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MICROBIAL POPULATION AND SHELF LIFE STUDY OF SPICED WATER MELON JUICE

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ABSTRACT: *The microbial quality of spiced watermelon juice and the effect of pasteurization on the juice were investigated. Watermelon juice samples were analyzed according to standard bacteriological methods. The present study indicated significant reduction in microbial activities in all the juice samples under review. However, the rate of decline in the samples was less in refrigerated samples as compared to that stored at ambient temperature. The result for aerobic mesophilic count for the different watermelon juice samples increased (unpasteurized) from 3.22 × 10^2 to 5.31 × 10^2 cfu/ml, while the pasteurized watermelon juice samples showed a decreased microbial load from 0.05 × 10^2 to 0.07 × 10^2 cfu/ml. Results pre and post pasteurization of the juices showed zero (0) count for mould and E.coli, while total coliform ranged from <3.0 × 10^2 to 9.0 × 10^2 for the pasteurized and unpasteurized samples (MPN Index). ^{0}Brix for all the samples showed a decreasing trend, with the control (sample E) having the highest brix value. As storage progressed over a period of four week, brix values were higher at refrigerated temperature than at room temperature storage for all the samples. Refractive index decreased with storage at room temperature for all samples but remained relatively stable at refrigeration temperature. The pH of samples decreased with storage and time over a period of four weeks, with the spiced samples having lower pH values, while total titratable acidity (TTA) increased over the same period. The study revealed that the combined effect of spice, pasteurization and refrigeration positively affected the shelf life of the juice samples.*

KEYWORDS: Water melon, juice, microbial analysis, shelf life.

INTRODUCTION

Fruit juices are nutritious drinks with great value, taste, refreshing nature, medicinal and health importance, Suaad and Hamed 2008). Juices produced from tropical fruits have increasingly gained global importance due to their health effect, (Nwachukwu and Ezeigbo 2013). According to Alan and Sutherland (1994), fruit juices have high levels of iron, calcium and sugars and they are excellent source of vitamins A and C.

Watermelon is used as a dessert fruit and a thirst quencher that is relished by most persons as a source of water, consumed as whole fruit or blended with other fruits as smoothie. They are usually consumed unpasteurized by fruit vendors and are more appealing for consumers due to its fresh flavor. Fruit juices including watermelon juice may be contaminated with microbes from raw materials, juicing machine, handling and unhygienic conditions. The low acidic nature and growing condition of watermelon makes it a potential hazardous food (FDA 2001).

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Processing therefore plays an important role in the conservation and better utilization of fruits and vegetables.

Spices have been used locally to improve the flavor and taste of different foods, however there is little or no information on spiced water melon juice and its storage. Therefore, the study is aimed at investigating the effect of spice on the microbiological characteristics of watermelon juice during storage.

MATERIALS AND METHODS

Materials

Watermelon fruits were obtained from Rumuokwuta Market and processed within three (3) hours of purchase and the spices (Uziza, Ehuru, Clove, and Garlic) were obtained from Mile 1 Market, all in Port Harcourt, Rivers State of Nigeria.

Chemicals

All the chemicals and equipments used in these analyses were of analytical grade and were obtained from the food chemistry laboratory of National Agency for Food, Drug Administration and Control (NAFDAC), Area laboratory Port –Harcourt.

Methods

Watermelon Juice Extraction (Eke-Ejiofor, *et al* 2016)

The watermelon fruits were washed in saline (30%), and allowed to drain. The fruits were then cut and the seeds removed. The edible pink portion was cut into small bits for extraction.

Juice was extracted from the cut bits using Master Chef Food Processor, Model No MC-JBL2102. The extracted juice was filtered using three fold muslin cloth.

Spices

The dry spices (Ehuru, Uziza and Clove) were washed in distilled water and dried in an electric oven at 105^0C for 2 hours, Ehuru was deshelled. Each of the spices was milled using Nakai Blender (Dry Mill) model No.442; 40g each of the milled spices was mixed in 500mls sterilized water, the mixture was boiled, and filtered using whatman filter paper (4).

