THE PRESERVATION OF KUNUN-ZAKI USING CHEMICAL PRESERVATIVES, REFRIGERATION AND PASTEURIZATION

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Abstract: The potential of three preservation techniques: the use of two chemical preservatives; sodium benzoate and sodium metabisulphite, pasteurization and refrigeration for the preservation of kunun-zaki was evaluated. The treated samples were stored for four weeks. Microbiological analyses and assay of physico-chemical parameters such as pH, titratable acidity, sugar content, mineral content and alcohol content were conducted at three-day intervals for the duration of storage. Changes in the quality attributes and overall acceptability of the stored samples were also monitored over the period. Results obtained indicate the presence of seven organisms comprising five bacteria and two yeasts in the kunun-zaki samples. They were Bacillus subtilis, Bacillus cereus, Leuconostoc mesenteroides, Micrococcus varians, Lactobacillus fermenti, Saccharomyces cerevisiae and Candida albicans. Bacterial counts ranged from $0.89x10^6$ to $14.9xl0^6$ CFU ml/^{1} while fungal counts ranged from $0.98xl0^6$ to $16.8xl0^6$ CFU mL-^{1}. The results of physicochemical analyses carried out on pre-storage showed that kunun-zaki had pH, titratable acidity, sugar content and alcohol content of 5.93, 0.137 ml, 6.40% and 0.0% respectively. There was a general reduction in pH, sugar content and mineral content of the kunun-zaki samples, with a general increases in the titratable acidity and alcohol contents during the storage period. Sensory evaluation tests indicated that kunun-zaki samples preserved using chemicals and stored at 5°C were acceptable to the 27th day of storage. This study revealed a new possibility of storage stability of kunun-zaki, without significantly affecting the organoleptic properties ($p<0.05$) for four weeks with combined processes of pasteurization, refrigeration (5°C) and the use of chemical preservatives.

Keywords: Cereal beverage, Chemical preservatives, Kunun-zaki, Pasteurization, Refrigeration.

INTRODUCTION

Kunun zaki is a popular and widely consumed non-alcoholic fermented millet based beverage in Northern Nigeria. The cereals used in its production are millet, sorghum and maize in decreasing order of preference (Gaffa *et al.*, 2002; Akoma *et al.*, 2013). Although *kunun-zaki* is mostly produced from millet and sorghum, maize can also be used in addition to millet and the beverage is normally flavored with ginger, with or without the addition of sugar as sweetener (Ayo *et al.*, 2010).

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The beverage is milky cream in appearance and is consumed within few hours of its production (Adeleke and Abiodun, 2010). *Kunun-zaki* is consumed mainly by the low income earners who cannot afford carbonated drinks. (Obadina *et al.*, 2008). The popularity of *kunun-zaki* which is consumed while still in active state of fermentation by adults and children is due to its sweet-sour taste and milky cream appearance. In addition, the general belief that it enhances lactation in nursing mothers has further increased its popularity (Efiuvwevwere and Akoma, 1995).

Various studies on *kunun zaki* have shown that the beverage has immense social, economic, nutritional and medicinal benefits

to its numerous consumers (Gaffa and Ayo, 2002; Omonigho and Osubor, 2002; Akoma *et al.*, 2006) and is rich in carbohydrates, B-vitamins and minerals but low in protein (Ayo and Okaka, 1998). Production of *kunun zaki* is essentially at village technology level and at present, no large-scale factory production is available (Elmahmood and Doughari, 2007; Adejuyitan *et al.*, 2008). Traditional production is characterized by chance inoculation and the use of rudimentary equipment which results in products of varying quality attributes. The sanitary quality of the product during production and sales is poor thereby giving rise to the short shelf life of *kunun zaki* which is a major concern (Elmahmood and Doughari, 2007). However, *kunun zaki* is considered intrinsically safe due to its low pH and high titratable acidity (Ogwaro *et al.*, 2002; Akoma *et al.*, 2006; Oshoma *et al.*,2009). The objective of this study was to determine the effects of some chemical preservatives and other preservation techniques such as refrigeration and pasteurization on the keeping quality of *kunun-zaki.*

MATERIALS AND METHODS

Preparation of Kunun zaki samples

Fresh samples of kunun-zaki were purchased from a local producer into sterile conical flasks covered with aluminum foil and analysed within 1 hour of collection. Kunun-zaki was also prepared using conventional methods under aseptic laboratory conditions which served as control.

Preservatives and Preservation Techniques

Sodium benzoate (0.1% concentration) and sodium metabisulphite (0.025%) was used for the preservation of the kunun-zaki as well as refrigeration ($5^0C \pm 1^0C$) and pasteurization (63^0 C for 30 minutes) were used for the preservation of the kunun-zaki as follows:

