been recent attempts at creating non-dairy probiotic fermented drinks using various substrates such as soy milk, whey, grains, and vegetables or fruit juices, which have been highly preferred (Marsh *et al.*, 2014). Research has shown significant links between microorganisms found in certain fermented products and numerous health benefits. These include weight management, lower risks of cardiovascular disease, anti-diabetic effects, relief from constipation, improved glucose and lipid levels, enhanced immune system function, anti-cancer properties, and most notably, a reduction in mortality (Cuamatzin-García *et al.*, 2022). Thus, fermented beverages are expected to remain a key segment of the functional food market.

A key aspect of the development of fermented beverage products is the pasteurisation process. Pasteurisation is a heat treatment method used to eliminate harmful microorganisms in foods and beverages, enhancing their safety and extending shelf life. Its main goal is to inactivate non-spore-forming pathogenic bacteria and most spoilage microorganisms, while also preventing microbial and enzymatic activities (Tibor, 2014). The two common pasteurisation techniques are low-temperature long-time (LTLT) and high-temperature short-time (HTST) (Rama & Jinal, 2019). This study focused on determining the optimal pasteurisation conditions to preserve the quality of fermented asparagus beverages, based on pasteurisation value, physicochemical characteristics, and bioactive compounds. The optimised process could support the development of nutrient-rich drinks in future research.

METHODOLOGY

Materials and equipment

High-quality *Asparagus officinalis* L. with no signs of physical damage or infestation, from My Thoi Ward, Long Xuyen City, An Giang Province, Vietnam was harvested. The heat-resistant variety UC 157 F2 supplied by Walker Brothers (USA) was used in this study. Green asparagus roots, measuring approximately one-third of the shoot's length, were obtained from the shoots that were harvested at a length of 25–30 cm (Nguyen *et al.*, 2024).

The green asparagus roots were sorted, cleaned, and drained. Each sample used 1 kg of asparagus roots mixed with water at an optimal water/material ratio of 2.5/1 and blended using a Philips HR2223 blender (China) for 5 minutes. The asparagus mixture was adjusted to 20°Brix and pH 4.5 using refined sugar and 10% citric acid (Jarun *et al.,* 2008), then heated at 85°C for 15 minutes to eliminate microorganisms while preserving the nutritional value of the asparagus for the next fermentation stage. The solution was poured into 5-litre glass jars and cooled to 40°C. The optimal fermented condition was based on the research of Giang *et al.* (2024), with a yeast concentration of 0.03% added. It was stirred well, sealed, and anaerobically fermented at room temperature for 5 days. After fermentation, the green asparagus liquid was filtered to remove solids, then adjusted for taste, clarified, filled into cans, and prepared for pasteurisation.

Experimental design

The pasteurisation process was conducted at different temperatures (80, 85, 90, and 95°C) and times (15, 20, 25, and 30 minutes) with triplicates. After pasteurisation, the product was collected and analysed to evaluate the physicochemical, microbiological, and sensory indicators.

Determination of the pasteurisation units (PU)

The determination of pasteurisation units (PU) is a way to quantify the effectiveness of the pasteurisation process in inactivating microorganisms, particularly those that are heat-sensitive, without compromising the product's quality (Binh & Phuong, 2011). The temperatures of the product were recorded at regular intervals during the pasteurisation process. PU is typically calculated using the following equation 1.

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

Where T: Temperature at time t (°C); 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).

Determination of total anaerobic microbes (ISO 4833-1, 2013)

Total aerobic bacterial count in food can be determined using surface culture methods on agar or the pour plate method by counting the number of colonies on a Petrifilm plate. Using a pipette, 1 mL of the test sample was transferred into a petri dish, followed by the addition of 10–15 mL of the prepared medium. The agar was left to solidify, the dish was inverted, and incubation was carried out at 37°C for 72 hours. The colony count was calculated using the following equation 2.

$$A\ (\text{CFU/g or CFU/ml}) = \frac{N}{n_1 V f_1 + \cdots + n_i V f_i} \quad (2)$$

Where A: Number of bacterial cells (colony-forming units, CFU) per gram (mL) of the sample; N: Total number of colonies counted on the selected plates; n_i: Number of plates inoculated at the i (times) dilution; V: Volume of the sample suspension (mL) inoculated onto each plate; f_i: Corresponding dilution factor for the i (times) dilution.

