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Effects of Storage Time on the Chemical and Microbiological Quality of Pasteurized Beetroot (*Beta vulgaris* L.) Juice with Enzyme Extraction and Clarification

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Abstract | Unprocessed beetroot juice has a short shelf life. Pasteurization had been one of the common techniques practised in beverage industry for prolonging shelf life of juice. This research was conducted to determine effect of storage time on the biochemical and microbiological quality of enzymatically pasteurized beetroot juice. A 3:1 ratio was used to blend the beetroot pulp with distilled water. After that, pectinase was added at a concentration of 1.5 U/mL for 4hrs and 40°C, and then clarified with pectinase and cellulase and their combination, at concentration of 1.0 U/ mL for 50°C and 15 min. After that, the mixtures were all kept at 90°C for five minutes in order to pasteurize the juice. Beetroot juice without treatment: fresh juice and juice with enzyme extraction but not pasteurised nor clarified served as control 1 and control 2, respectively. The juices were evaluated for pH, total titratable acidity, total soluble solids, vitamin C content, and microbial load at six-day intervals for 30 days. The pH and vitamin C content of the beetroot juices significantly ($p < 0.05$) decreased during storage. However, there was no significant influence on the total soluble solids over storage time. The total titratable acidity of the juices showed significantly ($p < 0.05$) increase over the storage time. The untreated beetroot juice showed be consumed in less than 6 days, while 24 days' storage for pasteurised enzyme-clarified beetroot juice is safe for human consumption. This research had contributed to the beverage industry in show-casing the shelf life of beetroot juice pasteurized and clarified with enzymes as well as the untreated beetroot juices.

Received | February 22, 2024; **Accepted** | September 06, 2024; **Published** | October 09, 2024***Correspondence** | Lee-Hoon Ho, Faculty of Bioresources and Food Industry, School of Food Industry, Universiti Sultan Zainal Abidin, 22200 Besut Campus, Malaysia; **Email:** holeehoon@yahoo.com, holeehoon@unisza.edu.my**Citation** | Funmilayo, A.M.Z. and L-H. Ho. 2024. Effects of storage time on the chemical and microbiological quality of pasteurized beetroot (*Beta vulgaris* L.) juice with enzyme extraction and clarification. *Sarhad Journal of Agriculture*, 40(Special issue 1): 69-80.**DOI** | <https://dx.doi.org/10.17582/journal.sja/2024/40/s1.69.80>**Keywords** | Pasteurised beetroot juice, Enzyme, Extraction, Clarification, Biochemical characteristics, Microbiology quality**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Beetroot (*Beta vulgaris*) is also referred to as the table beet, garden beet, dinner beet, or golden beet. In Malaysia, it is locally known as “bit.” Beetroot is included in the subfamily “Betoideae” of the family

“amaranthaceae” (including the chenopodiaceae) (Kadereit *et al.*, 2006; Romeiras *et al.*, 2016). According to a report by Industry ARC (2018), the global market for fruit and vegetable juices is projected to reach around US\$ 57 billion by 2025, growing at a compound annual growth rate of 5.93% over the forecast period.

This significant growing in the beverages industry is attributed to rising consumer demand driven by health and convenience considerations (Abid *et al.*, 2013). However, due to their water activity and high moisture content, fresh vegetables and fruit juices are subdued to rapid enzymatic and microbial spoilage during storage (Sharma *et al.*, 2014) thereby causing rejection of the juice by consumers and financial losses for the producers. Fresh beetroot can be transformed into juice with the aid of enzymes through thermal processing in order to preserve their quality (Zainab and Ho, 2021).

Among the cause of the vegetable juice contaminant with potentially harmful microorganisms is being due to consistent application of natural fertilizers in ecological agriculture (Libudzisz *et al.*, 2008). Moreover, depending on the environmental conditions, pathogenic bacteria survive in soil over a long duration (Justyna and Monika, 2018). Bacteria and molds may spoil beetroot juice either by direct action of microbes or indirectly by the uptake of metabolites as off-flavors and toxins (Beartriz de cassia *et al.*, 2014). Low acid vegetables are usually resistant to the growth of some harmful microbes such as *Listeria monocytogens*, *Campylobacter jejuni*, *Salmonella* species, *Escherichia coli* 0157:H7 or *Staphylococcus aureus* (Sokolowska *et al.*, 2011; Torres-vitela *et al.*, 2013; Aneja *et al.*, 2014). These leads to the lowering of vegetable juices pH by some manufacturers by using ascorbic acid for safety and extension of the fresh juice shelf life. Despite these efforts, coliforms and *Listeria* species are the most frequently found pathogens in unpasteurized fresh beetroot (Buck *et al.*, 2003; Sokolowska *et al.*, 2011). Some of these microbes may remain in juice even after extensive procedures (Beartriz de cassia *et al.*, 2014). Generally, spoilage is usually exhibited as an off- odour or flavor of a chemical or medicinal nature due to the formation of guaiacol and halo phenols (Yamazaki *et al.*, 1996; Chang and Kang, 2004; Chen *et al.*, 2006) leading to rejections by consumers (Zierler *et al.*, 2004).

