

Effect of Heating at Different Temperatures and Times on the Bacterial/Fungi Count and Sensory Properties of Bottled Watermelon (*Citrullus Lanatus*) Juice

*Umelo, M.C, *Ejesieme Wisdom, *Eluchie, C. N, *Odimegwu, E.N,
*Akajiaku, L.O, **Ehirim, F.N and **Nwachukwu, C.N

*Department of Food Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria.

**Department of Food Science and Technology, Imo State Univeristy, Owerri, Imo State-Nigeria.

Corresponding Author: Umelo, M.C

Abstract: The effect of heating at different temperatures and times on the microbial count and sensory properties of bottled watermelon juice (*Citrullus lanatus*) samples stored for 4 weeks were evaluated. Standard procedures were used for the evaluation. The results obtained after analysis and precise measurement shows that heat treatment of juice samples is important with respect to shelf stability of bottled water melon juice. Judging from the results of the analysis carried out on the microbial counts of the three samples, it was observed that watermelon juice samples heated at 75°C for 15mins coded sample (WMJ¹), watermelon juice heated at 60°C for 30 mins coded sample (WMJ²) and the control sample (WMJ³) (refrigerated watermelon juice at 9°C) and stored for four (4) weeks were significantly different ($P < 0.05$). The total bacteria count of the entire juice samples increased as storage time increased from the 1st to the 3rd week of storage and was at its peak in the 3rd week of storage with values of 53, 65 and 80 cfu/g counts for samples WMJ¹, WMJ² and WMJ³ respectively. The total fungi/yeast count followed a similar trend with the bacterial counts within the storage duration. The fungi/yeast count increased with increase in storage time up to the 3rd week of storage and decreased by the 4th week of storage. The bacteria and fungi/yeast growth observed was less in sample WMJ¹ and sample WMJ² when compared with the control sample. The sensory evaluation showed that sample WMJ³ (control-refrigerated at 9°C) scored least in taste with mean score of 4.28 ± 1.4 . Sample WMJ² (heated at 60°C for 30min) had the most appealing appearance with a mean score of 7.36 ± 1.15 while WMJ¹ (heated at 75°C for 15 min) was most generally accepted by panelists with a mean score of 6.92 ± 0.81 . The storage duration affected negatively the sensory attributes in sample WMJ³ (control sample) in terms of taste (sweetness) and aroma with mean scores of 4.36 ± 2.271 and 4.28 ± 1.4 respectively. Thus the aim of this study is to determine the effect of heating at different temperatures and times on the microbial growth, sensory properties and shelf stability of the bottled watermelon juice.

Key words: heating, temperature, sensory properties, bacteria and fungi counts

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I. Introduction

Water melon juice is a sweet juice extract from water melon fruit (*Citrullus lanatus*). Water melon juice could be taken prior to eating as to stimulate hunger, quench taste and hydration (Tarazona- Diaz and Aguayo, 2013). Water melon juice is made from watermelon fruit as its principal material. According to (Tarazona- Diaz and Aguayo, 2013). Watermelon juice is an excellent drink to reduce muscle soreness in athletes. Water melon juice is also taken as an alternative option to taking watermelon fruits. This is due to its ease to access and to drink. The juice also reduces the rate at which the fruit is wasted. Watermelon juice is a refreshing and nutritious drink. It is rich in L-citrulline, Lycopene, Potassium, sugar, low in fat and containing water in high level. (<https://articles.mercola>). Water melon juice colour and viscosity can be treated by high intensity pulsed electric heat. Aguilo-Aguayo *et al*; (2010) discussed the effect of high intensity pulsed electric field (HIPEF) processing (35kv/cm for 1727µs applying 4 µs pulses at 188Hz in bipolar mode) on colour and viscosity in a watermelon juice. The treatment of (HIPEF) processing inactivate deleterious micro-organisms and quality-related enzymes, it also helps juice keep their fresh characteristics. Today in water melon juice production, in order to reduce rate of waste of watermelon fruits as a result of spoilage, reduce unhygienic handling of fruit, meet microbial safety need and also aid easy access to fruits nutritious content, alternative options of processing and preservation methods were developed (Anonymous, 2016). Watermelon (*Citrullus lanatus*), the principal materials of the juice is a scrambling and trailing vine in the flowering plant family *Cucurbitaceae*. The specie originated in Kalahari Desert of Southern Africa with evidence of its cultivation in

