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Short Communication

Preliminary Study on Long-Term Storage of Bulk Watermelon Puree

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Abstract: Watermelon is one of the major fruits in Malaysia and it ranks fifth in terms of production in the country. The produce has a short shelf-life span when after it has been processed and stored in liquid form. Thus, a preliminary study on the storage of watermelon puree was conducted to examine the quality of the product when it is in frozen form. The condition of bulk puree was chosen for this study to replicate the existing practice of a local company that produces frozen watermelon puree. The study aims to investigate the effects of these two storage conditions on the quality of the frozen puree in terms of microbial load, physical properties and lycopene level. The purees were packed in a nylon-Polyethylene (PE) packaging material with 5 kg each. Preliminary study results exhibited no significant difference between the conditions in terms of qualities that were studied on 2nd-month retrieval. Thus, based solely on the result of the study, the industry would be sufficient to store the product without having to blast it before storage.

Keywords: watermelon puree; long term storage; frozen

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1. Introduction

Watermelon is one of the major fruits exported by Malaysia with 135, 682.48 mt produced in 2022. Among the issues with this fruit is that it is planted on huge acres of land and when the harvesting season occurs, the planters would have problems in selling the fruits. Yau *et al.* (2010) have reported that there should be a concern with the quality of the fruit if it is stored at room temperature. To overcome this issue, MARDI has conducted research to

process the fruit into puree hence creating a new industry that produces watermelon-based products. Once the fruits are processed into a puree, the aspect of storage of the puree is another huge factor that must be considered especially in the aspect of quality. Most of the literatures focus on the issue of short-term storage but none of them delves into the aspect of long-term storage. Thus, this paper will focus on the issue of long-term storage of bulk frozen watermelon puree.

2. Materials and Methods

The study used a processing system that was available in MARDI that comprised of TS-P80 vertical single-head watermelon peeler (Tengsheng Machinery, China), DRB-MN130 extractor (Daribo, China), and 100 L electric pasteurizer (Gems, China) to process watermelon flesh into puree. The puree was then packed into 5 kg of nylon-Polyethylene (PE) packaging. Once the purees were filled into the packaging material, it was then submerged into a water tank containing icy water to reduce the product temperature. Then, the final packaged purees were divided into two categories, 50% of the puree packages were treated with the blast freeze process and stored in the chest freezer while another 50% of the puree packages were stored in the chest freezer without going through the blast freezing process.

The study focuses on three aspects of puree product quality, which are microbiological quality, physical properties and lycopene level. The retrieval of the puree packages was set at several intervals (0 days, 1st month and 2nd month).

2.1. Microbiological Quality of Watermelon Puree

The first aspect of the product quality that is being investigated is the aspect of microbiological quality of the puree. The microbiological quality of the frozen watermelon puree was evaluated using the microbial total plate count (TPC), total coliform count, total yeast and mould count and also the occurrence of pathogenic group consisting of *Escherichia coli*, *Staphylococcus aureus*, *Listeria* spp. and *Salmonella* spp. The evaluation was done following the standard protocols for microbiological analysis for each type of microbes.

The samples (TP1 and TP2) were obtained from the bulk packaging using an aseptic technique following the standard methodology for preparation of sample homogenate for microbiological analysis. An amount of 10 mL of each sample were homogenised in 90 mL Ringer's solution (Oxoid, UK) using a stomacher (Seward Medical, UK) for 30s. Serial decimal dilution was performed where an amount of 1 mL of the diluent from the above homogenate was pipetted into 9 mL sterile Ringer's solution. A number of dilutions from

at least three consecutive dilutions that could provide single colony growth were chosen for plating in specific agar plates and Petrifilm™.

For evaluating the TPC, 1 mL aliquots of each dilution for each sample were transferred into the Petri dish culture plate using a micropipette. Approximately 15 mL of sterile Plate Count Agar (PCA, Oxoid, UK) that had been prepared earlier following the manufacturer's instruction and was cooled to 45 ± 2°C, was poured into plates using the sterile Pour Plate method and let to solidify. The plates then being inverted and placed in an incubator (Memmert, USA) for incubation at 37 ± 2°C for 24 to 48 h.