Determination of yeasts and moulds (ISO 21527-2, 2008)

The yeast and mould count per mL sample was determined based on the colony count obtained from Petri dishes containing 20–25 mL of Dichloran-rose Bengal Chloramphenicol (DRBC) agar medium, which were incubated aerobically at 25°C±1°C for five days. Results were expressed as CFU per gram (g) or millilitre (mL), based on the average count from three test plates.

The procedures for determining colour, saccharose, vitamin C, total phenolic, total flavonoid, and saponin were previously reported by the study of Giang & Khai (2024).

Data analysis methods

Data were collected and processed using STAGRAPHICS Centurion 16.1 software (Statistical Graphics Corp., USA). Multifactor variance analysis (ANOVA) and the Least Significant Difference (LSD) test were used to determine the difference between the average of

Table 1. Effects of different pasteurisation temperatures and times on the pasteurisation units (PU) value (minutes) of canned fermented drinks from green asparagus root

Time (minutes)	*Temperature (°C)*			
	80	*85*	*90*	*95*
15	0.37†	2.11	7.49	29.03
20	0.49	2.61	9.49	37.04
25	0.62	3.11	11.50	45.06
30	0.74	3.61	13.50	53.07

†Values are expressed as means of triplicate testing

experiments at 5% confidence (p=0.05). MS Excel 2016 software was used for calculation.

Results were presented using texts, tables, and figures, supported by statistical analyses including p-values, confidence intervals, and other relevant metrics to illustrate the data.

RESULTS AND DISCUSSION

PU values and the presence of microorganisms in the product

Fermented beverages typically have a pH range of 4.0-4.2 and a total soluble solids level (°Brix) of 15. For products with a low pH (below 4.5), the pasteurisation process parameters are set up with a pasteurisation unit (PU_o) of 5 minutes, a z-value (the temperature increase needed to reduce the microbial population by tenfold) of 8.3°C, and a reference temperature (T_{ref}) of 93.3°C (Binh & Phuong, 2011). Based on that, the pasteurisation process was carried out by recording the heating temperature over time and calculating the PU value of the whole process (Equation1). The PU value must be greater than PU_o to

Table 2. Presence of microorganisms in canned fermented drinks from green asparagus root at different pasteurisation temperatures and times

Temperatures (°C)	*Times (minutes)*	*Total number of anaerobic microorganisms (cfu/L)*	*Yeast and mold fungi (cfu/L)*
80	15	120†	55
	20	77	34
	25	24	19
	30	15	6
85	15	82	41
	20	49	28
	25	13	12
	30	5	3
90	15	-	-
	20	-	-
	25	-	-
	30	-	-
95	15	-	-
	20	-	-
	25	-	-
	30	-	-

- : Not detected

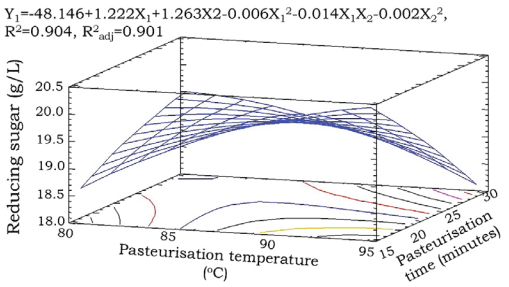
†Values are expressed as means of triplicate testing

ensure that all living microorganisms are inhibited within the intended product preservation time. The variation of PU according to the pasteurisation formula (Equation 1) is shown in Table 1. Results from Table 1 showed that the pasteurisation PU value depended on the temperature and pasteurisation time. As temperature rose from 80°C to 85°C, PU value increased from 4.88 to 5.70 times. Further increment of the temperature to 95°C led to a rise in the PU value from 71.72 to 78.46 times. The results showed that the pasteurisation temperature of 90°C for 15 to 30 minutes ensured microbiological safety. The analysis results confirmed that no anaerobic microorganisms, yeast, or mould fungi were detected after the products were pasteurised at 90°C for 15 to 30 minutes (Table 2).

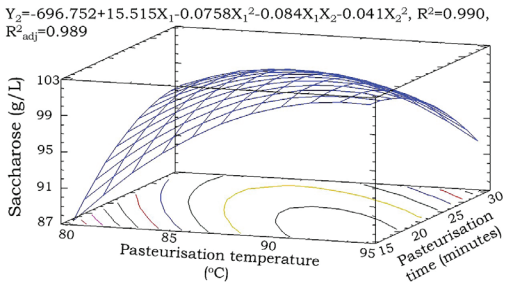
The impact of pasteurisation temperature and time on chemical composition

Results on the effects of different pasteurisation temperatures and times on the chemical composition of canned fermented drinks from asparagus root are illustrated in Figure 1.