Table A: Preservatives and Preservation Techniques

S/N	Preservation Techniques	Code Names
1	Kunun zaki samples purchased without preservatives+ room temperature ($26^0C \pm 1$)	Control 1
2	Laboratory-prepared Kunun zaki samples using sterile water and aseptic techniques without preservatives+ room temperature ($26^0C \pm 1$)	Control 2
3	Kunun zaki samples stored at 4^0C	Fridge
4	Kunun zaki samples pasteurized and stored at 4^0C	PFridge
5	Kunun zaki samples pasteurized and stored at 26 ± 1^0C	PRoom
6	Kunun zaki samples + 0.1% sodium benzoate and stored at 4^0C	BFridge
7	Kunun zaki samples + 0.1% sodium benzoate and stored at 26 ± 1^0C	BRoom
8	Kunun zaki samples + 0.025% sodium metabisulphite stored at $26^0C \pm 1$	MRoom
9	Kunun zaki samples + 0.025% sodium metabisulphite stored at 4^0C	MFridge
10	Kunun zaki samples pasteurized +0.1% sodium benzoate and stored at $26^0C \pm 1$	PBRoom
11	Kunun zaki samples pasteurized, + 0.025% sodium metabisulphite and stored at $26^0C \pm 1$	PMRoom
12	Kunun zaki samples pasteurized, + 0.025% sodium metabisulphite, stored at at 4^0C	PMFridge
13	Kunun zaki samples pasteurized, + 0.1%.sodium benzoate, stored at 4^0C	PBFridge
14	Kunun zaki samples pasteurized + 0.1% sodium benzoate and 0.025% sodium metabisulphite, stored at $26^0C \pm 1$	PBMRoom

15	Kunun zaki samples pasteurized + 0.01% sodium benzoate and 0.025% sodium metabisulphite, stored at 4°C	PBMFridge
16	Kunun zaki samples + 0.01% sodium benzoate and 0.025% sodium metabisulphite and stored at 4°C	BMFridge
17	Kunun zaki samples + 0.01% sodium benzoate and 0.025% sodium metabisulphite and stored at 26°C±1°C:	BMRoom

Microbiological Analysis

Total Viable Counts (TVC), fungal counts and microbial isolation were done using standard pour plate and streak plate techniques. Serially diluted Kunun zaki samples (10^{-5}) were inoculated into nutrient agar and potato dextrose agar plates for estimation of bacterial and fungal numbers respectively. Identification of isolated bacteria was done with the aid of the Bergey's Manual (Holt, 1994) using colonial and cellular morphologies and also various biochemical tests. Fungal identification was carried out using mycological atlas (Alexopolous and Mims, 1979; Beech *et al.*, 1986.; Kavanagh, 2005).

Physicochemical Analysis

The physicochemical analysis of the samples was carried on the onset of storage and monitored weekly throughout the four week storage period. The pH of the samples was determined using a pH meter (Philips PW9418); titratable acidity was determined using the method of Egan *et al.* 1981; the alcohol content was determined using an alcohol meter; total sugars were determined using a digital refractometer (TDR 095) using the method of AOAC (1990); Mineral content analysis was determined using the method of AOAC (1990). Analysis of variance (ANOVA) was carried out for the pH, titratable acidity, sugar content as well as overall acceptability for the kunun-zaki samples. The mean scores were computed and significant differences among the mean was determined ($p \leq 0.05$). The statistical analysis of the data was done using the Statistical Packages for Social Science for windows version 15.0 (SPSS, 2004)

RESULTS AND DISCUSSION

Seven organisms comprising five bacteria; *Lactobacillus fermenti, Micrococcus varians, Bacillus cereus, Bacillus subtilis* and *Leuconostoc mesenteroides* and two fungi *Saccharomyces cerevisiae* and *Candida albicans* were isolated from kunun-zaki samples. *Staphylococcus aureus, S. epidermidis, Micrococcus acidophilus, E. coli, Enterobacter aerogenes,* and *Klebsiella* sp. have been isolated from commercially prepared *kunun-zaki* (Essien *et al.*, 2009;Ejiogu *et al.*, 2010; Makut *et al.*, 2013). However, Efiuvwevwere and Akoma (1995) reported that *Lactobacillus fermentatum* and *Lactobacillus leichmannii* were dominant at the end of fermentation period. The distribution of the isolated microorganisms in the preserved *kunun-zaki* (Table 1) shows *Saccharomyces cerevisiae* and *Bacillus cereus* were predominant in all the preserved kunun-zaki.

The presence of yeasts in *kunun-zaki* samples is not surprising as yeasts play a prominent role in the fermentation of many African fermented beverages such as palmwine, pito, burukutu and agadagidi (Ekunsanmi and Odunfa, 1990), while the absence of moulds may be an indicator of the non-use of mouldy grains for *kunun zaki* production (Amusa and Ashaye, 2009). The microorganisms commonly associated with such beverages are members of the genera *Saccharomyces, Leuconostoc, Streptococcus* with *Bacillus* and *Micrococcus* occurring occasionally (Sanni and Oso, 1988; Sanni *et al.*, 1999, Ayo *et al.*, 2004, Amusa and Ashaye, 2009, Amusa and Odunbaku, 2009).

Results of the microbiological and physico-chemical analyses carried out on fresh samples of *kunun-zaki* reveal that the

fresh locally produced *kunun-zaki* samples had lower bacterial and fungal counts than the fresh laboratory-prepared *kunun-zaki* samples (Table 2). The higher microbial counts of the locally produced sample could result from unhygienic conditions of preparation, contaminated utensils and raw materials. The microflora of finished product depends on the processing and storage conditions it is subjected to (Oshoma *et al.*, 2009).

The pH of the fresh locally produced *kunun-zaki* samples was observed to be higher than the fresh laboratory-prepared *kunun-zaki* samples but lower amounts of titratable acids and sugars in the fresh locally produced samples (Table 2). No alcohol was found in both fresh laboratory-prepared *kunun-zaki* and fresh locally produced *kunun-zaki*.