Ancient Egypt (Wiki, 2018). Watermelon does not contain any fat or cholesterol and is an excellent source of vitamin A, B₆ and C, also contains fibre, potassium etc. Every part of watermelon is edible even the seeds and rinds, contains 92% of water, 6% of sugar (<https://cals.arizona.edu>). Watermelon has a sweet juicy flesh which is usually deep red or pink and has a distinctive watermelon scent, usually heavy (for its size), weighs from 2.3-22.7kg but not more than 41kg (<https://cals.arizona.edu>). Watermelon has about 50 varieties as well as cultivars. They vary appreciably in taste, size, shape, texture, colour, though most consumers eat every variety of watermelon not minding its varying difference. Watermelon is beneficial to human health as they function as antioxidants preventing cancer; they promote blood flow and improve circulation due to the presence of amino acids. Watermelon takes 3-4 months to mature and therefore has a high turnover of harvest and yield within the year (Onyenweaku, 2009). Watermelon has been recorded to be one of the fruits comparatively high in demand in the Nigerian market today, mostly for its health purposes. Watermelons are usually taken as snacks, supplements, appetizers in picnic, before the main course meal etc. (Onyenweaku, 2009). Watermelons are highly harvested and consumed in the society. This has proved to become a problem as the shelf stability of watermelon is low and hence it is a perishable fruits. This has led to numerous numbers of wastes and spoilage of watermelon fruits.

II. Materials And Methods

Procurement of Materials

The watermelon fruits (*Citrullus lanatus*) used for this work were purchased at Relief Market, Owerri, Imo State-Nigeria. The watermelon fruit specie used was “**crimson sweet**”. The other equipments including the kitchen knife, the bowls for washing the water melon, blender (extractor), weighing scale, muslin cloth, bottles for packaging, cork for bottle caps, the pasteurizer, water used and the analytical reagents were obtained from the laboratory of the department of Food Science and Technology, Federal University of Technology Owerri, Imo State-Nigeria and Laboratory of New Concepts Services, Obinze, Owerri, Imo State-Nigeria.



Plate 1.0: Materials used in production of Watermelon Juice

Processing of Watermelon

The watermelon juice production was carried out according to the steps described by Marks *et al.* (2003) as shown in Figure 1.0. The watermelon fruits were weighed in a weighing scale balance, after which the fruits were washed thoroughly in saline water, removing the dirt, dust and any other material of concern. With the use of the knife, the washed watermelon fruits were peeled and cut open into four (4) equal parts. The rinds and seeds were removed from the cut open watermelon fruit; which were further sliced into smaller chunks and sizes. The smaller chunks of the watermelon fruits were crushed and extracted into juice directly using a blender. This resulted to pulpy watermelon slurry. The watermelon slurry was filtered using a Muslin cloth. This resulted to the watermelon juice. A transparent, sterile glass bottle was used in the packaging of the watermelon juice obtained. The watermelon juice was filled manually, into the bottles and corked for proper sealing. The watermelon juice was heated to destroy micro-organisms and inactivate enzymic activities at different temperatures and time.