As for the total coliform count, total yeast and mould count and also *E. coli*, the 3M™ Petrifilm™ were used where 1 mL aliquots of each dilution for each sample were transferred onto 3M™ Petrifilm™ *E. coli* / Coliform Count Plate (USA) and 3M™ Petrifilm™ Rapid Yeast and Mould Count (USA), respectively. The Petrifilm™ then were incubated at 37 ± 2°C for 24 h (for *E. coli* and Coliform) while at 25 ± 2°C for 72 h (for yeast and mould count).

As for evaluating the occurrence of *Staphylococcus aureus* and Listeria spp., respectively, the Baird Parker Agar (Oxoid, UK) supplemented with Egg Yolk Tellurite Emulsion (Oxoid, UK) and also the Palcam Agar (Oxoid, UK) supplemented with PALCAM Selective Supplement (Oxoid, UK) were prepared earlier and poured into sterile Petri dish culture plate and left to solidify at room temperature. Later, 0.1 mL aliquots of each dilution for each sample were dropped onto the solidified Baird Parker Agar and PALCAM agar, respectively. The dropped aliquots were then spread onto the agar using a sterile plastic spreader. The plates then being inverted and placed in an incubator (Memmert, USA) for incubation at 37 ± 2°C for 24 to 48 h.

The occurrence of Salmonella spp. was evaluated using another protocol where 25 mL of each sample was aseptically transferred into Buffered Peptone Water (Oxoid, UK) and incubated for 18 h at 37 ± 2°C. Then, an amount of 0.1 mL and 1 mL of the culture were transferred into Rappaport-Vassiliadis (RV) Broth and Selenite Cystine (SC Broth), respectively. The RV Broth then was incubated for 24 h at 41.5 ± 2°C while SC Broth was incubated for 24 h at 37 ± 2°C. Later, a loopful of culture from RV and SC broth was streaked onto solidified XLD Agar (Oxoid, UK) and Brilliance Salmonella Agar (Oxoid, UK) and incubated for 24 h at 37 ± 2°C. After the incubation period, all data were recorded as colony forming unit (CFU)/g sample except for the Salmonella spp. where it was recorded as absent or present in 25 g of sample.

2.2. Physical Properties of Watermelon Puree

The second aspect of the product quality that was investigated is the analysis of the physical properties of the frozen watermelon puree. In this aspect, total soluble solids (TSS), pH, viscosity and colour were measured during storage. TSS (◦Brix) was measured with a digital refractometer (Model HI 96801, Hanna Instrument, USA). pH was measured using a pH meter (Metler Toledo, Switzerland). Analyses were performed in triplicates and the average weight was reported. To determine the colour of the puree, the colour of watermelon puree was evaluated using a Chroma meter (Model CR-400/410, Konica Minolta, Japan) by measuring the following colour parameters of the puree: L*= 0 indicates black and L* = 100 white), +a* indicates red and –a* = green, and +b* indicates yellow and –b* = blue was determined for the surface of each sample. Analyses were performed in three replicates of measurement. The last aspect of the analysis of the physico-chemical analysis is the determination of viscosity. The viscosity of the watermelon puree was determined with a viscometer (Brookfield, USA) using a spindle LV1 and 50 rpm. The frozen puree was left in an air-conditioned room overnight for the thawing process to happen. Analyses were performed in three replicates of measurement.