The *kunun-zaki* samples were found to undergo series of changes due to the presence of microorganisms in the beverage. These changes include increase in total bacterial and fungal counts, titratable acidity, pH, alcohol content, sugar content and mineral content. All these were due to the growth and activities of the microorganisms present in the *kunun-zaki* samples. Bacterial and fungal counts of the treated samples increased from an initial 0.89 and 0.98 x 10^6 CFU mL^{-1} to between 6.90-14.90 and 13.60-16.80 x 10^6 CFU mL^{-1} respectively. The fungal counts were found to be consistently higher than bacterial counts in all the stored samples (Fig.1, 2). This may be attributed to the low pH (from an initial 5.97±0.01 to between 1.97±0.06 and 2.51±0.01), levels of titratable acids (from an initial 0.058±0.002 to between 0.177±001 and 0.233±0.001) (Fig. 3, 4, 6) which favors the growth of fungi over bacteria (Jay *et al.*, 2005). In addition, the refrigerated samples had lower microbial counts than those stored at ambient temperature irrespective of treatment. This is due to the effect of temperature on the growth of the organisms. However, it was observed that the bacterial and fungal counts began to increase by the 21st and 18th day of storage respectively; this coincided with decrease in levels of pH and sugars (Fig. 3, 5). The decrease in pH from pre-storage levels of 5.97±0.01 to between 1.97±0.06 - 2.51±0.01 by end of storage with a corresponding increase in titratable acids from 0.058±0.002 to between 0.177±001 - 0.233±0.001 (Fig. 3, 4) is attributed to the activities of the lactic acid bacteria and other isolated organisms capable of acid production (Table 1). This acidic environment is unfavorable for other non acid tolerant organisms and this has also been reported by Adeleke and Abiodun (2010), Abel *et al.* (2011) and Omojasola *et al.* (2012) in the analysis of some local Nigerian beverages. In addition, this acidity creates an unfavorable environment for pathogenic bacteria and members of Enterobacteriaceae (Olotu *et al.*, 2009). However a number of researchers have reported the presence of *Escherichia coli, Enterobacter aerogenes, Enterobacter clocae, Streptococcus feaceum* from commercial samples of *kunun-zaki* which they attributed to poor sources of water and unhygienic handling by the retailers (Oranusi *et al.*, 2003; Essien *et al.*, 2009, Ejiogu *et al.*, 2010; Makut *et al.*,2013). The initial alcohol content of the *kunun-zaki* was 0% (Fig. 6). However, a consistent increase in alcohol content was observed in the preserved kunun-zaki samples. The production of alcohol is attributed the presence of yeasts such as *Saccharomyces cerevisiae* in the preserved samples which utilized the fermentable sugars contained in the *kunun-zaki* samples, hence converting the sugars into alcohol.

The reduction of the sugar content of the *kunun zaki* samples from an initial 6.40±0.20% to between 0.63±0.06 –

5.17±0.06% (Fig. 5) were more pronounced in samples other than those treated with sodium benzoate and stored in the refrigerator at 5^{0}C. The reduction of sugar coincided with a concomitant increase in acids and alcohol (Fig. 3, 4, 6). The reduction in sugar and mineral content (Table 3) is attributed to its utilization by the organisms' resident in the *kunun zaki* samples. This was also reported by Omale *et al.* (2011) who observed pH decreases with sugar reduction in *kunun zaki*. The samples that had less than 1.50% residual sugar by end of storage were the control samples and those stored at ambient temperature treated with preservatives other than sodium benzoate. This is very important as the retention of the traditional sweet sour taste of *kunun zaki* is a crucial factor in the acceptance of the preserved samples by consumers (Table 4). The shelf life of many products is determined by their taste and acceptance characteristics (Gimenez *et al.*, 2008). Changes in appearance, aroma and taste were also observed in the treated *kunun zaki* samples during storage. The refrigerated samples maintained their colour, general appearance and sweet sour aroma throughout the length of storage. However, the samples stored at ambient temperature acquired alcoholic off odours by 18th day of storage. The control samples and PRoom changed aroma after the 3rd day. The Control samples and treated samples stored at ambient temperatures (MRoom, PRoom, PMRoom, PBMRoom and BMRoom) developed a film layer on top of the samples. Observations on overall acceptability of the preserved *kunun zaki* samples stored at ambient temperatures declined significantly ($p<0.05$) by 12th day of storage, the refrigerated samples by 21st day of storage and the untreated control samples by the 4th day of storage (Table 4). A fresh, sweet- sour aroma were observed in most of the refrigerated treated samples up till 9th day of storage. The sensory shelf life of many food products determine their shelf life; therefore, consumers' overall acceptability rating of a food product could determine if such a food would still be acceptable after a certain period of storage (Gimenez *et al.* 2008). Hence, assessment of food products by consumers during storage could be appropriate tool for shelf-life determination of food product (Nkama *et al.* 2010). There was no significant difference between samples preserved with sodium benzoate at 5^{0}C (B fridge), pasteurization+sodium benzoate + sodium metabisulphite at 5^{0}C (PBM fridge) and sodium benzoate + sodium metabisulphite at 5^{0}C (BM fridge). This implies that kunun-zaki can be preserved efficiently for 27 days using only sodium benzoate at 5^{0}C (B fridge).

CONCLUSION

Kunun-zaki is an easily perishable product that cannot be kept for more than 48-72 hours after production without deteriorating significantly. This could be attributed to the high nutrient and moisture content of this non-alcoholic beverage. The result of this study showed that a combination of pasteurization, chemical treatment and refrigeration can extend the shelf life of *kunun-zaki* by 27 days. This is an indication that a highly perishable beverage like *kunun-zaki* with longer shelf life could be produced and preserved with combination of pasteurization, chemical treatment and refrigeration (5^{0}C). The results of this study suggest that the rapid deterioration of *kunun zaki* can be arrested and the shelf life extended from the traditional 1-2 days to 27 days and still find customer acceptability with the use of 0.1% sodium benzoate and refrigeration at 5^{0}C which this study recommends.