Plate 2.0 Watermelon Juice samples

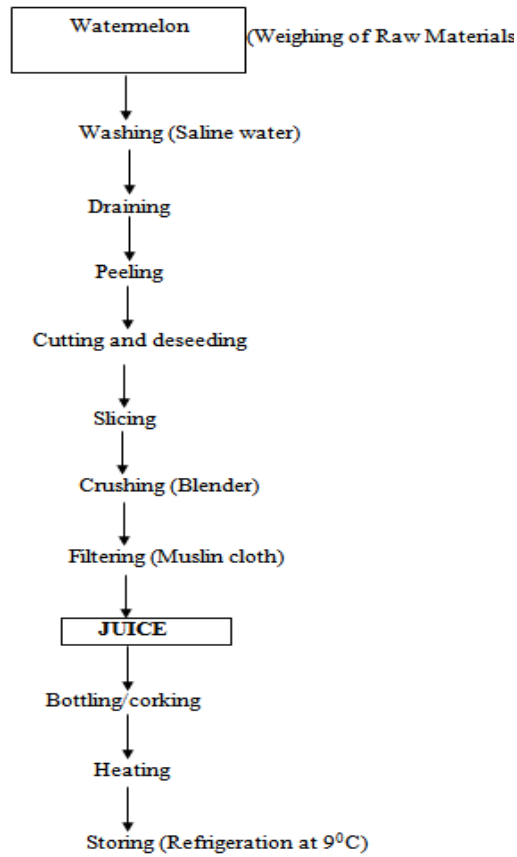


Fig. 1: Flow diagram of Watermelon juice production

Source: Marks *et al.* (2003)

The first bottled water melon juice was heated at 75°C for 15 mins and coded sample WMJ¹, the second was heated at 60°C for 30 mins and coded sample WMJ², the third was not heated at all (Control Sample-refrigerated at 9°C) and coded sample WMJ³. The bottled juice named, sample WMJ¹, WMJ², WMJ³ were stored in a refrigerator and maintained at the temperature of 9°C prior to analysis.

Microbiological Analysis of the Sample

Bacteria Count

The method used was adopted by Prescott (2002). About 1ml of the prepared group samples were diluted in 9ml of sterile distilled water and mixed vigorously by shaking. One (1) ml of the result mixture was aseptically transferred to 9mls of sterile water in a test tube. This action (serial dilution) was carried out under sterile aseptic conditions; the dilution was continued serially until the fourth dilution was attained. One tenth millilitre (0.1ml) of the third (3rd) and second (2nd) dilution were inoculated into sterile Nutrient Agar (NA) plates respectively. The spread plate technique as illustrated by Onwuka (2005) was used. A flamed glass rod was used to spread the inoculums evenly over the surface of the agar in the plate. The arrangements were done in triplicate for each of the group sample. The NA culture plates were inoculated at 37°C for 24 – 48 hours. All plates were observed daily. On establishing of the growth, the number of colonies formed in each plate was counted using Gallenkamp electronic colony counter. A mean of the count from the triplicate was obtained and multiply by appropriate dilution factor to obtain the microbial load as the total viable colonies per unit weight of the sample expressed as colony forming unit (CFU) per gram of the sample.

$$CFU/g = \frac{1}{w} \times N \times D$$

W = Weight of sample analyzed

N = Average number of colonies per plate

D = Diluent factor

In all cases, the microbial counts were taken from plates supporting not more than 300 colonies (Onwuka, 2018).

Fungi (Yeast count)

The method was adopted by Prescott (2002). About 1ml of the prepared group samples were diluted in 9ml of sterile distilled water (diluent) and mixed by shaking. One (1) ml of the result mixtures was transferred to 9mls of sterile water in a test tube. The serially dilution was carried out under aseptic condition until the 4th dilution was attained. About 0.1ml of the second (2nd) dilution was inoculated into sterile sabouraud dextrose agar (SDA) plate respectively. The spread plate technique as illustrated by Onwuka (2005) was employed. A flamed glass rod was used to spread the inoculums evenly over the agar in the plate. The arrangements were carried out in triplicate and incubated for 24 hrs. On establishing of growth, the number of colonies formed in each plate was counted using Gallenkamp electronic colony counter.

In all cases, the microbial counts were taken from plates supporting not more than 300 colonies (Onwuka, 2018).

N/B: Sabouraud dextrose agar (culturing media) was added chlorophenical during preparation of media.

Sensory Evaluation of Watermelon Juice

The sample (Watermelon juice) were evaluated by hedonic method for sensory characteristic and overall acceptability by a panel of 25 semi-trained judges selected; made up of students of Food Science and Technology Department, Federal University of Technology, Owerri, Imo State-Nigeria. They were served the coded samples of water melon juice chilled and asked to compare it by testing for taste, aroma (flavour), appearance (colour), mouth feel and overall acceptability. All tests were performed and rated on a 9 points structure hedonic scale described by Ihekoronye and Ngoddy (1985) as; 9 indicating like extremely, 8 indicating like very much, 7 indicating like moderately, 6 indicating like slightly, 5 indicating like or dislike, 4 indicating dislike slightly, 3 indicating dislike moderately, 2 indicating dislike very much, 1 indicating dislike extremely

Statistical Analysis

The sensory scores were subjected to analysis of variance (ANOVA) using one factor randomized test as described by Ihekoronye and Ngoddy (1985). The means were separated using the fisher's least significant difference (LSD) method at 5% level of significance.