Garlic was peeled, washed with distilled water and blended, 40g of the blended garlic was mixed in 500mls of sterilized water, the mixture was boiled and filtered using Whatman filter paper(4).

Formulation of spiced Watermelon Juice. (Eke- Ejiofor *et al* 2016)

Different volumes of the spice extracts (3.2.2) (30mls, 50mls, and 100mls) were, made up to 500mls each, with the watermelon juice (3.2.1), to produce the different samples of the spiced watermelon juice for sensory evaluation. The various juices were then bottled in a pre sterilized bottle and pasteurized at 72^0C for 15 minutes, cooled at room temperature and stored for analysis.

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Shelf Life Study

The pasteurized samples were stored at room and refrigeration temperatures for weekly analysis of pH, brix, refractive index, and total titratable acidity (TTA) for a period of four weeks.

The pH of the juices was determined using a digital pH meter (mettle Toledo mv/ord), while the percentage brix was determined using a digital sugar refractometer (Atago RX 7000k).Refractive index was measured using a refractometer (Abbe refractometer).

While the total titratable acidity was determined using the volumetric method described by food and drugs manual of chemical methods of analysis (Food and Drug 1982).

Microbiological Assay:

Aerobic mesophylic Bacteria, *E. coli* and Mould (Pour Plate Technique)

The pour plate technique was used to determine the number of microbes/ml of Aerobic mesophylic Bacteria, *E. coli* and mould (Fankhauser, 2005). The following media were used: Plate count Agar (PCA) was used for aerobic mesophylic Bacteria. Violet Red Bile Agar (VRB) for the isolation of *E. coli* and Potato Dextrose Agar (PDA) for mould. The dilute samples (1:9) were placed in empty sterile plates (Petri dishes), and 15mls of the melted agar (cooled to 45^0C) was poured to it. The content was gently swirled to mix well and allowed to cool for 10mins, for the agar to gel, (In case of VRB a second layer was poured after the gelatinization of the first layer). The dishes were than inverted and incubated at 37^0C for 48 hours for aerobic mesophylic bacteria and *E. coli*, and for 120hours for mould count. The readings were taken respectively and result calculated as follows:

Cfu/ml = Cfu/plate x dilution factor x 1ml/aliquote

Where cfu = colony forming unit.

Coliform (LSB)

LSB: Lauryl sulfate broth.

Preparation:

35.6g of LSB medium was suspended in 1 liter of demineralized water, and dispensed into test tubes fitted with fermentation tubes (Durham tubes) and was autoclaved for 15min at 121^0C.

The prepared broth was clear and yellowish – brown and the pH was 6.8 at 25^0C.

Inoculation.

1ml of the sample was inoculated into 10ml of the LSB containing an inverted fermentation tube. It was incubated at 45^0C for 48 hours

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RESULTS AND DISCUSSIONS

Microbiological results of spiced watermelon juice samples

Watermelon juice was analyzed in triplicate for aerobic bacteria and coliform. The result was determined using the most probable number procedure (USDA 2008). Positive result was indicated by gas and acid production, which is showed by bubbles on the fermentation tube and turbidity of the broth, as a result of lactose fermentation by coliform bacteria such as *E. coli*, while negative result indicated neither acid nor gas production with the broth remaining clear and yellowish – brown in color.

Table 1, shows the microbiological results of the samples before and after pasteurization. The result for aerobic mesophilic count for the different spiced watermelon juices ranged from 3.22×10^2 - 5.31×10^2 for the unpasteurized samples and 0.05×10^2 – 0.07×10^2 for the pasteurized samples. The results after pasteurization of the juices showed an appreciable reduction in total count of the samples. The results in the present study falls within the NAFDAC maximum allowable limit of microorganisms in juice (NAFDAC, 2004). Results after pasteurization of the juices showed 0.05×10^2 for total count, zero (0) for mould and *E.coli*, and $<3.0 \times 10^2$ for coliform. Total coliform ranged from <3.0 – 9.0 for the pasteurized and unpasteurized samples (MPN Index). There was no growth for mould and *E.coli* for both the pasteurized and unpasteurized samples.