Therefore, various physical treatments such as conventional pasteurization, microwave heating, Ohmic heating, pulsed electric fields (PEF), thermos sonication, hydrostatic pressure (HHP), light treatment, and super critical carbondioxide (Leneveu-jenvrin *et al.*, 2020b) have been in existence to inactivate both microbes and enzymes in order to extend the shelf-life of the product (Noci *et al.*,

2008; Leneveu-Jenvrin *et al.*, 2019) and preserve the freshness of beetroot juice. However, the aforementioned processing technologies requires high cost that might not be affordable for small and medium enterprises (Fryer and Versteeg, 2008; Madrid-Guijarro *et al.*, 2009). Nowadays, thermal pasteurization had been affordable to use for small scale processing units. However, the potential of this technique to produce quality juice is needed to be greatly investigated. Ubeira-Iglesias *et al.* (2019) investigated the microbiological, nutritional, and sensory aspects of beetroot juice processed under high hydrostatic pressure. It was discovered that the beetroot juice had a microbiological shelf life of 21 days (Ubeira-Iglesias *et al.*, 2019). Czynowska *et al.* (2020) investigated the microbial quality of stored fermented beetroot juice, the results indicated that lactic acid bacteria were predominant among the bacteria microbiota tested. However, there is no research yet on the storage studies on microbiological quality of enzyme extracted and clarified beetroot juice via pasteurization process. Therefore, this research has described the effect of storage time on biochemical and microbiological quality of unpasteurized and pasteurized enzyme extracted and clarified beetroot juices.

Materials and Methods

Sample preparation

Fresh and ripe beetroot (*Beta vulgaris* L) was acquired in Terengganu, Malaysia's Besut District's local wet market. Prior to procurement, the beetroot's physical quality was evaluated. The two commercial enzymes (pectinase (DIS-1030) and cellulase (DIS-1017) were purchased from Creative Enzymes (New York, USA).

Fruit juice preparation

After washing with water, the purchased beetroot was peeled and sliced into small pieces. A homogenous slurry was then achieved by blending it with distilled water in a 1:3 ratio. Following a 4 hours incubation period at 40°C in a water bath, the pulp was treated with pectinase at a concentration of 1.5 units per milliliter (U/mL). In order to clarify the extracted juice, each 50 mL of the juice was treated with 1 U/mL of pectinase, cellulase, or a combination of pectinase and cellulase. The juice was incubated for 15 minutes at 50 °C in a water bath. After that, each mixture was held at 90 °C for five minutes in order to pasteurize the juice (Padma *et al.*, 2017). The control

1 indicates fresh juice; control 2 shows pectinase extracted juice but not undergone pasteurisation nor clarification; PPTJ was the pasteurised juice clarified with pectinase; PCTJ indicates pasteurised juice clarified with cellulase; PPCTJ was the pasteurised juice clarified with an enzyme combination (pectinase + cellulase). Both the treated and untreated juices were stored at 4 °C for 30 days. Thereafter, all the stored juices were evaluated for biochemical properties and microbiological quality at six-day intervals of storage time.

Determination of pH value

Prior to the analysis, pH 4 and pH 5 buffer solutions were used to calibrate the pH meter. A pH meter was then used to ascertain the beetroot juice samples' pH value.

Total soluble solid content

A hand-held refractometer with a 0–20 °Brix scale was used to measure the total soluble solids content. Degrees Brix (°Bx) were used to express the value (Ho *et al.*, 2020).

Total titratable acidity

The beetroot juice samples' total titratable acidity was calculated using the methods outlined by Antony and Chandra (1997). A five millilitres sample of beetroot juice was titrated against sodium hydroxide (NaOH) at a concentration of 0.05 M. To ascertain the titration's end point, phenolphthalein indicator solution (~2–3 drops) were added to the mixture prior to titration. Oxalic acid (%) was used to express the total titratable acidity.

Analysis of vitamin C content

The AOAC Standard Method (2005) was used to determine the vitamin C content of the beetroot samples. A titration against a 0.1 M iodine solution was performed after beetroot juice sample (10 mL) was combined with distilled water (10 mL). 0.5 mL of starch indicator was added to the mixture prior to titration. The titration ended at the first trace of a dark blue-black colour that was still visible. The amount of vitamin C was given in mg/ 100 mL of juice.

Microbial analyses

According to standard AACC method, method 42-50 and 42-11, respectively of mould and yeast counts (MYC) and aerobic plate counts (APC) were carried out using the aerobic spread plate count method. A

1 to 1 × 10⁻⁴ sample dilution was made. To obtain homogenized sampling, each dilution was shaken. After that, 1 mL of each sample dilution was pipetted onto the appropriate petri dish for MYC and APC analyses, which contained potato dextrose agar and plate count agar (PCA Oxoid, England), respectively. By carefully rotating the plates, all of the diluted samples were distributed across the agar's surface. For the APC and MYC plates, the inoculated plates were incubated at 37 °C for 24 hours and at 25 °C for five days, respectively (AACC, 2000).