Table 1: Occurence of microorganisms in the kunun-zaki samples
+ = present, - = absent

Table 2: Physicochemical analyses and microbial counts of fresh samples of kunun- zaki

Samples	Total Bacterial Count ($x10^5$ cfu mL^{-1})	Total Fungal Count ($x10^5$ cfu mL^{-1})	pH	Titratable Acidity (cm^3)	Sugar Content (%)	Alcohol Content (% v/v)
$Kunun_1$	0.89	0.98	5.93	0.137	6.40	0
$Kunun_2$	0.60	0.82	5.97	0.135	6.28	0

The samples represented with subscripts $_1$ and $_2$ are fresh locally produced samples and

	Saccharom-yces cerevisiae	*Bacillus subtilis*	*Leuconostoc mesenteroides*	*Micrococcus varians*	*Bacillus cereus*	*Candida albicans*	*Lactobacillus fermenti*
Fresh sample	+	+	+	+	+	+	+
Control1	+	+	+	-	-	+	+
Control2	+	+	+	+	+	-	+
P room	+	+	+	+	+	+	+
Fridge	+	+	-	+	+	+	-
P fridge	+	+	-	-	+	+	-
M room	+	+	+	+	+	+	+
PM room	+	+	+	+	+	+	+
M fridge	+	+	-	-	+	+	-
PM fridge	+	+	-	-	+	-	-
BM room	+	+	+	+	+	-	+
PBM room	+	+	+	+	+	-	+
B room	+	+	+	+	+	+	-
PB room	+	+	+	+	+	+	+
BM fridge	+	+	-	-	+	-	-
B fridge	+	+	-	-	+	-	-
PBM fridge	+	+	-	-	+	-	-
PB fridge	+	+	-	-	+	-	-

laboratory-prepared samples respectively.

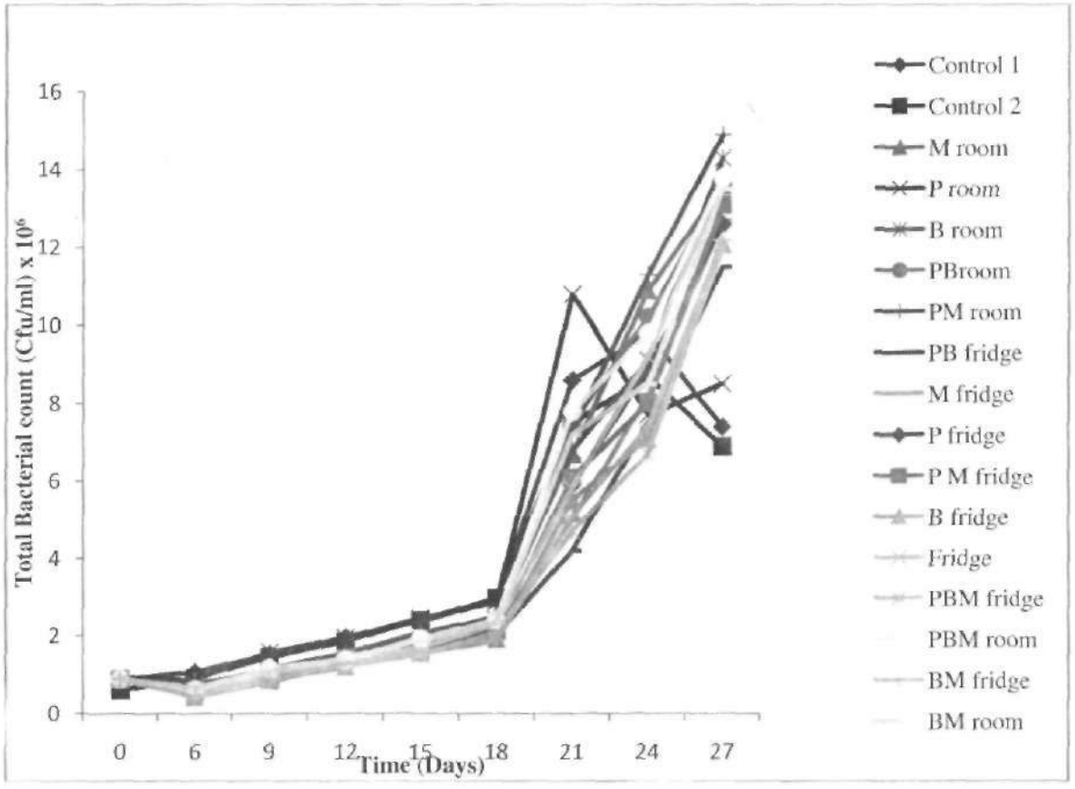

Figure 1: Changes in the Total Bacterial Count of kunun-zaki stored for 27 days.