Sample B_2 (0.8% Ehuru spiced watermelon juice) showed the highest value for aerobic mesophylic count (5.3×10^2 and 0.07×10^2) for unpasteurized and pasteurized samples respectively, while sample E (100% water melon juice) showed the highest value for coliform 9.0 for the unpasteurized sample using MPN index and at 95% confidence limit.

Table 1: Microbiological results of spiced watermelon juice samples before and after pasteurization

Sample	Aerobic count ($\times10^2$)		Mould		E.coli		Coliform	
	U	P	U	P	U	P	U	P
B_1	3.22	0.05	0	0	0	0	3.0	<3.0
B_2	5.31	0.07	0	0	0	0	3.0	<3.0
E	4.87	0.06	0	0	0	0	9.0	<3.0

Coliform result, MPN index and 95% confidence limit for various combination of positive tubes in a 3 tubes dilution series, using inoculum quantities of 0.1, 0.01, and 0.001 ml.

KEY:

B_1 0.5% Ehuru spiced Watermelon juice

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B_2 0.8% Ehuru spiced Watermelon juice

E 100% Watermelon juice

U Unpasteurized

P Pasteurized

Shelf Life Study on the Microbiological Status of the sample.

Figure 1, shows the effect of storage temperature and period on the total count of the samples. The result for the effect of storage temperature and time on the total mesophylic count of the juice samples showed a fluctuating growth pattern. Microbial growth is usually completely inhibited at <0°c, but the organism however remains alive and gets reactivated when the temperature rises above 0°c (Fitz *et al,* 2003).Some organisms inhibits the growth of other organisms and when the inhibiting organism is exhausted (reduced or eliminated) the inhibited organism will grow (Fitz *et al,* 2003), these may explain the inconsistent pattern in the results of the various samples. Samples B_1 and B_2 showed higher value at refrigerated temperature; the addition of the spice may have introduced an organism that is favored by lower temperature, sample E which does not contain any spice shows higher value at room temperature. The result showed that the microbiological quality of the samples on storage was affected by the addition of the spices. Similar fluctuating pattern in value of the result was noted by Abbo *et al,* (2006) and Giovanna *et al* (2009).

Figures 2 to 5 shows the effect of temperature and time on shelf life stability of spiced watermelon juice samples at room and refrigerated temperatures, over a period of four weeks.

The physicochemical properties and microbiological status are used as indices for the shelf life study of the samples.

Figure 2 shows the effect of storage time and temperature on ^{0}brix value of samples. ^{0}Brix ranged from 7.6 -4.4, 5.6 -4.9 and 9.9 -9.4 at room temperature and 5.8 -5.5, 5.6 -5.4 and 9.9 - 8.9 at refrigeration temperature for samples B_1, B_2,and E respectively. All the samples showed a decreasing trend, with the control (sample E) having the highest brix. The result showed that brix was highest in the control sample which had no spice and at room temperature at week zero. As storage progressed over a period of four week, brix values were higher at refrigerated temperature than at room temperature storage for all the samples. The result also showed that the lower brix values observed in samples B_1 and B_2 may be due to the addition of the different levels of spices. The result agreed with the trend reported by Bhardwaj and Pandeys (2011).

Figure 3, shows the effect of storage temperature and time on the refractive index of spiced watermelon juice in samples; B_1, B_2, and E. Refractive index decreased with storage at room temperature for all samples but remained relatively stable at refrigeration temperature.

Figure 4, shows the effect of spice and temperature on pH of watermelon juice samples. pH ranged from 4.9 – 4.3 in sample B_1, 4.9 -4.0 in sample B_2 and 5. 9 -4.2 in sample E, decreasing with storage and time over a period of four weeks. This indicates that storage of spiced watermelon juice under refrigeration temperature reduced pH changes. Furthermore, the spiced juice samples had lower pH than the control. Mgaya-Kilima *et el (*2014), reported

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a pH range of 2.34-4.37 and 2.24- 3.34 for roselle fruit juice blends at room and refrigeration temperature respectively. Makanjuola *et al* (2013) reported a baseline pH for watermelon juice to range from 5.4- 4.0, and further reported much lower value of 3.11- 3.65 at room temperature and 3.09- 3.72 at refrigerated temperature, for some tropical fruit juices.