Statistical analysis

Experimental data were processed using IBM SPSS Statistics (IBM Corp, Armonk, N.Y., USA), version 25.0. A one-way analysis of variance (ANOVA) was performed, followed by a Tukey test, to analyze the significant differences between the sample means at a significance level of $p < 0.05$.

Results and Discussion

pH, total titratable acidity, and total soluble solids of beetroot juices during 30 days of storage at 4 °C

The results of the pH, total soluble solids (°Bx), and total titratable acidity (%), of beetroot juice after storage at 4°C for 30 days are tabulated in Table 1. Considering the pH value for each sample during 30 days of storage, the pH for all the samples (i.e., control 1, control 2, pasteurised pectinase treated juice (PPTJ), pasteurised cellulase treated juice (PCTJ), pasteurised pectinase + cellulase treated juice (PPCTJ)) were significantly ($p < 0.05$) different among the days of storage within the same sample. This may be due to spoilage of the juice from days to days by microorganisms (Cortes *et al.*, 2008). Shamsudin *et al.* (2020), reported similar result where they had noticed a decrease in pH value of an untreated pineapple + mango juice blend through 25 days' storage in a refrigerator at 4±2 °C at 5 days' interval. Begum *et al.* (2018) experienced a decline in pH value of mango + pineapple + orange juice blend pasteurized for 5 min at 80 °C and then stored at -20 °C during 30 days of storage and recorded that the juice might be affected by microbial activities due to the carbohydrate degradation available in the juice blend. The rise in population/colony of microorganisms has cause more acid release due to fermentation, then cause pH to value drop. According to the authors, the juice might be affected by microbial activities as a result of the carbohydrate degradation available in the juice blend

(Begum *et al.*, 2018). Nidhi *et al.* (2007) also reported a decline in pH value during storage of untreated mango + papaya + pineapple juice and bael + guava juice.

Table 1: pH, total titratable acidity, and total soluble solids of beetroot juices during 30 days of storage at 4 °C¹.

Sam- ples ²	Days of storage	pH	Total titratable acidity (% of oxalic acid)	Total soluble solids (°Bx)
Control 1	0	6.32±0.01 ^{fC}	0.15±0.01 ^{bA}	4.83±0.06 ^{bA}
	6	5.07±0.03 ^{cB}	0.19±0.02 ^{dA}	4.97±0.06 ^{bA}
	12	5.15±0.02 ^{dB}	0.19±0.01 ^{dA}	4.97±0.06 ^{bA}
	18	4.97±0.03 ^{bB}	0.20±0.01 ^{dA}	5.00±0.00 ^{bA}
	24	5.28±0.01 ^{eB}	0.16±0.01 ^{cA}	5.00±0.00 ^{bA}
	30	4.84±0.05 ^{aB}	0.07±0.01 ^{aA}	4.17±0.29 ^{aA}
Control 2	0	6.14±0.01 ^{eB}	0.20±0.01 ^{aB}	4.80±0.00 ^{bA}
	6	4.87±0.02 ^{dA}	0.22±0.01 ^{aA}	5.00±0.00 ^{bA}
	12	4.77±0.02 ^{cA}	0.32±0.01 ^{bB}	5.00±0.00 ^{bA}
	18	4.69±0.01 ^{bA}	0.37±0.02 ^{cB}	5.17±0.29 ^{bA}
	24	4.70±0.02 ^{bA}	0.33±0.01 ^{bB}	5.00±0.00 ^{bA}
	30	4.63±0.01 ^{aA}	0.36±0.02 ^{bcB}	4.33±0.29 ^{aAB}
PPTJ	0	6.07±0.02 ^{cA}	0.40±0.01 ^{bC}	4.93±0.01 ^{aA}
	6	6.20±0.01 ^{dD}	0.36±0.01 ^{aC}	5.00±0.00 ^{aA}
	12	6.04±0.01 ^{bD}	0.42±0.02 ^{bC}	5.00±0.00 ^{aA}
	18	6.01±0.01 ^{bD}	0.47±0.01 ^{cC}	5.00±0.00 ^{aA}
	24	6.02±0.01 ^{bD}	0.45±0.02 ^{cC}	4.97±0.01 ^{aA}
	30	5.98±0.01 ^{aC}	0.48±0.01 ^{cD}	4.67±0.29 ^{aAB}
PCTJ	0	6.06±0.01 ^{cA}	0.42±0.01 ^{abC}	4.93±0.01 ^{aA}
	6	6.20±0.02 ^{dD}	0.41±0.02 ^{aD}	5.00±0.00 ^{bA}
	12	6.03±0.00 ^{bD}	0.41±0.01 ^{abC}	5.00±0.00 ^{bA}
	18	6.02±0.01 ^{bD}	0.42±0.01 ^{abC}	5.00±0.00 ^{bA}
	24	6.01±0.01 ^{abD}	0.43±0.01 ^{bC}	5.00±0.00 ^{bA}
	30	5.99±0.00 ^{aC}	0.44±0.01 ^{bC}	5.00±0.00 ^{bB}
PPCTJ	0	6.06±0.00 ^{dA}	0.41±0.10 ^{bC}	4.83±0.01 ^{aA}
	6	6.14±0.01 ^{eC}	0.33±0.15 ^{aB}	5.00±0.00 ^{aA}
	12	5.97±0.00 ^{cC}	0.42±0.20 ^{bC}	5.00±0.00 ^{aA}
	18	5.95±0.01 ^{abC}	0.44±0.40 ^{bC}	5.00±0.00 ^{aA}
	24	5.96±0.00 ^{bcC}	0.43±0.01 ^{bC}	4.97±0.01 ^{aA}
	30	5.94±0.01 ^{aC}	0.45±0.01 ^{bC}	4.83±0.29 ^{aAB}

