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ORGANIC PRESERVATION AND SHELF-LIFE EVALUATION OF LIQUID KUNU ZAKI FOOD DRINK, WITH EXTRACT OF WEST AFRICAN BLACK PEPPER (*PIPER GUINEENSE*)

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ABSTRACT: Organic extract from West African black pepper that contains antimicrobial/antioxidant agents, was prepared and used to preserve liquid kunun zaki along side control sample unpreserved. The extract was applied at four (4) concentration levels (5.0, 10.0, 15.0 and 20.0g extract/L kunun) making four experimental samples and one control properly packaged in plastic bottle (all coded). The samples were stored under ambient conditions, subjected to weekly routine inspection, physical, sensory, microbiological evaluation, shelf-life assessment and data analysis using standard methods in each case. The brix level, specific gravity, pH and titratable acidity of kunun samples were maintained for four weeks in 15.0g extract/Litre sample, found similar to the freshly prepared samples, showing no significant difference ($p \geq 0.05$). While samples containing 5.0, 10.0 and 20.0g extract/L could maintain these physical properties for less than 3 weeks, before deterioration of sensory properties set in. Samples 10.0, 15.0 and 20.0g extract/L maintained initial high colour and taste ratings for 4 weeks under storage, while unpreserved control and 5.0g extract/L samples experienced rapid decline in colour and taste found to be significantly different ($p \geq 0.05$). Flavour scores declined in all the samples as storage progressed, while samples 15.0 and 20.0g extract/L experienced the least decrease over 4 weeks duration, not significantly different ($p \geq 0.05$) from the original high rating, while other samples were found objectionable/rejected at the end of week 3. Sample 15.0g extract/L maintained high mouth – feel rating throughout 4 weeks storage, while the control, 5.0 and 10.0g extract/L samples developed objectionable mouth – feel after week 3 and 20.0g extract/L sample had poor rating throughout storage over 4 weeks. Detected microbiological contents in samples under 4 weeks storage, included total plate count (9.8×10^{I} cfu/mL, max), lactic acid bacteria (7.2×10^{I} MPN, max), Geotricum spp (6.8×10^{I} MPN/mL), Saccharomyces cerevisiae (8.8×10^{I} MPN, max) and coliform (NIL). The values of each microbiological parameter fall

within acceptable/ permissible limits for fermented food beverages, to guarantee safety for consumption. Apparently, 15.0g extract/L sample has the most desirable and acceptable quality attributes under 4 weeks storage at the ambient conditions (i.e. physical, sensory properties and safety), and therefore the extract of West African black pepper is a suitable organic preservative that can replace chemical/synthetic preservation of kunun zaki food drink.
Key words: Organic preservative, Black pepper extract, kunun zaki drink, antimicrobial/antioxidant agents.

INTRODUCTION

Kunun zaki is food beverage, watery cereal – based non-alcoholic, which is indigenous to Northern Nigeria, and widely consumed in some West African countries due to its refreshing and nutrition qualities. It is prepared majorly from millet, sorghum and corn/maize. It has low viscosity, sweet – sour taste and milk – cream appearance, but characterized with short shelf-life, not more than 2 – 3 days under tropical ambient conditions (Adeyemi, 2011). It has been severally reported that synthetic/chemical preservatives are rampantly used to extend shelf/storage life of foods, most especially food beverages/drinks, fruits/vegetables, cookies, cordials (Orishagbemi, et al., 2018). In most instances, prohibited, expired and over dosage of these preservatives are used which leads to health risks, apart from the long run negative effects of cumulative residual chemical additives to the body causing various ailments including, cancer, kidney/liver/heart failures and turmoils (Pecklam, 2009, WHO, 2009). Nowadays, food and related products chemically preserved are avoided by consumers for health reasons, which necessitates need for alternative safe, organic preservatives to replace the chemicals used in food beverages, cookies, soft and malted drinks. Natural, organic food additives/preservatives are obtained from plants and animals, and not by chemical reactions. They exist in crude extract form, which is purified/refined for use. Many organic preservatives have been extracted, such as from Baobab, Chilli pepper, lime, alligator pepper, ginger, garlic, black pepper (Ascorbic/citric acid as antioxidant and nutrient, and