Fruit juices have a low pH because they are comparatively rich in organic acids (Tasnim *et al* 2010). The result agrees with the trend reported by Giovanna *et al*, (2009); Majundar *et al*, (2011) and Abbo *et al*, (2006). pH controls microbial growth in foods by directly inhibiting microbial growth and reducing the heat resistance of the microorganisms (Frasier and Westhoff 1998)

There was a gradual increase in titratable acidity (TTA) as storage period increased over the weeks (fig 5).The acidity of sample E (100% watermelon juice) at refrigerated temperature became constant after week one. The rate of increase was higher at room temperature, indicating that more acids were produced at room temperature than at refrigeration temperature during storage. TTA values of juice for room and refrigeration storage were significantly different ($p<0.05$). The increase in titratable acidity was more than in those stored in the fridge (12^0C). The result generally show that the lower the pH the higher the acidity of the juice. The increase in acidity might be ascribed to rise in the concentration of weakly ionized acids and their salts during storage (Safdar *et al* 2012). Increase in acidity might also be due to formation of acids by degradation of polysaccharides and oxidation of reducing sugar (lqbal *et al* 2001).

CONCLUSION

The addition of 0.8% Ehuru shows a measure of stability on the refractive index, total titratable acidity and pH of the juice.

The result on microbiological status indicates a high microbial activity at week 3; this may be due to the kind of microorganisms present. The pasteurization process was able to take care of the pathgenic organisms,

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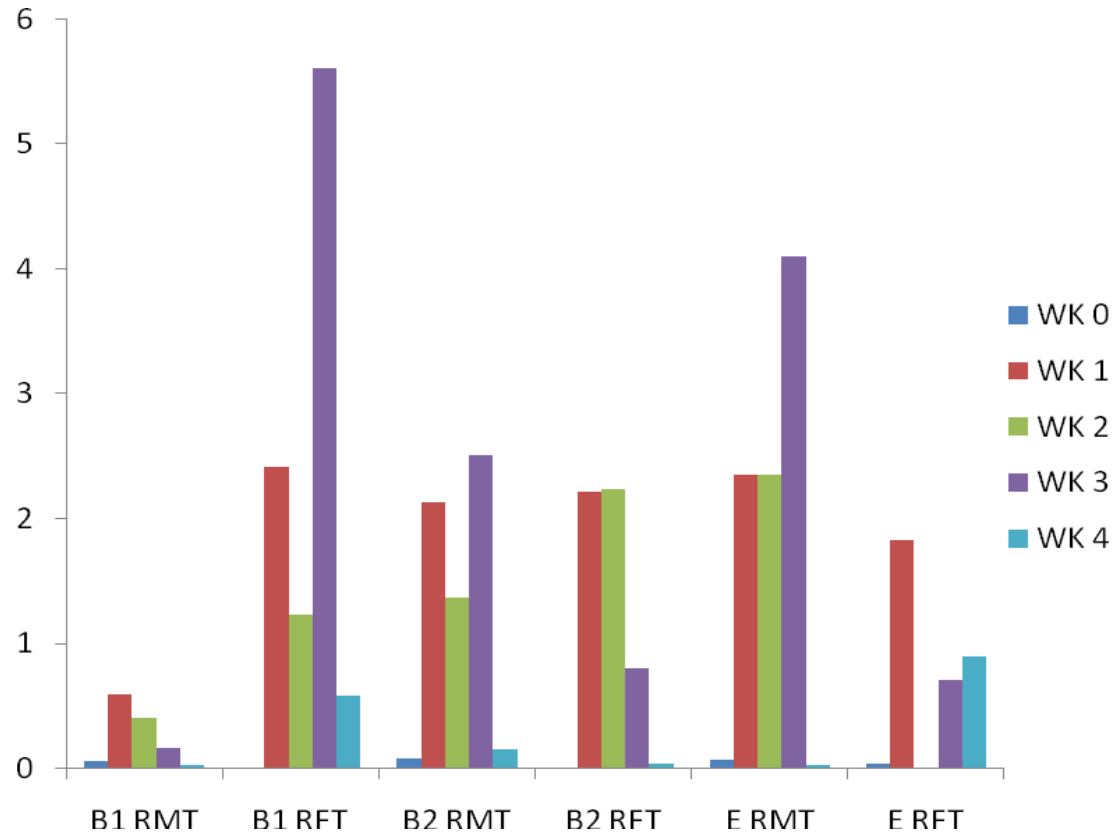

Fig 1: Effect of storage temperature and time on total mesophilic count of juice samples.