Different superscript lowercase letters in the same column indicate significant ($p < 0.05$) difference for each beetroot juice sample in proportion to storage time (0–30 days). Different superscript upper-case letters in the same column indicate significant ($p < 0.05$) difference between beetroot juices at the same storage day. ¹Data are presented as mean ± standard deviation ($n = 3$). ²Control 1: Fresh juice, non-enzyme treated nor pasteurised; Control 2: Pectinase extracted but not pasteurised nor clarified juice; PPTJ: Pasteurised pectinase clarified juice; PCTJ: Pasteurised cellulase clarified juice; PPCTJ: pasteurised pectinase + cellulase clarified juice

For the total titratable acidity, the result shows an increasing trend. The rise in total titratable acidity during storage might be due to the decline in pH, as acidity and pH are inversely proportional to one another (Islam *et al.*, 2014). Shamsudin *et al.* (2020)

also reported higher total titratable acidity in pineapple + mango juice during 25 days of storage refrigerated at 4 °C, they recorded that the changes in total titratable acidity during storage might be due to the release of acids from the juice particles. Begum *et al.* (2018) revealed that the increase in the total titratable acidity for 30 days storage of pasteurized pineapple + mango juice blend at -20 °C might also be due to microbial activity, especially the acid producing microorganisms (Seema, 2015). Furthermore, Desrosier and Singh (2018) explained that yeast promote fermentation in fruits by breaking down of sugars into carbon dioxide and alcohol. Moreover, moulds cause lots of vegetable rots by changing their texture, colour and acidic products. Moulds grow at range of pH 3–8 in which some of them are able to grow in refrigerators due to different species having different optimal growth temperatures (Seema, 2015).

In comparing the total soluble solids of storage days for each sample (Table 1), The differences in all the beetroot juice samples that had undergone pasteurisation process, during the 30 days storage period were not significant, except sample PCTJ. This is in support of a research by Leneveu-Jenvrin *et al.* (2020a) on the storage studies of pasteurised and unpasteurised pineapple juice, whereby the difference in the total soluble solids of the juice samples (pasteurised and unpasteurised) were not significant. Moreover, control 1 and control 2 did not have significant difference from day 0 until day 24, thereafter, a significant decreased on day 30 was observed. This is due to the utilization of the sugar by yeast and mould as a result of massive growth of microbes on day 30 (Shamsudin *et al.*, 2014).

In comparing the pH value among the samples (control 1, control 2, PPTJ, PCTJ, and PPCTJ) at storage days. Pasteurized enzyme clarified beetroot juice (PPTJ, PCTJ, PPCTJ) had significant ($p < 0.05$) higher pH value than the unpasteurised beetroot juice samples (control 1 and control 2) except for the first day of storage, whereby the unpasteurized juices had higher pH value than the pasteurized and clarified juices. Also, control 2 (enzyme extracted juice but not pasteurised) had significant ($p < 0.05$) higher pH value than control 1 (fresh untreated nor pasteurised juice) through storage period. The decline in the pH of the pasteurised juices and control 2 as compared to control 1 on the day 0 of storage might be as a result of the release of carboxyl groups and galacturonic acid from

polysaccharides and pectin after enzyme treatment of the juices (Reddy *et al.*, 2018). The significant lower pH of the unpasteurised beetroot juice as compared to the pasteurised juice was due to the spoilage of the unpasteurised juice. Yeasts and mould have the ability to grow at low pH and high sugar concentration (Kamal *et al.*, 2014). Pasteurization at temperature of 90-95 °C for 4-10 seconds has been able to achieve Food and Drug Administration (FDA) recommendation of $5\log_{10}$ reduction of infection pathogens in a fruit juice (ICMSF, 2005; Mosqueda-Melgar *et al.*, 2012). Temperature at 95 °C is very effective against *Salmonella* and *Escherichia coli* (Danyluk *et al.*, 2011). However, it was as well noted that PPTJ and PCTJ had significant ($p < 0.05$) higher pH value as compared with PPCTJ from day 6 until day 24. This shows that PPTJ and PCTJ are significantly quality as compared with PPCTJ.