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

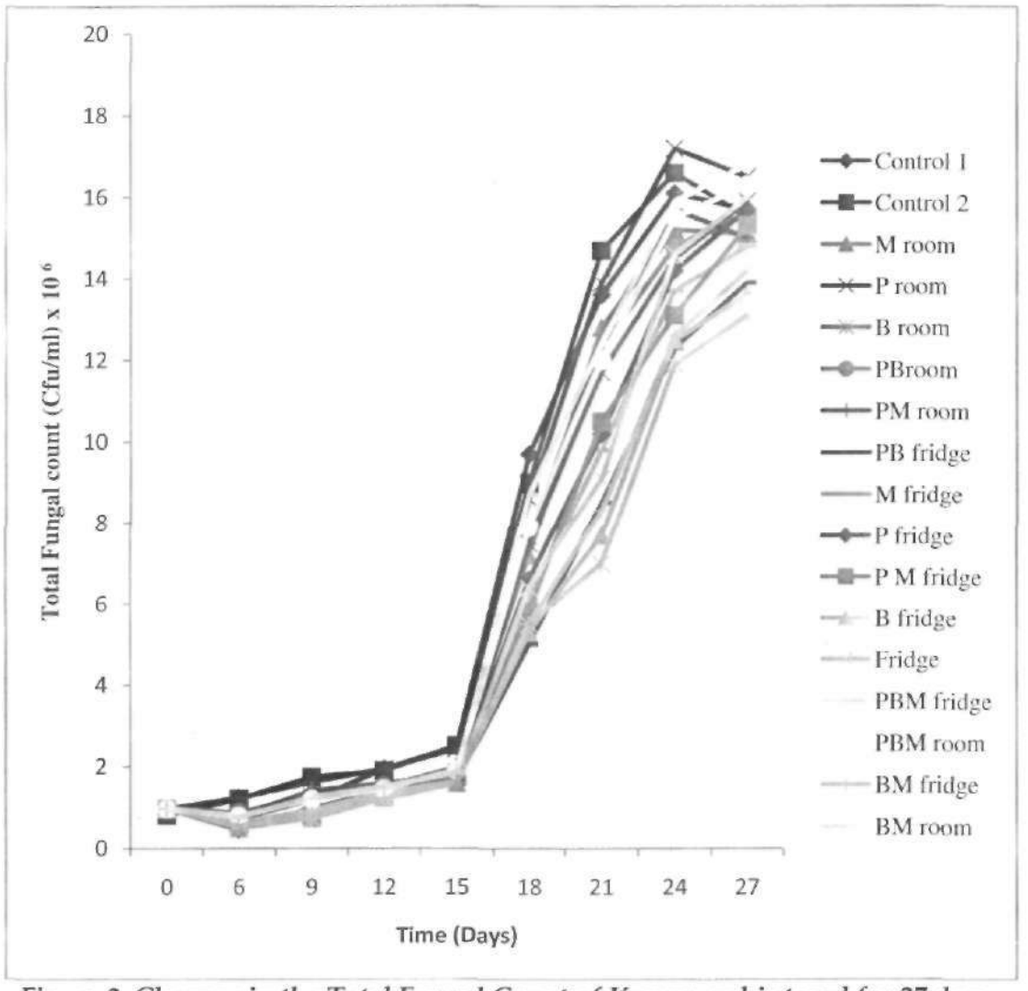

Figure 2: Changes in the Total Fungal Count of Kunun- zaki stored for 27 days

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

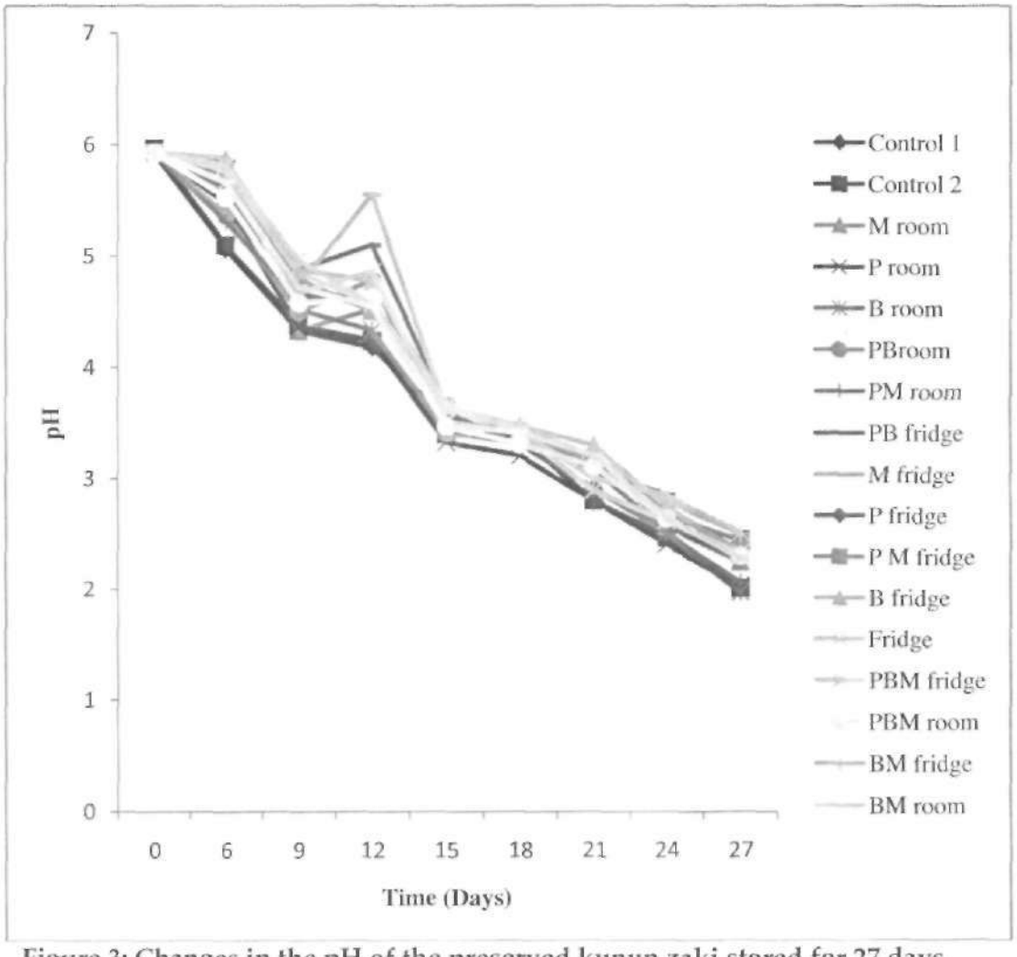