antimicrobial agents/phytochemicals), prune, cinnamon, clove (sources of benzoic acid as antimicrobial agent), rock salt, Chile petre, cryolite (sulphur dioxide derivative, K, Na, Us, Ca as antimould/bacteria), (Annunciata, et al., 2013). These organic extracts have been investigated and found not to constitute harmful effect, potent and desirable preservation effect on food products, cheap, health friendly but requires refining, dosage settling, permissible limits or levels (Behall, 2006). The main objective of this study was to prepare and use desirable organic extract obtainable from African black pepper seed (containing antibacterial, antifungal and antioxidant agents) to preserve liquid kunun zaki and evaluate sensory, physico-chemical properties and shelf-life under tropical storage conditions.

MATERIALS AND METHODS

Sources of Raw Materials

White sorghum bicolour, sweet potatoes (white flesh) were obtained from Anyigba, Kogi State and African black pepper seeds from Ogbete market, Enugu, Nigeria, and used for this work.

Black Pepper Extract Preparation

The organic black pepper preservative extract was prepared according to method described (Anyanwu and Nwosu, 2014). Black pepper seed sample was washed, dried (60°C, 2hr) cooled and reduced into powder (150 microns) by dry-milling and then packaged into heat sealed high density polyethylene. Powder was divided into 2 parts, one part for qualitative tests for benzoic acid (antimicrobial agent) according to method described (BDH, 2014) and ascorbic acid (antioxidant) as described (Pearson, 2009) and second part for incorporation into kunun food beverage sample.

KUNUN zaki Preparation with Added Organic Preservative

Liquid kunun was prepared in the laboratory according to standard method described (Adeyemi, 2011) using the raw materials, white sorghum (16.5%), sweet potato (40%), ginger as flavoring (1.5%) and de-ionized water (42%). Raw kunun was divided into 5 equal parts, one part as control sample, and 4 parts as experimental samples incorporated 5.0, 10.0, 15.0 and 20.0g pepper extract powder/L Kunun respectively. Each was packaged in PET plastic bottles (25CL capacity, in five places, with 5 pieces in each place), then pasteurized (at 70°C, 30 mins) in water bath, cooled and kept at ambient temperatures (28±2°c) for storage (4 weeks duration). They were subjected to weekly routine inspection and analysis (physical, chemical, sensory and microbiological).

Shelf-life Study on Preserved Kunun Food Drink

Packaged Kunun samples stored under ambient conditions were subjected to weekly inspection and analysis (physical, chemical, sensory and bacteriological and fungal) for four weeks.

PHYSICAL AND CHEMICAL EVALUATION

Specific gravity (SG) and Brix level determination: They were determined according to methods described (AOAC, 2010),

pH determination: pH was determined at room temperature with a pH meter (TECPEL, model 705) that has been previously standardized with buffer solutions of 4.0 and 7.0 pH values as described (Onwuka, 2005).

Total Titratable acidity: It was determined using the method described (Orishagbemi, *et al.*, 2018).

Sensory Evaluation

The organoleptic attributes of Kunun samples in storage (colour, taste, flavour, mouth - feel) were evaluated by fifteen (15) panelists, untrained but familiar with Kunun drink, based on 9 – point

hedonic scales rating (Orishagbemi *et al.*, 2019). Data were subjected to analysis of variance and separation of means, using standard technique (SPSS version 17).

Microbiological Analysis

Bottled samples of kunun drink in storage at the ambient temperatures (28±2°c) were subjected to determination of total plate count (TPC), Lactic acid bacteria (LAB), Saccharomyces cerevisiae (SC), Geotricum species (GS) and coliform (CF) counts using standard microbiological procedures and instruments (Ogbo, 2005 and Orishagbemi, *et al.*, 2019).