Key:

B1 = 0.5% Ehuru spiced watermelon juice

B2 = 0.8% Ehuru spiced watermelon juice

E = 100% watermelon juice

RMT = Room temperature

REFT = Refrigerated temperature

Wk = Week

Fig 2 to 5 shows the shelf life stability result of the samples at room and refrigerated temperatures, over a period of four weeks.

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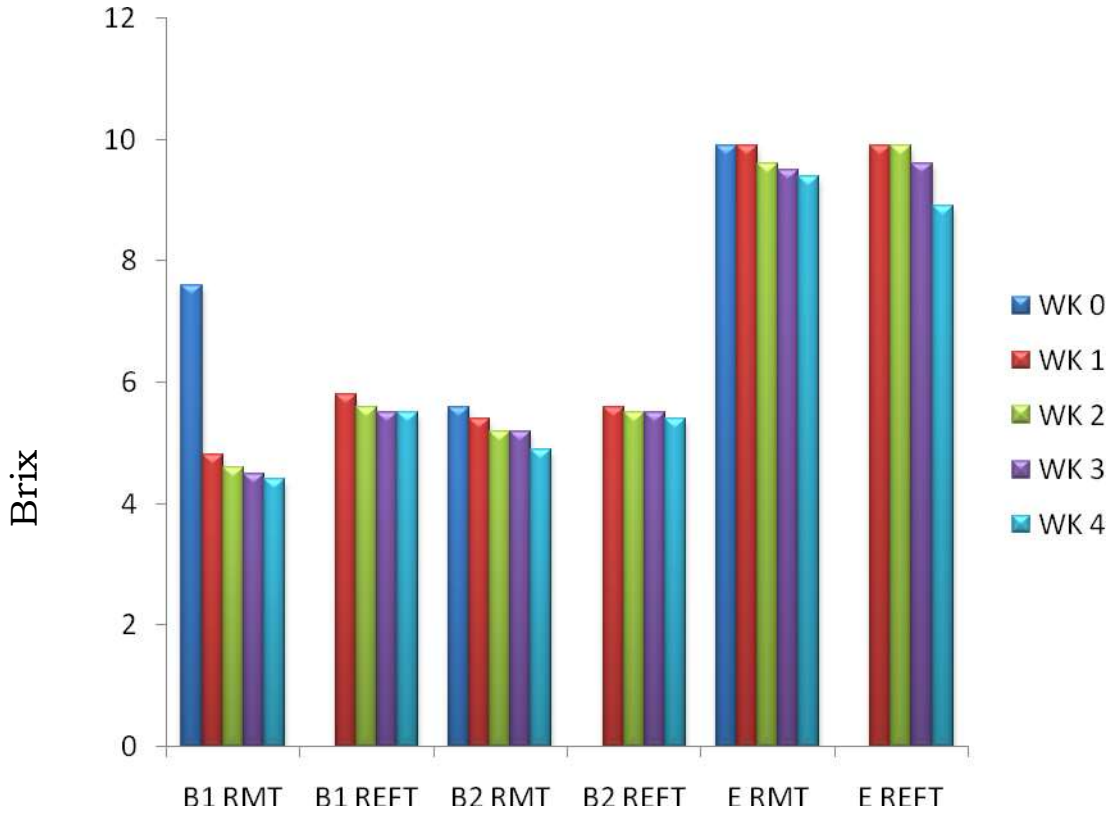

Fig 2: Effect of storage temperature and time on the brix Value of the juice samples.