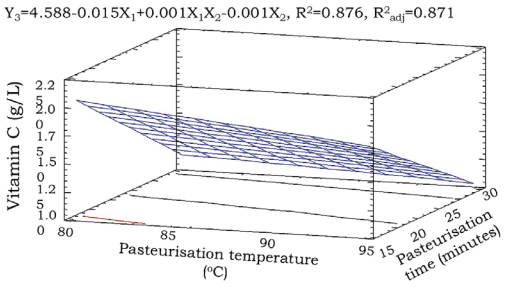
The results indicated that as pasteurisation temperature increased, the levels of reducing sugar, saccharose, and total acidity tended to increase to an optimal value, then decreased gradually. Specifically, the levels of reducing sugar, saccharose, and total acid (expressed per litre) reached their highest values when pasteurised at 90°C, at 19.51 g, 100.47 g, and 0.063 g, respectively, which were statistically significant compared to the remaining samples. As the pasteurisation temperature



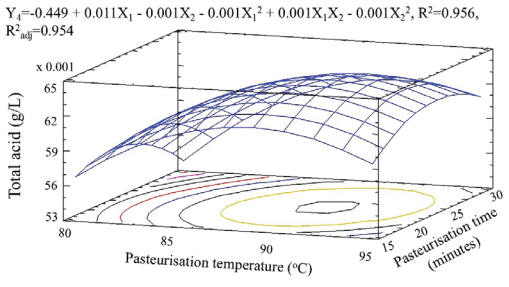
a. Reducing sugar



b. Saccharose



c. Vitamin C



d. Total acid

Figure 1. Impact of pasteurisation temperature and time on (a) Reducing sugar, (b) Saccharose, (c) Vitamin C, and (d) Total acid of canned fermented drinks from green asparagus root;
Y: objective functions; X_1: pasteurisation temperature (°C); X_2: pasteurisation time (minute)

continued to increase to 95°C, the levels of reducing sugar, saccharose, and total acid decreased.

Results in Figure 1 showed that vitamin C content decreased as pasteurisation temperature increased. Similarly, with increasing pasteurisation time, the levels of reducing sugar and vitamin C tended to decrease, while the levels of saccharose and total acid increased to an optimal value, then decreased gradually. The highest levels of saccharose and total acid were reached when pasteurised for 20-25 minutes (no statistically significant difference at these two time points). During the pasteurisation process, the levels of reducing sugar and saccharose increased due to the decomposition of polysaccharides into disaccharides and monosaccharides (Zhang *et al.*, 2014). However, as pasteurisation temperature and time continued to increase, the levels of these chemical substances decreased due to non-enzymatic browning reactions. Additionally, vitamin C is a highly temperature-sensitive substance; heating or holding phases of the pasteurisation process can lead to losses of vitamin C content (Hong, Tuan & Hung, 2019).

The analysis results also showed that ethanol content (% v/v) remained unchanged during the pasteurisation process (5% v/v). However, when pasteurised at 90°C for 30 minutes or 95°C for 15-30 minutes, the canned fermented drinks experienced foaming. The ethanol content remained stable during pasteurisation because the product was tightly sealed, preventing the evaporation of ethanol. However, high temperatures increase molecular motion rates, especially for CO_2 and ethanol molecules present in fermented beverages (Day & McSweeney, 2016). Additionally, ethanol molecules have a lower boiling point than water (ethyl alcohol boils at around 78.5°C), making them easier to evaporate (Destanoğlu & AteŞ, 2019). Therefore, when pasteurising the product at temperatures above 90°C for a relatively long period of 15-30 minutes, foaming occurred due to the evaporation of these molecules (Figure 2).