RESULTS AND DISCUSSION

Changes in physical and chemical properties of preserved kunun drink under storage, 28±2°C (4 weeks)

Table 1 shows the quantitative changes in the brix level, specific gravity, pH and titratable acidity of kunun samples under storage for 4 weeks. The brix levels of kunun samples during week 1 ranged from 12.5 – 19.0°B, the control, 0.0g extract/L (Coded AAA) with lowest value and sample 15.0g extract/L (Coded DDD) highest, showing variation. However, brix level of each of the 4 samples in fresh state (day 1) did not change throughout week 1. Difference in brix level seemed not to follow any pattern regarding the 4 concentration levels of the organic extracts (5.0, 10.0, 15.0, 20.0g extract/L). There were no significant differences ($p \geq 0.05$) in brix level of samples 10.0 and 15.0g extract/L (CCC and DDD samples respectively) up till week 4 in storage while significant reduction changes occurred in other samples (control, 5.0 and 20.0g extract/L) and were even discarded at the end of week 3 in storage. The reduction in brix level is attributable to fermentation especially the control sample (0.0g extract/L) and 5.0g extract/L sample showing that antimicrobial content available in the extract incorporated was not enough to inhibit growth of microorganisms

that survived pasteurization for long duration in storage. This is similar to several reports that fermentation of preserved fruit juices resulted into brix level reduction, reduced pH and increased acidity (Orishagbemi, *et al.*, 2018). Specific gravity of kunun samples (for week 1) were 1.14 (control sample), 1.12, lowest value (5.0g extract/L kunun), 1.18, highest (10.0g extract/L), 1.17 (15.0g extract/L sample) and 1.16 (20.0g extract/L kunun). These values seemed not to depend on the concentration of organic extract in the kunun samples and did not experience any significant change ($p \geq 0.05$) during storage (4 weeks) for 2 samples (10.0 and 15.0g extract per litre), while others could not keep as long. This shows that both concentration of preservative extract and storage duration at the ambient have no effect on the specific gravity of liquid kunun food drink.

pH values of freshly prepared kunun samples ranged from 6.7 (samples 0.0, 5.0, 15.0, 20.0g extract/L) to 6.8 (sample 10.0g extract/L), showing no significant difference ($p \geq 0.05$) among the samples, and not affected by concentration of incorporated extract. The pH of samples *CCC* (10.0g extract/L) and DDD (15.0g extract/L) did not experience any significant change up till 4 weeks in storage, while samples *AAA* (control, 0.0g extract/L), BBB (5.0g extract/L) and EEE (20.0g extract/L) experienced slight pH reduction under 3 weeks storage, and could not keep beyond, due to unacceptable sensory attributes (taste, flavour and mouth-feel). pH reduction was as a result of fermentation of sugar components in kunun samples at the end of 4 weeks, in which the organic extract incorporated at 0.0 and 5.0g/L concentration could not inhibit responsible microorganisms. Total titratable acidity (%) in fresh samples (week 1), *AAA* (0.0g extract/L), BBB (5.0g extract/L), *CCC* (10.0g extract/L), DDD (15.0g extract/L) and EEE (20.0g extract/L) were 3.8, 1.2, 4.0, 5.8 and 5.7% respectively. There was slight increase in TTA of all samples under storage, which showed no significant

difference ($p \geq 0.05$) among samples uptill 3 weeks in storage, while increase was negligible in samples CCC and DDD uptill 4 weeks storage at the ambient. Similar reasons are adduced to reduction in pH and increase in TTA, while 10.0 and 15.0g extract/L samples showed near zero pH value change due to antimicrobial agent in extract.