Key:

B_1 = 0.5% Ehuru spiced watermelon juice

B_2 = 0.8% Ehuru spiced watermelon juice

E = 100% watermelon juice

RMT = Room temperature

REFT = Refrigerated temperature

Wk = Week

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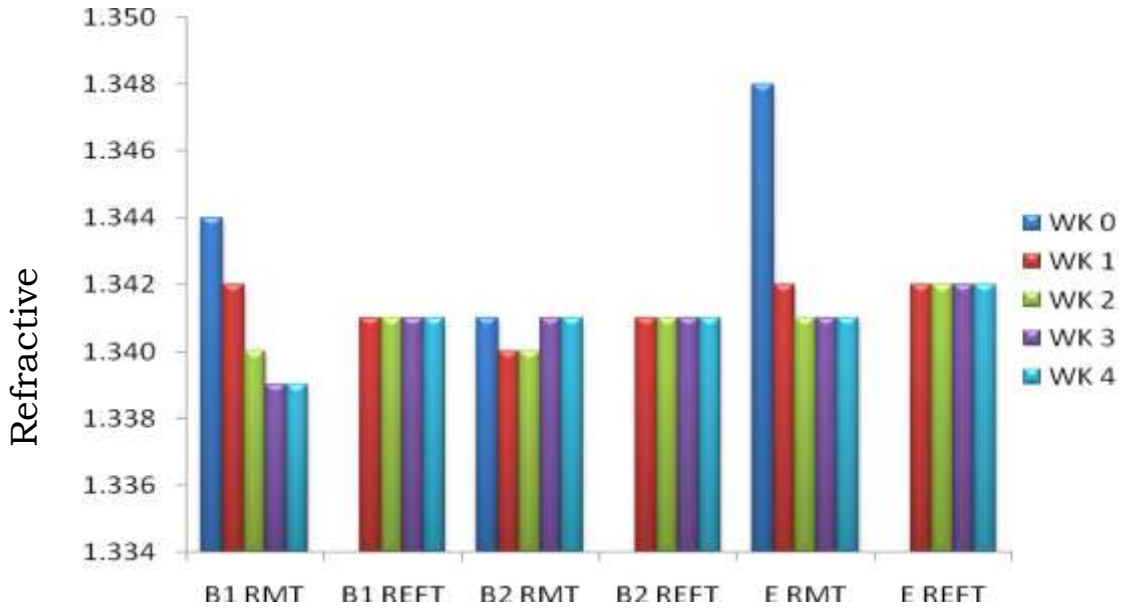

Fig 3: Effect of storage temperature and time on the refractive index of the juice samples,

Key:

B_1	=	0.5% Ehuru spiced watermelon juice
B_2	=	0.8% Ehuru spiced watermelon juice
E	=	100% watermelon juice
RMT	=	Room temperature
REFT	=	Refrigerated temperature