III. Results And Discussion

Bacteria Count

The total bacteria count

The total bacteria counts of the watermelon juice samples increased as storage time increased up to the 3rd week of storage but decreased at the 4th week of storage as presented in Table 1.0. However, sample WMJ¹ (heated at 75°C for 15 minutes.) had the lowest bacterial count of 19 cfu/g in the first week of storage and 40 cfu/g at the fourth week of storage. Sample WMJ² (heated at 60°C for 30mins) had a mean bacterial count of 23 and 48 cfu/g for the 1st and 4th week storage duration respectively. The refrigerated sample WMJ³ (control) had the highest bacterial count of 28 and 69 cfu/g for 1st and 4th week storage duration respectively. The total bacteria count of the entire juice samples increased as storage time increased from the 1st to the 3rd week of storage and was at its peak in the 3rd week of storage with values of 53, 65 and 80 cfu/g counts for samples WMJ¹, WMJ² and WMJ³ respectively. This observation agrees with the report of Splittstoesser *et al.*, (2000) that *Bacillus* is a major spoilage organism in juices.

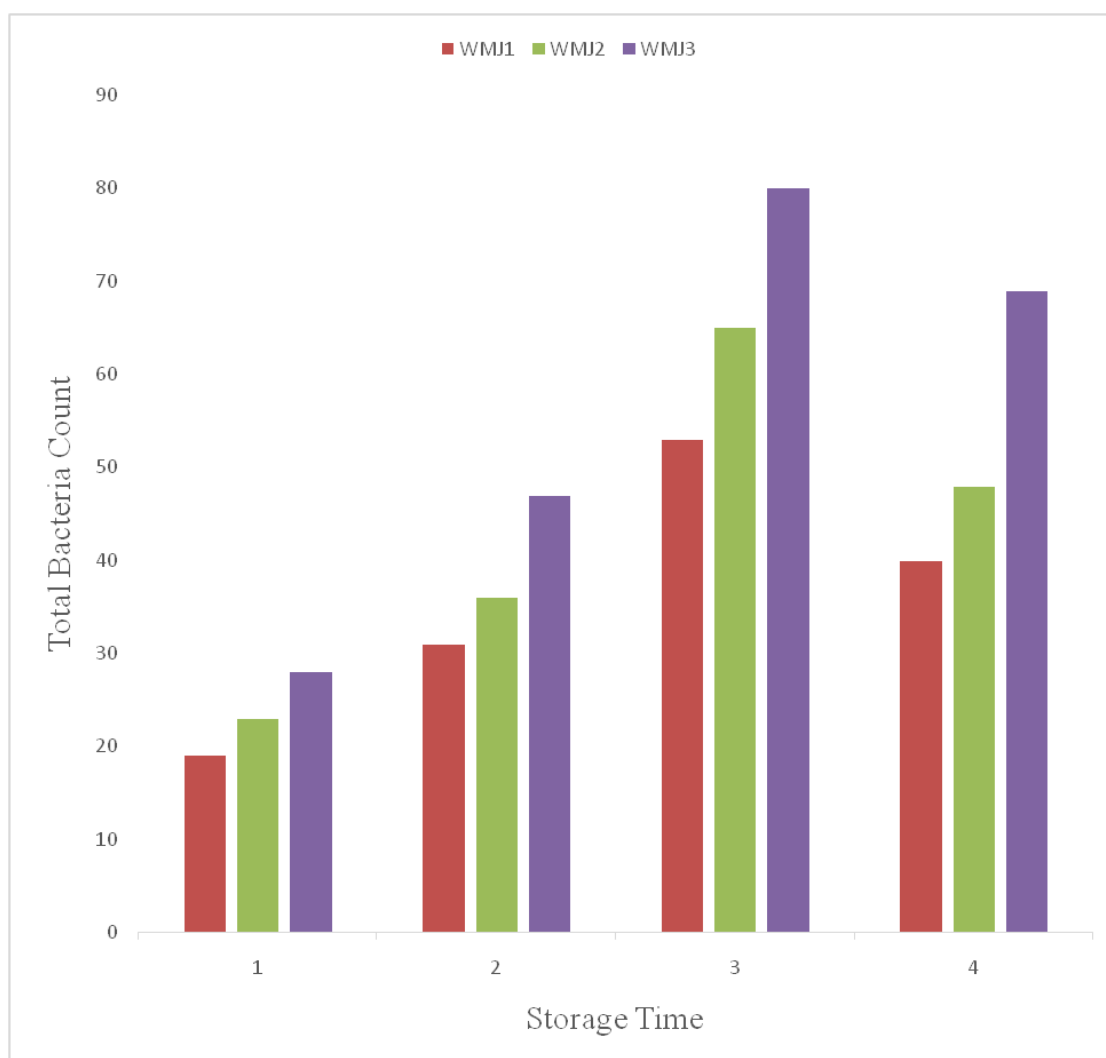


Fig. 2.0: Effect of Storage time on the Total Bacteria Count

According to Onwuka (2018), *Bacillus* is a food borne bacteria which produces spores with considerable heat resistance. It was observed that there was a decline (decrease) in the bacteria count on the 4th week of storage compared to the 2nd and 3rd week. This could be as a result of the highly acidic environment of the watermelon juice samples as storage time increases being inhibitory for the growth of the bacteria. This is in concordance with the findings of Onwuka (2018), which reports that microorganisms grow until the environment of which it grows becomes inhibitory for its growth. According to Weiser and Reddy (2001) organisms are inhibited from further growth by its own acidity. From all the observations made, it implies that heat processing of the watermelon fruit juice samples decreased the values of bacterial significantly when the heated juice samples were compared to the control sample (refrigerated juice). Thus, heat treatments could