Sensory/Organoleptic Properties of Preserved Kunun Under Ambient Storage

The mean sensory scores of experimental samples under storage (4 weeks) are shown in figures 1-4. The mean colour scores of fresh kunun samples (fig.1) were found to reduce with increase in the concentration of extract powder (5.0, 10.0, 15.0, 20.0g extract/L). The control sample (0.0g extract/L) had highest colour score, which showed no significant difference ($p \geq 0.05$) among other samples. However, samples 10.0, 15.0 and 20.0g extract/L maintained initial colour for 4 weeks storage duration, and significantly different ($p > 0.05$) from the control with deteriorated colour (fig.1). Change in colour occurred as the result of the availability of pigment components of the extract powder, of which the quantity increased with increase of extract concentration. The taste scores of fresh samples (fig.2) were 8.0, 8.5, 8.0, 7.0 and 6.0 for the control, 5.0, 10.0, 15.0 and 20.0g extract/L samples respectively. These scores were maintained for week 1. Also, samples 10.0, 15.0 and 20.0g extract/L maintained high taste scores for 3 weeks and slightly declined for 15.0 and 20.0g extract/Litre samples at the end of 4 weeks. On the contrary, the control (0.0g extract/L), 5.0g extract/L samples had rapid decline in taste from week 2 and discarded at the end of week 3. The maintenance of original taste for longer duration was found to depend on the quantity of organic extract incorporated, containing more of the antimicrobial agents to prevent taste loss. This observation is similar to the report of Orishagbemi *et al.* (2018) in their work on chemical preservation (using sodium

benzoate as antimicrobial agent) of dietary protein drink in which the preservative did not seem to affect taste of the drink, even at high concentrations (0.03 – 0.05g/100ml).

The perceived flavour scores of fresh kunun drink (fig. 3) are high for samples 5.0, 10.0, 15.0, 20.0g extract/L). The flavour scores generally reduced in all the samples as storage progressed up to 4 weeks. Kunun samples 15.0 and 20.0g extract/L experienced the least decrease (5.9 and 6.1 scores respectively) at the end of week 4, while others had been discarded at the end of week 3 storage due to deteriorated flavour. Flavour loss with increased storage duration was found to reduce with increased concentration of organic extract showing greater quantity of antioxidant and antimoud agents in the extract, able to prevent off-flavour development as storage increased. Increased concentration of chemical preservative, such as sodium benzoate has also been shown to reduce flavour deterioration in protein dietary juice (Orishagbemi, *et al.*, 2018), similar to the observed outcome of this study on organic preservation of kunun zaki drink. Mouth – feel attribute scores are represented (fig 4). Fresh samples of 0.0, 5.0, 10.0 and 15.0g extract/L have high mouth – feel scores (7.8 – 8.2 ratings), which were maintained for 2 weeks, and slightly declined to 6.1 – 6.3 range during week 3, which was maintained by sample 15.0g extract/L up to week 4. While the control and 5.0g extract/L samples had completely deteriorated mouth – feel after week 1 and week 3 respectively. Fresh sample of 20.0g extract/L had poor mouth – feel rating (4.5 score) which occurred throughout storage (4 weeks). Apparently, sample 15.0g extract/L has desirably acceptable mouth-feel at the end of week 4 under ambient storage. Deteriorated mouth-feel is attributable to insufficient antioxidant which is below desirable level to prevent off – flavour (control and 5.0g/L samples) and too much preservative agent beyond desirable level (sample 20.0g extract/L) which contributed to poor/objectionable mouth-feel. This also agreed with

the reports of previous researches that synthetic preservatives applied below and above desirable/permissible limits constitute poor mouth-feel, flavour and taste to preserved protein dietary juice kept beyond the optimum storage/shelf life (Orishagbemi *et al.*, 2018, 2019).