Figure 3: Changes in the pH of the preserved kunun zaki stored for 27 days

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

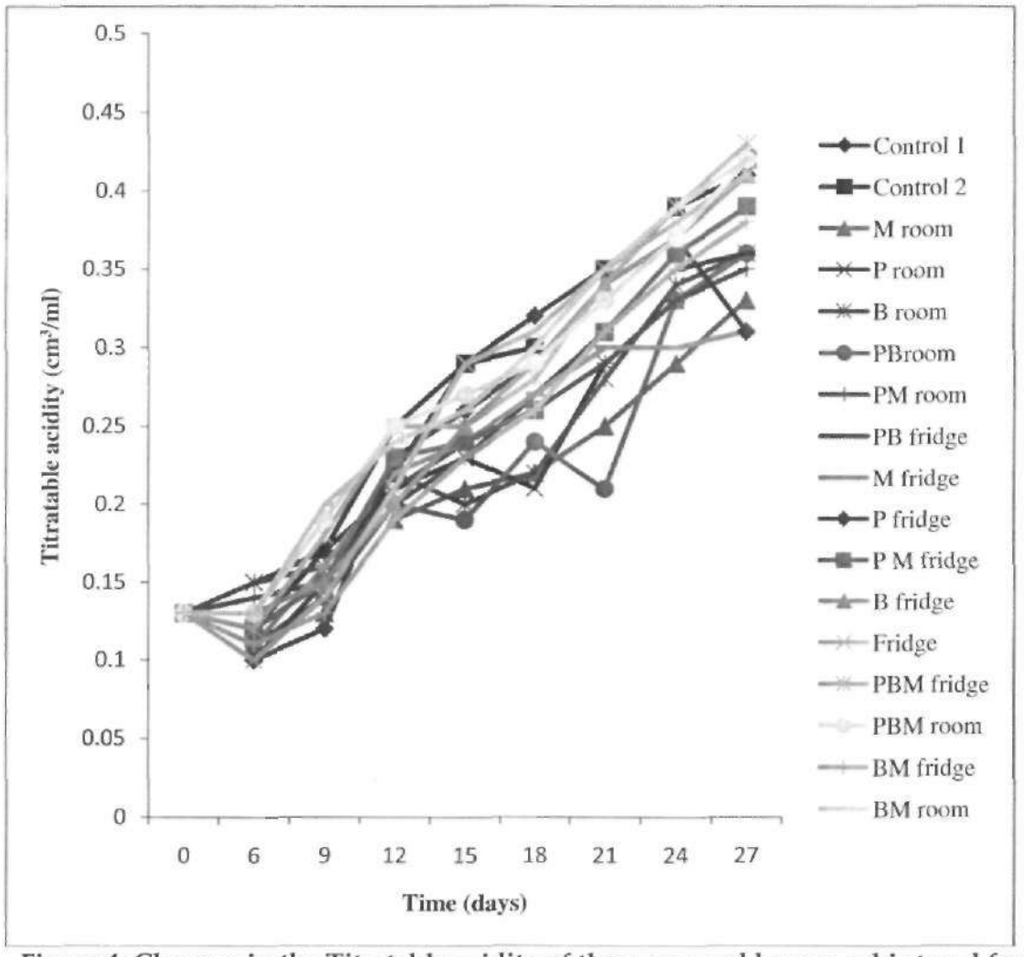

Figure 4: Changes in the Titratable acidity of the preserved kunun zaki stored for 27 days