For total titratable acidity, all the pasteurised samples (PPTJ, PCTJ, and PPCTJ) had a significant ($p < 0.05$) higher total titratable acidity value than the unpasteurised (control 1 and control 2) juice samples, likewise, Control 2 had significant ($p < 0.05$) higher total titratable acidity value as compared to Control 1 except at day 6 of storage, whereby both showed insignificant difference in total titratable acidity (0.19% and 0.22% for control 1 and control 2, respectively). This might be due to the release of carboxylic acids and galacturonic acids as a result of the enzymatic treatment of the juice in, as pectinase facilitates pectin hydrolysis through de-esterification and the breakdown of polysaccharides (Joshi *et al.*, 2011; Akesowan and Choonhahirun, 2013). Hence, the rising of oxalic acid released during enzyme activity. Moreover, Seow *et al.* (2015) reported that high titratable acidity means high organic acid contents. Furthermore, these organic acids contribute to palatability and flavour of fruit juices (Maria Rita *et al.*, 2017). Juice with greater acidity is more resistant to spoilage by microorganisms and thereby having higher shelf life (Pimentel *et al.*, 2015). This might explain to the results obtained for pasteurised beetroot juice that had higher titratable acidity than unpasteurised beetroot juice throughout the storage days. On the other hand, for the total soluble solids, all the samples had no significant difference. Similar result was observed by Leneveu-Jenvrin *et al.* (2020a), they recorded an insignificant difference in the total soluble solids of mild heat treatment of pineapple juice during 14 days of storage period.

Vitamin C content of beetroot juices

Vitamin C provides protection against free radicals. It is an important element that possesses antioxidant ability (Esteve *et al.*, 2005). The results of the vitamin C (mg/ 100 mL) content of the beetroot juices over 30 days' storage period are presented in Figure 1. The results revealed that all the samples had a decreasing trend in vitamin C content during 30 days of storage period. This was due to the oxidation of the juice as ascorbic acid is sensitive to heat, oxygen, and light. Degradation of ascorbic acid during storage might be due to several reasons such as formation of dehydro-ascorbic acid, type of processing of the juice, packing materials and/ or storage temperature (Ayhan *et al.*, 2001; Byanna and Gowda, 2012). This result is in correlation with the report by Shamsudin *et al.* (2020), a decreasing trend was observed for the vitamin C content of pineapple + mango juice blend along the storage days. Similarly, Islam *et al.* (2014) reported a decrease in vitamin C content of juice prepared from orange + pineapple during the 35 storage days. Furthermore, vitamin C is considered as a point reference for the nutritional quality of juices (Bull *et al.*, 2004) and as one of the criteria to determine a product shelf life. The shelf life of juice is being considered for juices with as 50% retention of their initial vitamin C content (Shaw, 1992; Torregrosa *et al.*, 2005). All the beetroot juice samples had below 50% retention of their initial vitamin C upon 12th day of storage. However, at day 6, PPTJ had 86.29% of its initial vitamin C value; PCTJ, 82.26%; PPCTJ, 71.79%; control 2, 55.38%; and control 1, 52.04%. The vitamin C content of all the samples significantly remained constant from day 12 until day 30 of storage.

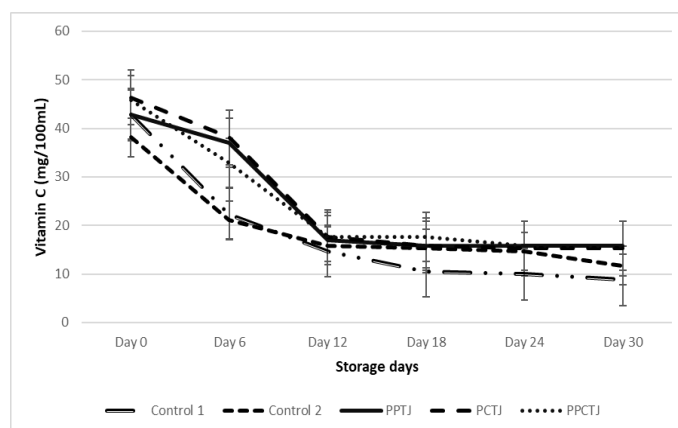


Figure 1: Effect of storage time on the vitamin C content of beetroot juice during 30 days' storage period at 4 °C. Control 1: Fresh juice, non-enzyme treated nor pasteurised; Control 2: Pectinase extracted but not pasteurised nor clarified juice; PPTJ: Pasteurised pectinase clarified juice; PCTJ: Pasteurised cellulase clarified juice; PPCTJ: pasteurised pectinase + cellulase clarified juice.