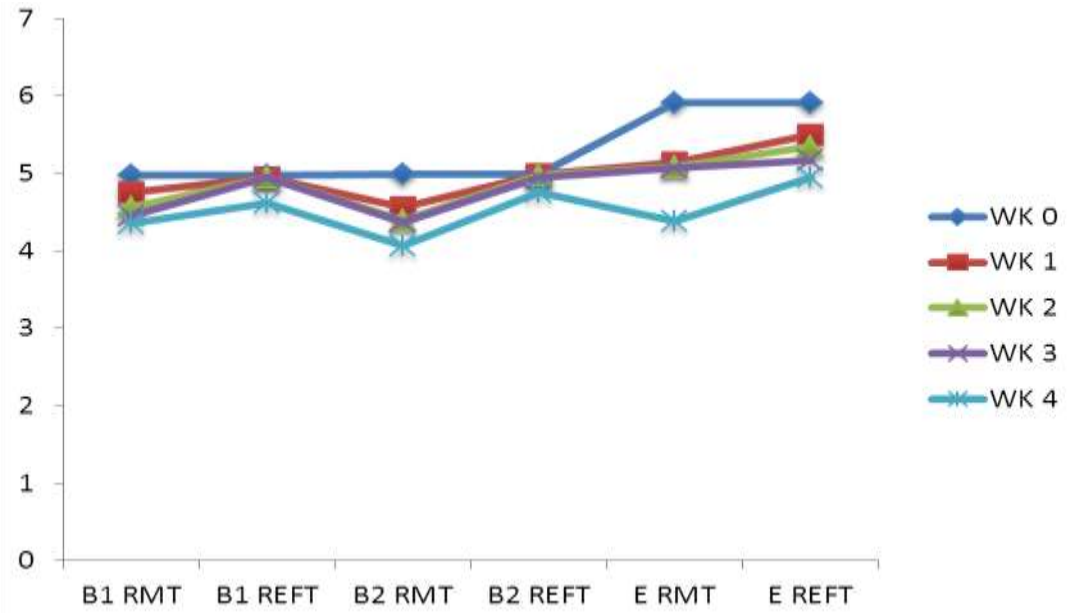

Fig 4: Effect of storage temperature and time on the pH of the juice samples

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Key:

B_1 = 0.5% Ehuru spiced watermelon juice

B_2 = 0.8% Ehuru spiced watermelon juice

E = 100% watermelon juice

RMT = Room temperature

REFT = Refrigerated temperature

Wk = Week

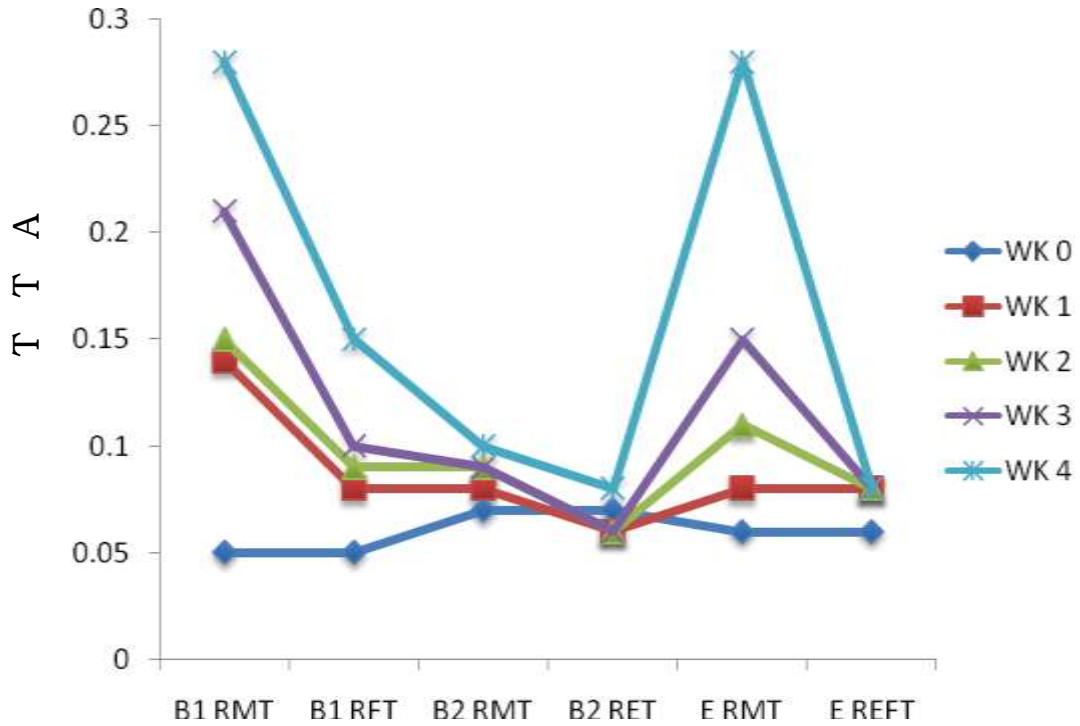

Fig 5: Effect of storage temperature and time on the total titratable acidity(TTA) of the juice samples

Key:

B_1 = 0.5% Ehuru spiced watermelon juice
B_2 = 0.8% Ehuru spiced watermelon juice
E = 100% watermelon juice
RMT = Room temperature
REFT = Refrigerated temperature
Wk = Week

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