2.3. Lycopene Content of Watermelon Puree

The last aspect of the watermelon puree that was studied was the level of lycopene content. In order to perform the analysis, Butylated hydroxytoluene (BHT) from Merck KGaA (Darmstadt, Germany), analytical grade of acetone (J.T.Baker, USA), ethanol 95% (Systerm, Malaysia), and n-hexane (Fisher Scientific, UK) were used in this assay. Lycopene was extracted using hexane: ethanol: acetone (2:1:1) and determined using a UV-vis spectrophotometer according to the method of Anthon and Barret (2007) and Kong and Ismail (2011). An amount of 1 mL of watermelon puree was weighed into an amber test tube Followed by 5 mL of acetone (containing 0.05% BHT), 5 mL of ethanol (95%) and 10 mL of hexane were added into the test tube and vortexed for 1 min. The mixture was then put into the ice bath and shaken for 20 min (200 rpm) in an orbital shaker. Then 3 mL of deionised water was added into the test tube and shaken (200 rpm) for 5 min. The mixture was left for phase separation (approximately 5 min). The upper layer (hexane layer) was then read in quartz cuvette using Eon Microplate Reader (BIOTEK GEN5 Vermont, USA) at 503 nm (hexane as the blank). The lycopene content was estimated and reported as mg/kg puree based on the following Equation (1) where the molecular weight of lycopene is 537 g/mole and the molar extinction coefficient of lycopene in hexane was 172 mM^{-1} (Zechmeister *et al.*, 1943). A_{503} is the absorbance of the hexane layer at 503 nm, W is the sample weight, 20 mL is the

volume of mixed solvent and 0.55 mL is the volume ratio of the upper layer in the mixed solvent.

$$\text{Lycopene}\left(\frac{\text{mg}}{\text{kg}}\text{fresh wt.}\right) = \frac{A_{503} \times 537\ \text{g/mole} \times 20\ \text{mL} \times 0.55\ \text{mL}}{W \times 172\ \text{mM} - 1} = \frac{A_{503} \times 537 \frac{\text{g}}{\text{mole}} \times 20\ \text{mL} \times 0.55\ \text{mL}}{W \times 172\ mM - 1} \quad (1)$$

3. Results and Discussions

3.1. Microbiological Quality of Watermelon Puree

Table 1. Microbiological analysis for watermelon puree on Day 1 (Month 0)

	Microbiological analysis						
	Total plate count	**Total yeast and mould count**	**Total coliform**	***Escherichia coli***	***Staphylococcus aureus***	***Listeria spp.***	***Salmonella***
TPa	3.70 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TPb	3.00 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TPc	1.00 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND

Notes: <1.0 x 10 indicates the microorganisms tested were not detected in the analysed samples, *Salmonella* spp. was not detected in the 25 mL sample, and triplicate sampling was done from the same bottle. The alphabet a, b, c represents a triplicate from the same bottle.
The result shows the microbial load of the sample is in the accepted range.

Table 2. Microbiological analysis for watermelon puree on Day 25 (Month 1)

	Microbiological analysis						
	Total plate count	**Total yeast and mould count**	**Total coliform**	***Escherichia coli***	***Staphylococcus aureus***	***Listeria* spp.**	***Salmonella* spp.**
TP1a	4.90 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP1b	4.10 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP1c	2.20 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2a	2.20 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2b	3.15 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2c	3.50 x 10^2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND

Notes: <1.0 x 10 indicates the microorganisms tested were not detected in the analysed samples, *Salmonella* spp. was not detected in the 25 mL sample, and triplicate sampling was done from the same bulk packaging. TP1 went through blast freezing. The alphabet a, b, c represents a triplicate from the same bulk packaging.

The result shows the microbial load of the sample is in the accepted range.

Table 3. Microbiological analysis for watermelon puree on Day 47 (Month 2)

Sample	Microbiological analysis						
	Total plate count	**Total yeast and mould count**	**Total coliform**	***Escherichia coli***	***Staphylococcus aureus***	***Listeria* spp.**	***Salmonella* spp.**
TP1a	1.14 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP1b	1.10 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP1c	1.06 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2a	1.07 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2b	1.00 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND
TP2c	1.11 x 10^3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10	ND

Notes: <1.0 x 10 indicates the microorganisms tested were not detected in the analysed samples, *Salmonella* spp. was not detected in the 25 mL sample, and triplicate sampling was done from the same bulk packaging. TP1 and TP2 are frozen sample. TP1 went through blast freezing. The alphabet a, b, c represents a triplicate from the same bulk packaging.