Microbiological Contents of Organically Preserved Kunun Zaki, Under Ambient Storage (4 weeks)

Table 2 shows the microbiological contents of experimental kunun samples preserved with extract of piper guineense and control, kept for 4 weeks. During week 1 total plate count (TPC) range included control (50 cfu/mL), 5.0g extract/L, BBB (58 cfu/mL); 10.0g extract/L, *CCC* (44 cfu/mL), 15g extract/L, DDD (40 cfu/mL) and 20g extract/L, EEE (48 cfu/mL). TPC for all the samples increased as storage duration (weeks) increased to 4 weeks. Sample *AAA* (control) experienced the most increase ($2.2x10^2$ cfu/mL), followed by BBB ($1.52x10^2$), *CCC* ($9.8x10^1$ cfu/mL), DDD ($8.6x10^1$) and EEE ($8.0x10^1$ cfu/mL). It was found that TPC increased as concentration of incorporated organic extract increased, which showed that the extract contained anti bacterial agent which also depended on the quantity applied. Apparently, TPC for experimental kunun samples at the end of storage (4 weeks) were within allowable limits for safety, while the control exceeded this limit and therefore not fit and safe for consumption. Fungi (as Saccharomyces cerevisiae and Goetricum spp) were detected in all the samples ($2.2x10^1$ – $8.8x10^1$ MPN/mL) which also increased with storage time, but found to be within acceptable limit for fermented food beverages. Both Saccharomyces cerevisiae and Geotricum spp were responsible for slight fermentation that occurred naturally during kunun preparation. Lactic acid bacteria (LAB) were detected in all the kunun samples under storage (control, $4.1x10^1$ – $1.70x10^2$ MPN/mL range; BBB – $3.6x10^1$ – $1.48x10^2$ MPN/mL, *CCC*, 3.2 – $8.0x10^1$ MPN/mL which were also responsible for fermentation and they were found to be within

allowable limits. Coliform counts were not detected in any sample, which is an indication of hygienic preparation and water used free from faecal contaminations.

Acceptable limits or levels of TPC, fungi, LAB, and zero level coliform in liquid kunun beverage preserved and kept for 4 weeks under ambient storage, revealed strict adherence to hygienic and good manufacturing practices procedures, and use of clean water free from faecal contamination. Hence, all samples, except the control were found to be microbiologically/ bacteriologically wholesome and safe for human consumption. Nevertheless, on the basis of sensory attributes, samples BBB (5.0g extract/L; and 20.0g extract/L) were undesirable at the end of 4 weeks storage.

CONCLUSION

Extract of West African black pepper (piper guineense) has been prepared and used as organic preservative (antibacterial, antimould and antioxidant) to extend shelf-life of liquid kunun zaki under ambient storage (28±2°C) from 2 days to 28 days (4 weeks), which maintained the original qualities of fresh kunun (i.e nutritive, physical, sensory attributes) and acceptable limits of microbiological contents to guarantee safety of the consumer. Preservation involved the use of 10.0 – 15.0g extract piper guineense/L kunun, (samples CCC and DDD) found appropriate for shelf-life extension up to 4 weeks under tropical ambient storage temperature conditions (28±2°c), with adequate packaging; which is better than using synthetic/chemical preservatives. It was found to retain the nutritive, physical, sensory quality properties and also ensures safety of freshly prepared kunun beverage cherished by consumers.

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Table 1: Changes in Brix level, specific gravity, pH and total titratable acidity of preserved kunu samples during storage (28±2°c)