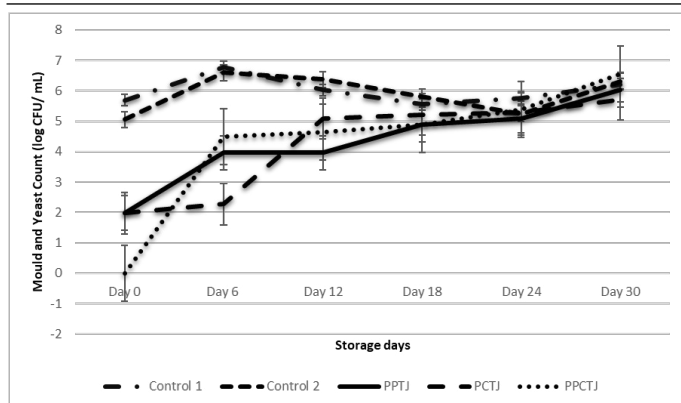


Figure 2: Mould and yeast count of beetroot juice during 30 days' storage period at 4 °C. Control 1: Fresh juice, non-enzyme treated nor pasteurised; Control 2: Pectinase extracted but not pasteurised nor clarified juice; PPTJ: Pasteurised pectinase clarified juice; PCTJ: Pasteurised cellulase clarified juice; PPCTJ: pasteurised pectinase + cellulase clarified juice.

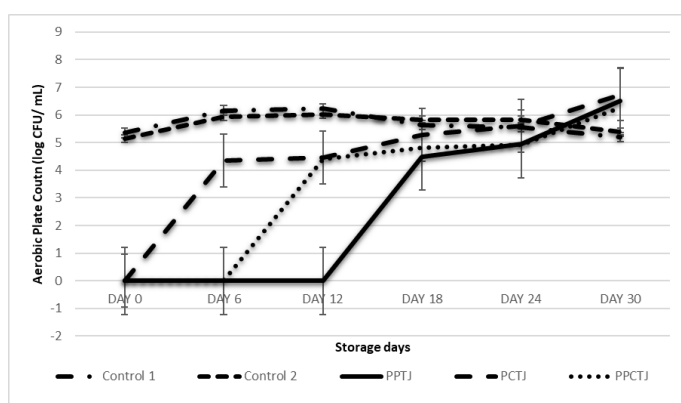


Figure 3: Aerobic plate count of beetroot juice during 30 days' storage period at 4 °C. Control 1: Fresh juice, non-enzyme treated nor pasteurised; Control 2: Pectinase extracted but not pasteurised nor clarified juice; PPTJ: Pasteurised pectinase clarified juice; PCTJ: Pasteurised cellulase clarified juice; PPCTJ: pasteurised pectinase + cellulase clarified juice.

Microbiological activities of beetroot juices

Bacteria, yeast and moulds were examined to access the microbiological quality of the beetroot juice samples. The results of Mould and Yeast Count (MYC) and Aerobic Plate Count (APC) of beetroot juices are presented in Figures 2 and 3, respectively. Mould and yeasts are the most notable group of microbes involved with spoilage of fruit juices. They can lead to alteration in smell, taste and appearance of the juice due to their metabolic by products such as carbon dioxide, acid and tainting compounds. Acid-tolerant bacteria can also cause spoilage of juice, even though most of the spoilage are usually caused by yeasts and mould in products related to fruits (Hocking and Jensen, 2001; Jay and Anderson, 2001). Results on the MYC showed unpasteurised beetroot juice (control 1 and control 2) to contain 5.69 logCFU/ mL and 5.06 logCFU/ mL respectively of colony before storage (Day 0)

(Figure 2). Leneveu-Jenvrin *et al.* (2020a) reported a similar result of MYC (5 logCFU/ mL) for pineapple juice during zero day of storage. They reported the most likely involvement of yeast and mould in the untreated juice spoilage. Similarly, Oranusi *et al.* (2012) reported incidence of 5 logCFU/ mL in total fungal count (TFC) of selected commercially packed fruit juices, such as: Citrus burst juice was reported to have 1.0×10^5 CFU/ g, orange juice, 1.4×10^5 CFU/ g, pineapple juice, 1.8×10^5 CFU/ g, and guava juice, 2.6×10^5 CFU/ g of the TFC. Fresh fruits and fruit juices contain a wide variety of microbes that can be environmental pollutants and raw material (Egemen, 2019). Juice contamination usually results from using inferior fruits (Drusch *et al.*, 2003). Fungi can infest fruits during various stages, including cultivation, harvesting, handling, transport, post-harvest storage as well as during marketing. Fruits storage at lower temperature usually significantly slows down fungal growth, hence, extends fruit shelf life. Refrigeration slows down the spoilage of food and therefore extend the product shelf-life (Salwa *et al.*, 2023). However, many microorganisms can grow at low temperature and cause significant damage to fruits, particularly if they are stored for extended periods (Arias *et al.*, 2002) before being processed to juice. Therefore, unpasteurised products are more contaminated to the pasteurised juice.