Figure 2. Canned fermented beverage from green asparagus roots

The impact of pasteurisation temperature and time on bioactive compounds

Figure 3 indicates the effects of pasteurisation parameters (temperature and time) on the bioactive compositions in fermented beverages from green asparagus roots. The levels of phenolic, flavonoid, and saponin all tended to increase to an optimal value, then decreased gradually with increasing pasteurisation temperature. Specifically, at a pasteurisation temperature of 80°C, the levels of phenolic, flavonoid, and saponin (expressed per litre) were 0.88 g TAE, 0.33 g QE, and 2.34 g SE, respectively. When the pasteurisation temperature increased to 90°C, the levels of phenolic, flavonoid, and saponins (expressed per litre) increased and reached values of 0.94 g TAE, 0.34 g QE, and 2.37 g SE, respectively. However, there were no statistically significant differences compared to samples pasteurised at 85°C. Similarly, with increasing pasteurisation time, the levels of phenolic and saponin also increased to an optimal value, then decreased gradually. Specifically, the

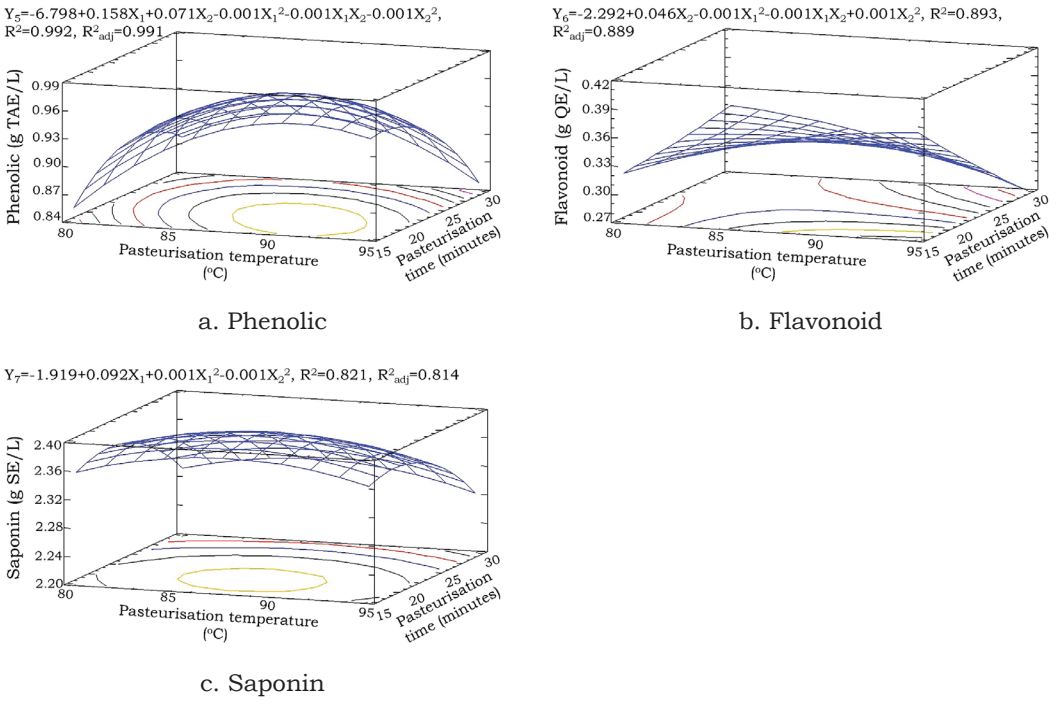


Figure 3. The impact of pasteurisation temperature and time on (a) Phenolic, (b) Flavonoid, and (c) Saponin in fermented beverage from green asparagus roots
Y: objective functions; X_1: pasteurisation temperature (°C), X_2: pasteurisation time (minute)

level of phenolic increased from 0.92 g TAE to 0.94 g TAE per litre, while the level of saponin increased from 2.37 g SE to 2.38 g SE per litre when the pasteurisation time rose from 15 to 20 minutes (p<0.05). However, when the pasteurisation time was further increased to 30 minutes, phenolic and saponin contents were only 0.88 g TAE and 2.30 g SE per litre, respectively. Meanwhile, the level of flavonoid decreased with increasing pasteurisation time, reaching its highest value of 0.36 g QE per litre when pasteurised for 15 minutes. This phenomenon is because flavonoids are less stable at high temperatures compared to other phenolics and saponins. Heat can break glycosidic bonds, leading to the degradation of flavonoid structures and a reduction in the total content during processing (Velázquez-Barreto *et al.*, 2020).

The stability of bioactive compounds (phenolic, flavonoid, and saponin) depends on the temperature and time of pasteurisation and their molecular structures (Velázquez-Barreto *et al.*, 2020). The stability of bioactive compounds in food matrices is influenced by various biochemical and chemical reactions, with key factors including pH, temperature, light, enzymes, and proteins. Among these, pH and temperature are the most critical (Hui *et al.*, 2021). Acidic conditions help stabilise these compounds. During heat processing, phenolics, flavonoids, and saponins can rapidly transform into different derivatives; thus, lower temperatures are more effective in preserving their integrity (Xiao, 2022). Additionally, the increase in bioactive compound content can also be affected by pasteurisation time (Sharma *et al.*, 2015).