contribute appreciably to the shelf stability of the fruit juice samples mostly at high temperature short time (HTST) were the count was decreased the most.

Fungi Count

The total fungi/yeast count

The total fungi/yeast count followed a similar trend with the bacterial counts within the storage duration. The fungi/yeast count increased with increase in storage time up to the 3rd week of storage and decreased by the 4th week of storage of the bottled water melon juice as shown in Table 1.0. However, samples WMJ¹ (heated at 75°C for 15mins) had the lowest yeast count of 20 cfu/g observed for the 1st week and 35 cfu/g for the 2nd week of storage compared to other samples. Sample WMJ² (heated at 60°C for 30mins) had a yeast count of 25 and 40 cfu/g for the 1st and 2nd week of storage respectively. The refrigerated juice (control sample) WMJ³ was observed to have the highest fungi count of 38 cfu/g on the 1st week and 54cfu/g on the 2nd week of storage respectively. Proliferation and increase of the fungi/yeast count was at its peak on the 3rd week of storage. They were 56, 62 and 90 counts for samples WMJ¹, WMJ², WMJ³ (control sample) respectively. This could be that the food environments (juice samples) were favourable for yeast growth at this point and there were much available nutrients for their metabolism. According to Onwuka (2018), appropriate environment (acidity), available nutrients, storage temperature, support microbial growth and the presence of viable microorganisms capable of growing under the prevailing condition. In addition, Ogubanwo *et al.*, (2013) reports that fungi/yeasts have the ability to survive in acidic juice environment at both ambient and refrigerated temperature.