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

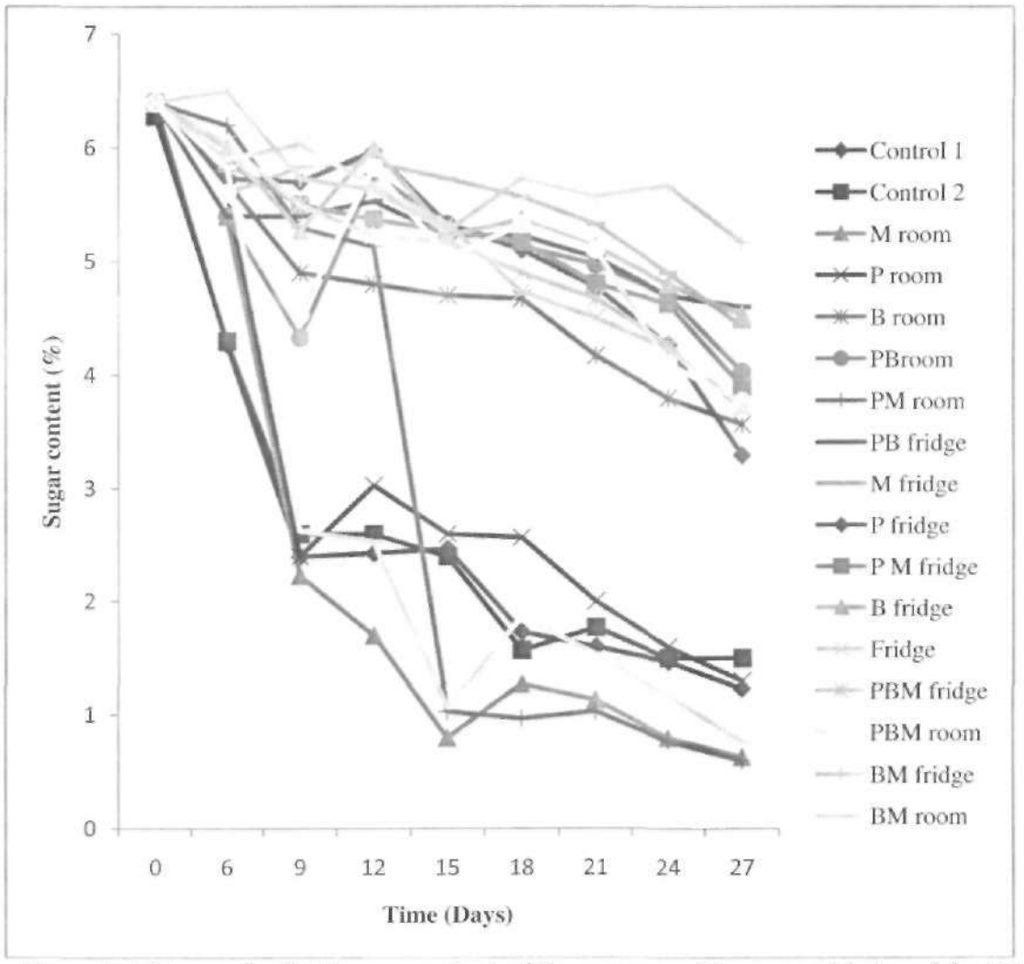

Figure 5: Changes in the Sugar content of the preserved kunun zaki stored for 27 days

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

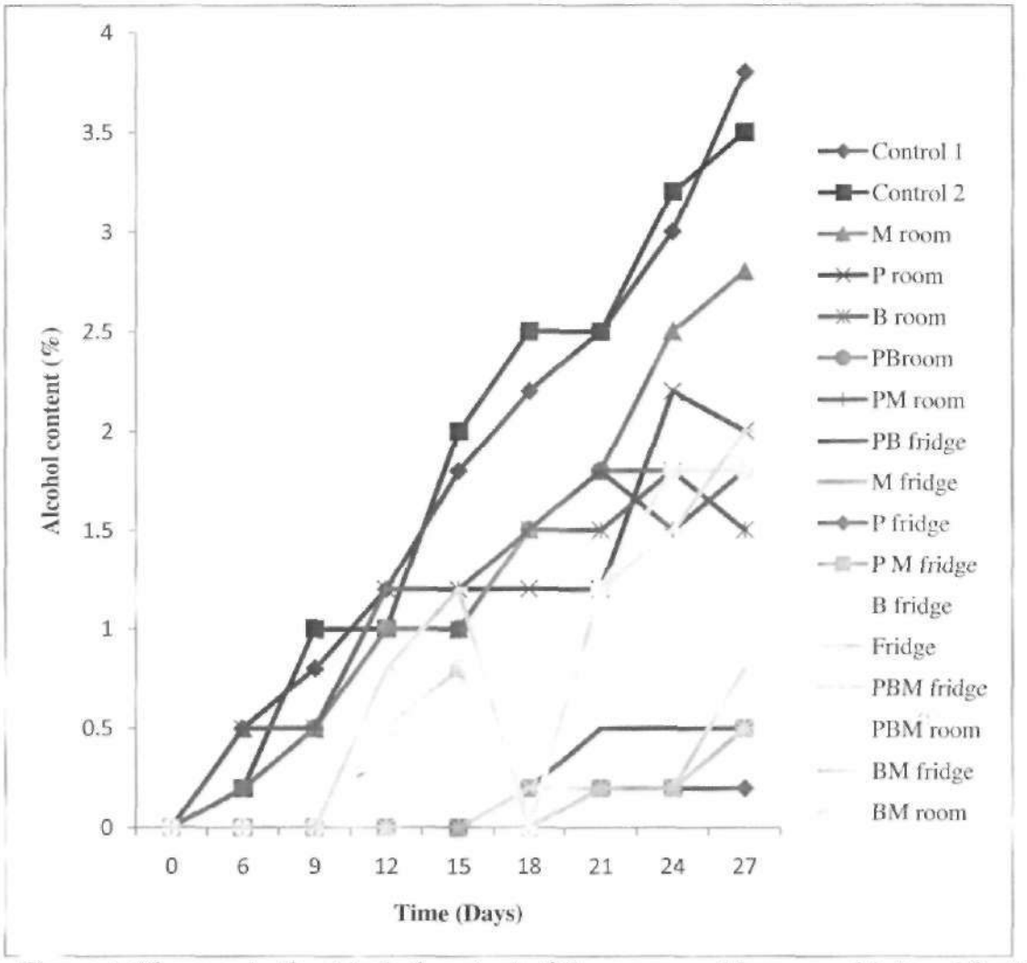

Figure 6: Changes in the Alcohol content of the preserved kunun zaki stored for 27 days

Control1= sample without any preservative at room temperature, **Control2**= laboratory prepared sample without any preservative at room temperature. **M room**=sodium metabisulphite +room temperature, **P room**= Pasteurized +room temperature, **B room**= sodium benzoate +room temperature, **PB room**=Sodium benzoate+pasteurization+room, **PM room**= sodium metabisulphite+Pasteurization+room, **PB fridge**= Sodium benzoate+pasteurization+4^{0}c, **M fridge**= sodium metabisulphite+4^{0}c, **P fridge**= pasteurization+4^{0}c, **PM fridge**= pasteurization+sodium metabisulphite+4^{0}c, **B fridge**= sodium benzoate+4^{0}c, Fridge=4^{0}c, **PBM fridge**= pasteurization+sodium benzoate= sodium metabisulphite+4^{0}c, **PBM room**= pasteurization+sodium benzoate+ sodium metabisulphite+room temperature, **BM fridge**= sodium benzoate+sodium metabisulphite+4^{0}c, **BM room**= sodium benzoate+ sodium metabisulphite+ room temperature.