Table 3. Effects of different pasteurisation temperatures and times on the colour parameters (*L, a, b*) of canned fermented drinks from green asparagus root

	L	*a*	*b*
Pasteurisation temperature (°C)			
80	39.38†b	-0.70b	5.49c
85	39.55a	-0.68b	5.50c
90	39.57a	-0.58b	5.53b
95	39.24c	-0.32a	5.57a
The level of significance	**	**	**
Pasteurisation time (minutes)			
15	39.38a	-0.51a	5.54a
20	39.47a	-0.36a	5.54a
25	39.46a	-0.52a	5.53a
30	39.42a	-0.90b	5.50b
The level of significance	**	**	**
The significance of interaction	ns	ns	**

†Average data (n=3); different letters on means in the same column indicate statistically significant differences at **p<0.01
ns: p>0.05

However, both higher temperatures and prolonged pasteurisation reduce the content of bioactive compounds, as they are sensitive to heat (Rawson *et al.*, 2013; Sharma *et al.*, 2015).

Results in Table 3 showed the effects of different pasteurisation temperatures and times on colour parameters. *L* value tended to increase to an optimal value, then decreased gradually with increasing temperature and pasteurisation time. Specifically, the *L* value increased slightly from 39.38 to 39.56 when pasteurisation temperature increased from 80 to 90°C; when pasteurisation temperature continued to increase to 95°C, the *L* value decreased to 39.24. Similarly, when pasteurisation time increased from 15 to 20 minutes, the *L* value increased from 39.38 to 39.47. The differences were not statistically significant compared to samples pasteurised at 25 and 30 minutes. The *a* value showed a similar trend with increasing pasteurisation time and reached its highest value (-0.36) when pasteurised for 20 minutes. Both *a* and *b* values increased with increasing pasteurisation temperature, while b decreased with increasing pasteurisation time. Heat processing is an important factor affecting the formation and the increasing or decreasing of aroma, colour, and flavour components (Hofmann & Schieberle, 2000). Furthermore, the increase in colour is also related to the decrease in total phenolic and flavonoid components (Recamales *et al.*, 2006). Additionally, browning due to the oxidation of vitamin C also contributes to the darkening of fermented drinks.

The study also developed regression equations to predict the levels of phenolic, flavonoid, saponin, vitamin C, and saccharose of canned fermented drinks from asparagus root at different pasteurisation temperatures and times (Figure 1 and Figure 3). Factors or interactions with non-significant influences were removed from the established model. The obtained equations have correlation coefficients R^2 and R^2_{adj}>0.81; thus, they can be used to predict changes in the levels of bioactive and chemical compounds, as well as the antioxidant capacity of

the extracted solution based on the pasteurisation temperature and time investigated. In addition, the purpose of finding the most efficient pasteurisation method is crucial for balancing operational efficiency (energy and time saving), maintaining product quality (including product quality preservation), and microbial safety assurance. By identifying and controlling the most effective pasteurisation, this helps maintain the product's nutritional, sensory, and functional properties, as well as ensures microbial inactivation of the product (Singh *et al.*, 2024). Optimising pasteurisation parameters reduces energy use and minimises waste from over-processing or damaged products, which contributes to overall cost savings in production and brings environmental benefits. These features satisfy both consumer demand and business profitability (Rama & Jinal 2019).

CONCLUSION

In this study, the optimal pasteurisation parameters for fermented drinks derived from green asparagus roots were identified as a temperature of 90°C for 20 minutes. These conditions ensured that they were microbiologically safe, maintained high levels of bioactive compounds, and were economically beneficial. This study not only highlighted broader implications for nutrition, public health, and sustainable economic development, but also offered potential for the production of nutritionally enhanced, antioxidant-rich formulations, serving as a foundation for future research and product development.

Acknowledgement

This research received financial support from the Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number "C2022-16-12".

Authors' contributions

Giang NTN, designed, carried out the experiment, analysed the data, wrote, reviewed, and edited the manuscript; Khai TV, carried out the experiment and analysed the data.

Conflict of interest

The authors declare no conflict of interest.

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