The result shows the microbial load of the sample is in the accepted range.

As for the aspect of microbiological properties of watermelon puree, the finding shows that the samples contain a satisfactory microbial count following the microbiological limit for intermediate product for the 2 months period.

3.2. Physical Properties of Watermelon Puree

Table 4. Changes of physical properties (pH, total soluble solid and viscosity) of watermelon puree during storage

Storage time (month)	**pH**		**Total soluble solid (º Brix)**		**Viscosity (cP)**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**
1	5.40 ± 0.01bB	5.43 ± 0.02abB	12.87 ± 0.15abA	13.00 ± 0.00aA	10.81 ± 0.26aB	3.72 ± 0.40bB
2	5.60 ± 0.01A	5.69 ± 0.01A	9.57 ± 0.06aB	9.10 ± 0.00bB	14.80 ± 0.35aA	11.27 ± 0.36bA

Data is shown in mean ± standard deviation.

Different lowercase letters in the same row are significant differences ($p < 0.05$).

Different uppercase letters in the same column are significant differences ($p < 0.05$)

Table 5. Changes of colour (L*, a*, b*) of watermelon puree during storage

Storage time (months)	L*		a*		b*	
	S1	S2	S1	S2	S1	S2
1	29.52 ± 0.46bB	31.26 ± 0.41aB	7.05 ± 0.04aA	5.47 ± 0.15bB	4.41 ± 0.04aA	3.99 ± 0.03bB
2	37.44 ± 0.04aA	31.96 ± 0.00bA	3.11 ± 0.08bB	5.69 ± 0.02aA	3.16 ± 0.06bB	4.57 ± 0.02aA

Data is shown in mean ± standard deviation.

Different lowercase letters in the same row are significant differences ($p < 0.05$).

Different uppercase letters in the same column are significant differences ($p < 0.05$)

As for the physical properties of frozen watermelon puree, for the aspect of pH, the pH value of S2 was higher compared to S1 ($p < 0.05$). However, the pH values of SI and S2 increased significantly ($p < 0.05$) after two months of storage, from 5.40 to 5.60 (SI) and from 5.43 to 5.69 (S2), respectively. In the analysis of dissolved sugar content in the watermelon puree, S2 exhibited the highest Brix value (13 ◦Brix) as compared to S1 ($p < 0.05$), which means that S2 was the sweetest. After being kept for 2 months, the Brix value of SI and S2 dropped dramatically ($p <0.05$). In the aspect of viscosity, the viscosity of the S1 sample was more viscous (10.81 cP), as compared to S2. However, the value increased after being stored for 2 months from 10.81to14.80 (S1) and from 3.72 to 11.27 (S1), respectively. As for the aspect of colour, the colour for sample S1 exhibited the highest red with the a* value (7.05) followed by sample S2 ($p < 0.05$). However, the colour dropped significantly ($p < 0.05$) and vice versa with sample S2 ($p < 0.05$).

3.3. Lycopene Level of Watermelon Puree

Table 6. Lycopene content of the frozen watermelon puree

Watermelon puree	Lycopene (mg/L)		
	Month 0	Month 1	Month 2
	60.54 ± 0.68		
Blast freezer + chest freezer		*63.55 ± 0.71*	*62.26 ± 1.58*
Chest Freezer		*64.20 ± 0.66*	*63.02 ± 0.22*

As for the aspect of lycopene content, the trend of lycopene level was rising from 0 month to 1 month of storage (4.9 % increase) but the trend seems to have went downward after 2 months of storage study (2.0 % decrease).

4. Conclusions

This study on long-term storage of bulk watermelon puree shows that there is no need for the puree to be blast freeze before being frozen. The industry player who is looking

forward to producing high-value watermelon products from the watermelon will benefit from this paper.

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Conflicts of Interest: The authors declare no conflict of interest.

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