Table 1.0 Total Bacteria (TBC) and Fungi Counts (TFC) of samples

| TIME | SAMPLE | TBC(cfu/g) | TFC(cfu/g) |
|--------|------------------|-----------------------|-----------------------|
| WEEK 1 | WMJ ¹ | 19 ± 0.0 ^a | 20 ± 0.0 ^b |
| | WMJ ² | 23 ± 0.0 ^b | 25 ± 0.0 ^c |
| | WMJ ³ | 28 ± 0.0 ^c | 38 ± 0.0 ^a |
| | LSD | 0.0 | 0.0 |
| WEEK 2 | WMJ ¹ | 31 ± 0.0 ^b | 35 ± 0.0 ^c |
| | WMJ ² | 36 ± 0.0 ^c | 40 ± 0.0 ^a |
| | WMJ ³ | 47 ± 0.0 ^a | 54 ± 0.0 ^b |
| | LSD | 0.0 | 0.0 |
| WEEK 3 | WMJ1 | 53 ± 0.0 ^c | 56 ± 0.0 ^a |
| | WMJ2 | 65 ± 0.0 ^a | 62 ± 0.0 ^b |
| | WMJ3 | 80 ± 0.0 ^b | 90 ± 0.0 ^c |
| | LSD | 0.0 | 0.0 |
| WEEK 4 | WMJ1 | 40 ± 0.0 ^a | 31 ± 0.0 ^b |
| | WMJ2 | 48 ± 0.0 ^b | 38 ± 0.0 ^c |
| | WMJ3 | 69 ± 0.0 ^c | 59 ± 0.0 ^a |
| | LSD | 0.0 | 0.0 |

Values with different superscript on the same column are significantly different at (P<0.05).

Key:

WMJ¹ = Water melon juice heated at 75°C for 15mins

WMJ² = Water melon juice heated at 60°C for 30mins

WMJ³ = Water melon juice refrigerated at 9°C (control)

LSD = Least significant difference

TBC= Total Bacteria Count

TFC= Total Fungi Count

Also water and environment may play a major role in fungi contamination of watermelon juice especially washing of the fruit during production. The presence of the yeast *Saccharomyce spp* is expected due to its preference for sugar and besides lowering of pH, highly favours yeast proliferation (Adams and Moss 1995). A decline was also observed on the 4th week of storage in fungi/yeast count. Samples WMJ¹, WMJ², WMJ³ recorded values of 31, 38 and 59 counts respectively. This could be as a result of the number of microorganisms outnumbering the available nutrients in the food environment for metabolism. According to Onwuka (2018), microorganisms have their metabolism to the food environment. Therefore, when nutrients to metabolize become unavailable the survivals of the microorganisms are threatened leading to a decline (Adams, 1996). According to Weiser *et al.*, (2001), metabolism of nutrients in the food environment by yeast leads to reduction in quality of product, reduction in sugar quantity and development of off flavour.