Mould and yeast count for sample control 1 (fresh juice) had no significant difference for the whole storage period (0 day to 30 days) except at day 6 which had significant increase in mould and yeast growth (optimum growth), thereafter a constant trend (decreasing) was observed. Similarly, control 2 (Pectinase extracted but not pasteurised nor clarified juice) had a significant increase in mould and yeast growth on the day 6 (optimum growth), but a significant decrease on the day 18 and 24. This indicates the spoilage of the beetroot juice began at earlier processing before storage, while a decrease in the growth was attributed to the population dying (the death phase). This obtained results can be supported by the results obtained for pH value during storage (Table 1), whereby, there was a significant change in the pH value of the juice during storage period. This indicates an increase in the growth rate of yeast and mould. Begum *et al.* (2018) experienced decline in pH of mango+ pineapple +orange juice blend during 30 days of storage and recorded that the juice might be affected by microbial activities

as a result of the carbohydrate degradation in the juice blend. The increase of population/colony of microorganisms has caused more acid release as a result of fermentation. However, the MYC was shown to significantly increase from 5.24 log CFU/ mL to 6.31 log CFU/ mL of colony at day 30 of storage. This was attributed to the fact that most of the cells die (population decrease), then there is less competition among the survive microorganisms for food. The survive microbes then continue to reproduce. The significant increase in mould and yeast growth on the 30 day was attested by significant reduction in the total soluble solids on the day 30 of storage (Table 1). A larger utilization of sugar by the microorganisms was due to the massive growth of yeast and moulds (Shamsudin *et al.*, 2014). Similar result was observed by Justyna *et al.* (2018), whereby, beetroot treated with high hydrostatic pressure (HHP) and then stored for 28 days in the refrigerator had decrease in the growth of *Escherichia coli* ATCC7839IP80.11T (5.42 log CFU/mL) and *Escherichia coli* 61/14 strain (3.10 log CFU/mL), compared to the initial microbial counts. After 21 days of storage, both the *Escherichia coli* strain population decreased to approximately 1.0 log CFU/ mL, but on prolonging the storage time to four weeks, there was increased growth rate of these bacteria (Justyna *et al.*, 2018).

The pasteurised juices samples had 1.98 log CFU/ mL of MYC for pasteurized juice clarified with pectinase (PPTJ) or cellulase (PCTJ) and no colony was indicated for the pasteurised juice clarified with pectinase + cellulase (PPCTJ) on the zero day of storage. This shows that the pasteurization process was effectively in inhibiting the growth of the mould and yeast. Similar result was reported by Beartriz de cassia *et al.* (2014), whereby apples at the reception before processing into juice had mould and yeast growth of 1.3×10^5 CFU/g of colony and washed apples had as high as 1.3×10^7 CFU/g of colony but significantly decrease to 1.5×10^2 CFU/g of colony after undergoing pasteurization.

Furthermore, there was no significant difference in the MYC for PPTJ and PCTJ throughout the storage days. For the pasteurised juice clarified with pectinase + cellulase (PPCTJ), a significant increase in colony was observed started on the 6th. This is in conjunction with the pH which also had significant decrease during these days. The increase in the acidity might be due to the releasing of metabolic by products (such

as carbon dioxide, lactic acid, ethanol and acetic acid) by microorganisms during their growth (Hocking and Jensen, 2001; Jay and Anderson, 2001). All the pasteurised and enzymatic clarified beetroot juice samples (PPTJ, PCTJ, and PPCTJ) had reached their growth peak on the 30th day at 6 logCFU/ mL. Similarly, Tournas *et al.* (2006) reported that during their studies on the microbiological quality of packaged fruit salads and pasteurised fruit juices, 22% of fruit juice samples were contaminated with fungi, ranging from <1.0 to 6.83 log₁₀ CFU/mL. According to “good manufacturing practices” (GMP), the standard limit for yeasts in unpasteurized fruit juices is < 10³ CFU/ mL and <10 CFU/mL for pasteurised fruit juices but the maximum acceptable level is set to be 10⁶ CFU/ mL (Stannard, 1997; Oranusi *et al.*, 2012). Moreover, Juice containing below 10⁶ CFU/ mL or 6 log CFU/ mL of microbial load is within acceptable limit for human consumption (ICMSF, 1994; Stannard *et al.*, 1997; Leneveu-Jenvrin *et al.*, 2020a). Therefore, 24 days of storage was the maximum storage day for pasteurised beetroot juice.

For the aerobic plate count, the result in Figure 3 shows that the unpasteurised juice samples (control 1 and control 2) reached 5.35 log CFU/ mL of colony and 5.14 log CFU/ mL of colony, respectively on the zero day of storage. This indicates a significant presence of bacteria in the juice. Similarly, Justyna and Monika (2018) reported a slightly higher result of 6 log CFU/ mL of colony in untreated beetroot juice for survival of *L. innocua* strains and *E. coli* strains on the zero day of storage. Juice containing below 10⁶ CFU/ mL or 6 log CFU/mL of microbial load is within acceptable limit for human consumption (ICMSF, 1994; Stannard *et al.*, 1997; Leneveu-Jenvrin *et al.*, 2020a).