Physical/ Chemical Parameter	Samples (g Extract/Litre) and storage period (weeks)																			
	Week 1					Week 2					Week 3					Week 4				
	0.0AA	5.0BB	10.0CCC	15.0DDD	20.0EEE	0.0AA	5.0BB	10.0CC	15.0DD	20.0EE	0.0AA	5.0BB	10.0CC	15.0DD	20.0EE	0.0AA	5.0BB	10.0CC	15.0DD	20.0EE
Brix (°B)	12.5±0.13	15.0±0.07	11.0±0.02	19.0±0.10	16.0±0.12	12.5±0.05	15.0±0.00	11.0±0.11	19.0±0.02	16.0±0.11	12.4±0.10	14.6±0.06	11.0±0.03	19.1±0.01	15.5±0.02	-	-	11.1±0.01	18.8±0.03	-
Sp Gravity	1.14±0.03	1.12±0.01	1.18±0.03	1.17±0.02	1.16±0.04	1.14±0.01	1.13±0.04	1.18±0.00	1.17±0.01	1.16±0.10	1.14±0.01	1.13±0.12	1.18±0.02	1.17±0.01	1.12±0.05	-	-	1.18±0.01	1.16±0.04	-
pH value	6.7±0.12	6.7±0.10	6.8±0.06	6.7±0.10	6.7±0.05	6.7±0.10	6.7±0.02	6.8±0.04	6.7±0.00	6.7±0.04	6.7±0.03	6.65±0.02	6.80±0.10	6.7±0.01	6.5±0.03	-	-	6.7±0.06	6.6±0.01	-
Titratable acidity (%)	3.8±0.02	1.2±0.1	4.0±0.11	5.8±0.00	5.7±0.3	3.75±0.10	1.29±0.05	4.1±0.00	5.8±0.02	5.7±0.13	3.9±0.07	1.3±0.02	4.0±0.00	5.75±0.10	5.6±0.30	-	-	4.70±0.00	5.75±0.01	-

Values represent means, n = 3 (± standard deviation).

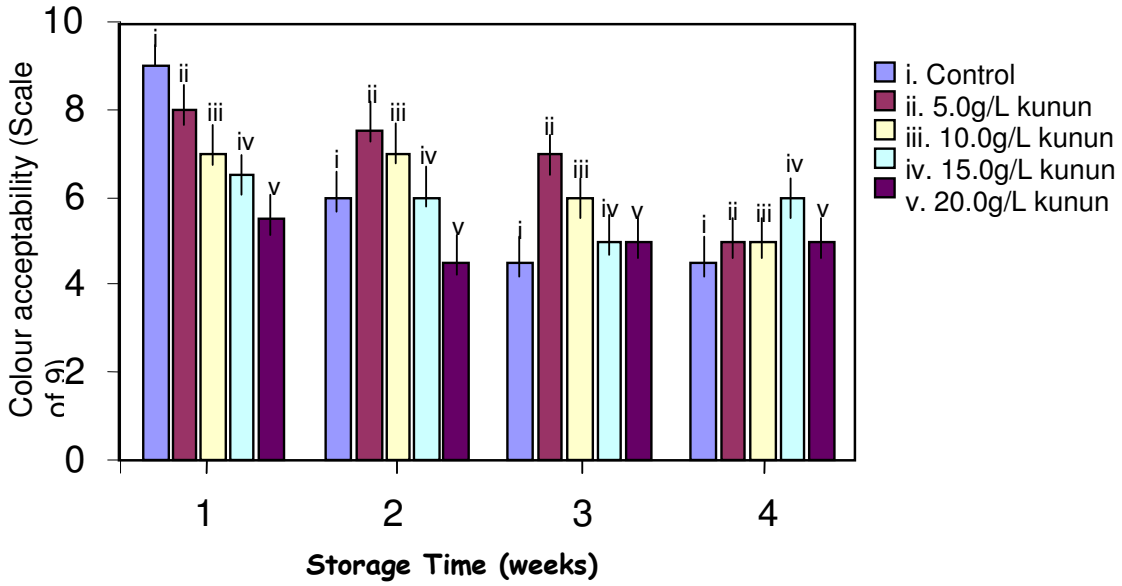

Fig.1: Mean colour scores of kunun zaki beverage with piper guineense extract powder as organic preservative.