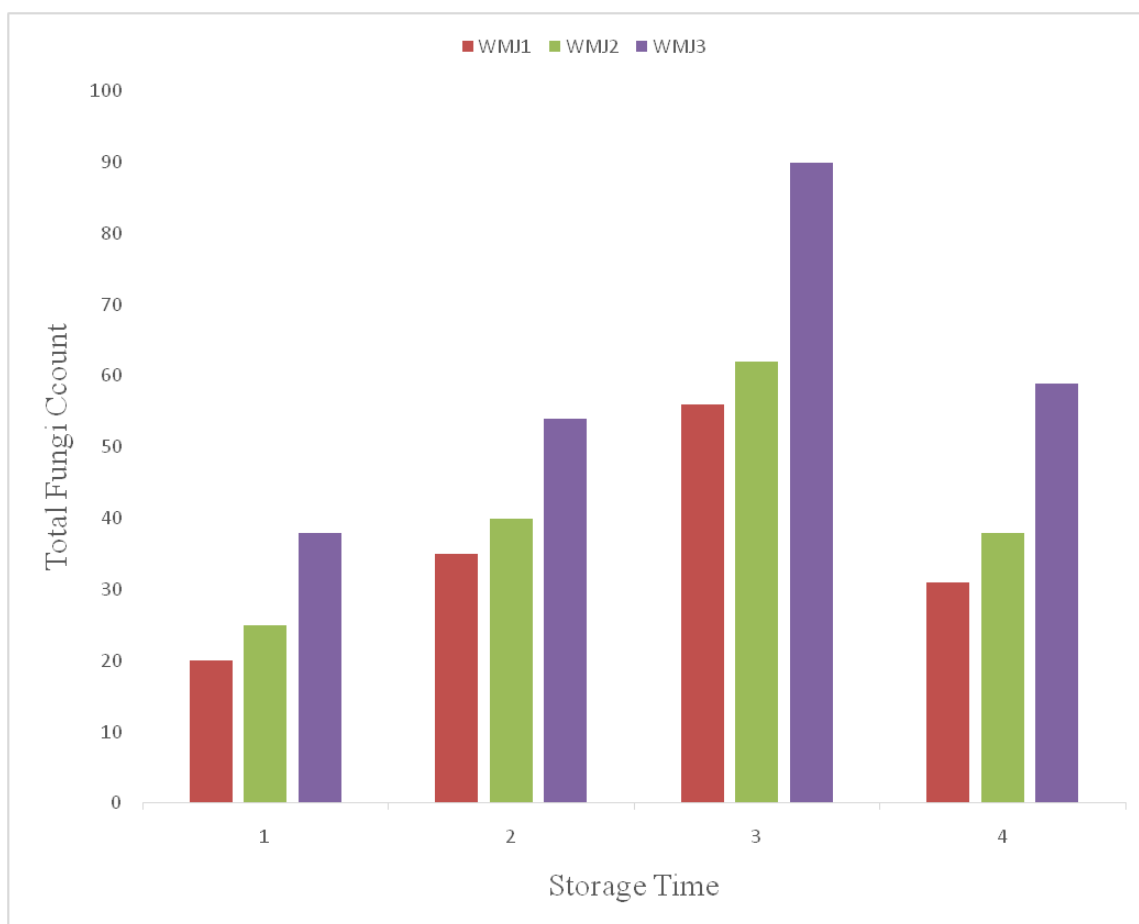


Fig. 3.0: Effect of Storage time on Total Fungi Count

Table 2.0: Mean Sensory Scores of Bottled Watermelon Juice Samples

| SAMPLE | AROMA | APPEARANCE | TASTE | MOUTHFEEL | GENERAL ACCEPTABILITY |
|------------------|-------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| WMJ ¹ | 6.4 ± 1.29 ^a | 6.68 ± 1.11 ^{ac} | 6.92 ± 0.91 ^b | 7.2 ± 1.22 ^c | 6.92 ± 0.81 ^a |
| WMJ ² | 5.2 ± 1.78 ^a | 7.36 ± 1.15 ^a | 6.4 ± 1.61 ^b | 6.52 ± 1.39 ^c | 6.56 ± 1.33 ^a |
| WMJ ³ | 4.28 ± 1.4 ^a | 5.48 ± 1.12 ^c | 4.36 ± 2.271 ^b | 5.4 ± 1.94 ^c | 5.08 ± 1.47 ^a |
| LSD | 2.44997 | 1.83477 | 2.751217 | 2.51814 | 2.0109 |

Values with different superscript on the same column are significantly different at (P<0.05).

KEY:

WMJ¹ = Water melon juice heated at 75°C for 15mins

WMJ² = Water melon juice heated at 60°C for 30mins

WMJ³ = Water melon juice refrigerated at 9°C

LSD = Least significant difference

Aroma (Flavour)

The result shows that sample WMJ¹ (heated at 75°C for 15mins) had the highest score in terms of the aroma (flavour) with mean score of 6.4±1.29 Table 2.0. It was closely followed by sample WMJ² (heated at 60°C for 30mins) with a mean score of 5.2±1.78 and sample WMJ³ (control-refrigerated sample) with a score of 4.28±1.40. The decrease in the mean score for aroma could be as a result of gradual spoilage as a result of microbial activities in the watermelon juice as storage time increased. According to Ogubanwo *et al.* (2013), microorganism such as bacteria, yeasts and mould implicated in spoilage of fruit drinks causes reduction in the sensory and quality of the drink. This affects the acceptance of the juice by consumers. Thus heat treatment of the juice samples WMJ¹ and WMJ² made the flavour more preferable compared to sample WMJ³ (control) over the period of storage. Hence, heat treatment helped in the reduction of the effect of spoilage by microorganisms.

Taste

In terms of the taste, sample WMJ¹ (heated at 75°C for 15mins) had the highest mean score of 6.92±0.91. Sample WMJ² (heated at 60°C for 30mins) closely followed with a mean score of 6.46±1.61 and sample WMJ³ (control) had a mean score of 4.36±2.27. The decrease in the mean scores as storage time increased could be due to fermentation and conversion of the sugars in the juice samples to alcohol, carbondioxide (CO₂), organic acids etc. According to Alam *et al.*, (2013), conversion of sugar affects the quality and sweetness of juice. Also Kaddumukasa *et al.*, (2017), stated that conversion of sugars to organic acids leads to reduction in quantity of sugar, spoilage and shortened shelf stability. Thus, conversion of sugars in watermelon juice could be the reason for its unpleasant taste during evaluation.