Furthermore, from the results depicted in Figure 3, it was noted that the bacteria colony had significant increase from 5.35 log CFU/ mL to 6.16 log CFU/ mL for control 1 and 5.14 log CFU/ mL to 5.94 log CFU/ mL for control 2 during storage day of zero and 6th, respectively. Aerobic bacteria in both control 1 and control 2 samples had reached their optimum growth on day 12 and thereafter, significantly decrease in colony on the day 30th. The significant decrease of colony was due to population dying (the death phase). Justyna and Monika (2018) also experienced bacteria population dying during the storage studies of unpasteurised and high hydrostatic

pressure (HHP) treated beetroot and carrot juice. Furthermore, beetroot juice stored at 5 °C had growth of *Listeria innocua* (CIP8.11T) by 5.37 log CFU/mL of colony and *Listeria innocua* Wild strain 23/13 by 5.15 log CFU/mL of colony on the zero day of storage but declined to zero on the 28th day. On the other hand, growth of *Escherichia coli* ATTC7839 at 6.53 log CFU/mL of colony on zero day of storage was observed and decline to zero on the day 28th for beetroot juice stored at 25°C (Justyna and Monika, 2018).

All the pasteurised beetroot juice (PPTJ, PCTJ, and PPCTJ) had no bacterial growth on the zero day of storage. These shows that the pasteurization was effective in inhibiting the vegetative cell of bacteria in the juices. For pasteurised beetroot juice clarified with pectinase (PPTJ), no bacterial growth was observed until the 12th day of storage but significantly increased on the day 18 of storage. *Erwinia carotovora*, several *Pseudomonas* species and lactic acid bacteria are important bacteria that causes spoilage. During spoilage, starches and sugars are metabolised producing lactic acid and ethanol. *Pseudomonas* are gram negative bacteria that need a high-water activity for growth (0.95 or higher). During spoilage, starches and sugars are metabolised, producing lactic acid and ethanol and leading to unpleasant odors and flavors. Some of its species are psychophilic which grow at refrigerator temperatures. *Pseudomonas fluorescens* and *Pseudomonas viridiglava* are two *pseudomonas* species that account for up to 40% of the naturally occurring bacteria on the surfaces of fruits and vegetables (Seema, 2015). *Erwinia carotovora* is the most common bacterium responsible for spoilage; it has been detected in nearly every type of vegetable and can grow at refrigeration temperatures (Tournas, 2005). This cool condition triggered the growth of psychophilic on day 18. Furthermore, a significant rise in bacteria growth was noted on the 6th day of storage for pasteurised beetroot juice clarified with cellulase (PCTJ), This was due to the significant increase in the total soluble solids (Table 1) particularly monosaccharides of the juice during this period which might be caused as a result of the degradation of polysaccharides such as starch, pectin, and cellulose, and its conversion into simple sugar like fructose and glucose (Mohd-Hanif et al., 2016) and available for the use of microorganisms.

Nevertheless, pasteurised beetroot juice clarified with

pectinase + cellulase (PPCTJ) showed no bacteria growth until the 6th day of storage, thereafter, a significant sharply increase of bacteria colony at day 12th of storage. This might be due to the growth of acidophiles (acid loving bacteria). These current obtained results can be associated to the pH value that was recorded in Table 1, whereby there was significant decline in the pH value of the beetroot juice (from pH value of 6.14 to 5.97) during this period, thereby significantly becoming more acidic, causing growth of acid loving bacteria.

Conclusions and Recommendations

Overall, pasteurization had significantly influenced the stored beetroot juices clarified with or without enzymes. In general, the values of pH and total soluble solids decrease proportionally to the increase of total titratable acidity during 30 days of storage. Both pasteurization and clarification processes are able to increase the shelf life of the beetroot juice up to 24 days under the cold storage in the refrigerator at 4 °C. However, for the better retention of nutrient such as vitamin C, all the juice samples are best consumed before 12 days of storage. Pasteurised pectinase clarified beetroot juice (PPTJ) or pasteurised cellulase clarified beetroot juice (PCTJ) alone was recommended, considering being cost effective and more stable in compared to pasteurised pectinase + cellulase clarified beetroot juice (PPCTJ).

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Novelty Statement

Beetroot juice was produced using an innovative method that combines enzyme treatment and pasteurization to enhance its quality and shelf life. The study found that while untreated juice lasts less than 6 days, the pasteurized and enzyme-clarified juice remains safe for consumption for up to 24 days, providing valuable insights for improving shelf life in the beverage industry.

Author's Contribution

Abdussalam Muhyideen Zainab Funmilayo: Con-

ducted analysis, performed data analysis, wrote the original draft, and edited the writing.

Lee-Hoon Ho: Handled conceptualization, project coordinator, supervision, validation data and reviewed and edited the writing.

Conflict of interest

The authors have declared no conflict of interest.

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