Table 3: Changes in the mineral content of the kunun-zaki samples before and after storage

Samples	Pre- Storage (ppm)					Post Storage (ppm)				
	Ca^+	Mg^+	K^+	P^+	Na^+	Ca^+	Mg^+	K^+	P^+	Na^+
Control 1	60	120	320	12.6	250	20	80	20	6.02	20
Control 2	60	120	320	12.6	250	10	70	30	6.02	20
M room	60	120	320	12.6	250	20	80	180	6.88	50
P room	60	120	320	12.6	250	20	80	120	6.60	20
B room	60	120	320	12.6	250	20	80	120	8.60	50
PB room	60	120	320	12.6	250	30	80	90	8.60	80
PM room	60	120	320	12.6	250	20	70	180	6.88	50
PB fridge	60	120	320	12.6	250	30	80	170	7.74	130
M fridge	60	120	320	12.6	250	20	80	120	8.60	100
P fridge	60	120	320	12.6	250	20	70	100	6.60	140
P M fridge	60	120	320	12.6	250	30	80	160	7.74	110
B fridge	60	120	320	12.6	250	10	80	180	6.60	180
Fridge	60	120	320	12.6	250	20	80	130	6.60	210
PBM fridge	60	120	320	12.6	250	20	80	260	6.60	210
PBM room	60	120	320	12.6	250	30	70	160	7.74	80
BM fridge	60	120	320	12.6	250	20	80	290	9.46	140
BM room	60	120	320	12.6	250	20	80	180	8.60	110

Table 4: Changes in Overall acceptability of the preserved kunun-zaki samples stored for 27 days

	Period of storage (Days)								
	0-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27
Control 1	4.80±0.45^{a}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
Control 2	4.80±0.45^{a}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
M room	4.80±0.45^{a}	4.80±0.45^{a}	3.40±0.55^{b} ^{c}	2.80±0.45^{c}	1.40±0.55^{d} ^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
P room	4.80±0.45^{a}	1.60±0.54^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
B room	4.80±0.45^{a}	4.80±0.45^{a}	4.60±0.55^{a}	4.00±0.00^{b}	2.20±0.45^{c} ^{d}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
PBroom	4.80±0.45^{a}	4.80±0.45^{a}	4.20±0.45^{a} ^{b}	4.20±0.45^{a} ^{b}	2.40±0.55^{c}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
PM room	4.80±0.45^{a}	4.80±0.45^{a}	3.80±0.45^{b} ^{c}	2.80±0.45^{c}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
PB fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.00±0.00^{b}	4.00±0.00^{b}	2.80±0.45^{c}	2.20±0.45^{c} ^{d}
M fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	3.80±0.45^{b} ^{c}	3.20±0.45^{b} ^{c}	2.60±0.55^{c}	2.60±0.55^{c}	1.40±0.55^{d} ^{e}	1.00±0.00^{e}
P fridge	4.80±0.45^{a}	4.60±0.54^{a}	2.40±0.55^{c} ^{d}	1.60±0.55^{d}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
P M fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.00±0.00^{b}	3.80±0.45^{b} ^{c}	3.20±0.45^{b} ^{c}	2.80±0.45^{c}	2.80±0.45^{c}	1.80±0.45^{c} ^{d}	1.00±0.00^{e}
B fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.20±0.45^{a} ^{b}	4.00±0.00^{b}	3.60±0.55^{b} ^{c}	3.20±0.45^{c}
Fridge	4.80±0.45^{a}	4.60±0.55^{a}	3.00±0.00^{c}	1.80±0.45^{d}	1.20±0.45^{d} ^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}	1.00±0.00^{e}
PBM fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.20±0.45^{a} ^{b}	4.00±0.00^{b}	3.60±0.55^{b} ^{c}	3.20±0.45^{c}
PBM room	4.80±0.45^{a}	4.80±0.45^{a}	4.60±0.54^{a}	3.20±0.45^{b} ^{c}	3.00±0.00^{c}	2.60±0.55^{c}	1.80±0.45^{c} ^{d}	1.20±0.45^{d} ^{e}	1.00±0.00^{e}
BM fridge	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.80±0.45^{a}	4.40±0.55^{a}	4.40±0.55^{a}	3.60±0.55^{b} ^{c}	3.40±0.55^{c}	3.20±0.45^{c}
BM room	4.80±0.45^{a}	4.80±0.45^{a}	4.40±0.55^{b}	4.00±0.00^{b}	3.60±0.55^{b} ^{c}	3.00±0.00^{c}	2.60±0.55^{c}	1.80±0.45^{d}	1.40±0.55 ^{de}

Each value is the mean ± SD of 5 panelists where 5 = very good, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

Different letters within each column are significantly different ($p<0.05$).

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