Mouthfeel

The result shows that sample WMJ³ (control) had the least score of 5.4±1.9 in terms of mouthfeel compared to sample WMJ² (heated at 60°C for 30mins) and WMJ¹ (heated at 75°C for 15mins) with mean scores of 6.52±1.39 and 7.2±1.22 respectively. The mouthfeel of the juice samples were liked slightly because of activities of microorganism and gradual spoilage which had began in the samples as storage time increased. According to Ogbanwo *et al.*, (2013), microorganism such as bacteria, yeasts and mould implicated in spoilage of fruit drinks causes reduction in the sensory and quality of the drink. This affects the acceptance of the juice by consumers. Hence, the control sample WMJ³ tends to experience more presence of microbial activities and sporadic spoilage compared to samples WMJ² and WMJ¹ heated at different temperatures and times. In addition, the pectin which could have decreased as storage time increased could be responsible for the lower score for mouthfeel.

Appearance (colour)

The sensory results shows that sample WMJ² (heated at 60°C for 30mins) scored highest in terms of appearance having a mean score of 7.36±1.15 Table 2.0. Sample WMJ¹ (heated to 75°C for 15mins) closely followed with mean score of 6.68±1.11 and sample WMJ³(control) with score of 5.48±1.12 .The decrease in the colour could be due to degradation of lycopene as well as decrease in vitamin A concentration of the samples. According to Alam *et al.*, (2013), watermelon juices are preferred in terms of colour due to the presence of lycopene which gives it, its natural colour. The lycopene content of the juice are degraded by processing methods, high temperature as well as long increase in storage duration. Thus, due to the heating of samples WMJ¹ at a high temperature (heated at 75°C for 15mins), the lycopene pigment which gives the juice its reddish colour was degraded. Sample WMJ³ (control) had a detestable appearance as a result of the effect of long time storage on lycopene content of the juice sample as well as microbial break down of the pigment. Sample WMJ² (heated at 60°C for 30mins) is most pleasing in appearance. This is because the juice sample was heated at lower temperature. This implies that the lycopene pigments were not readily impaired by mild heating, thus, giving the juice sample a nice appearance when compared with the control.

General acceptability

The result shows that sample WMJ³ (control) scored the lowest in terms of general acceptability having a mean score of 5.08±1.47, closely followed by WMJ² (heated at 60°C for 15mins) with score of 6.56±1.33 and sample WMJ¹ (heated at 75°C for 15mins) with score of 6.92±0.81 table 2.0. This could be due to the decrease in all the sensory attributes and the presence of increasing microbial activity in all the juice samples. According to Ogbanwo *et al.* (2013) microorganisms implicated in spoilage of fruit drinks causes reduction in organoleptic attributes and quality of the substances making them unacceptable by consumers.

IV. Conclusion.

This study has demonstrated that processing methods, storage temperature and storage duration significantly combine to affect the shelf stability and sensory attributes of the watermelon juice samples. The total bacteria count of the entire juice samples increased as storage time increased from the 1st to the 3rd week of storage and was at its peak in the 3rd week of storage and later decreased at the 4th week. The total fungi/yeast count followed a similar trend with the bacterial counts within the storage duration. The bacteria and fungi/yeast growth observed was less in sample WMJ¹ and sample WMJ² when compared with the control sample. The sensory evaluation showed that sample WMJ³ (control-refrigerated at 9°C) scored least in taste. Sample WMJ² (heated at 60°C for 30min) had the most appealing appearance while WMJ¹ (heated at 75°C for 15 min) was most generally accepted by consumers. The storage duration affected negatively the sensory attributes in sample WMJ³(control sample) in terms of taste (sweetness) and aroma. Thus heat treatment should be considered a better option in terms of extending the shelf life and improving the sensory attributes of bottled watermelon juice.

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