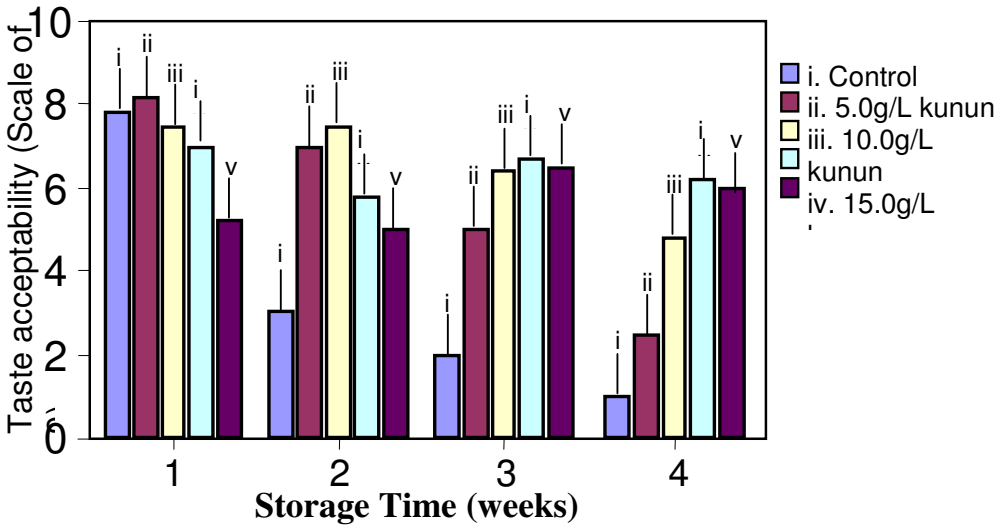

Fig.2: Mean taste scores of kunun zaki beverage incorporated with Piper guineense extract as

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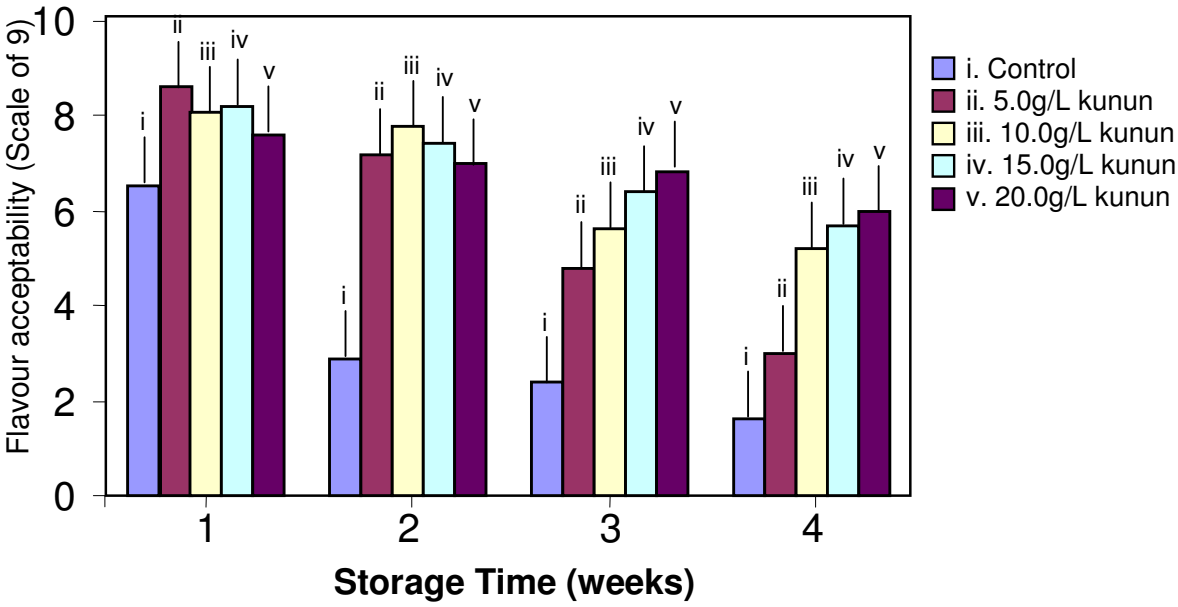

Fig.3: Mean flavour scores of kunun zaki beverage incorporated with Piper guineense extract as organic preservative

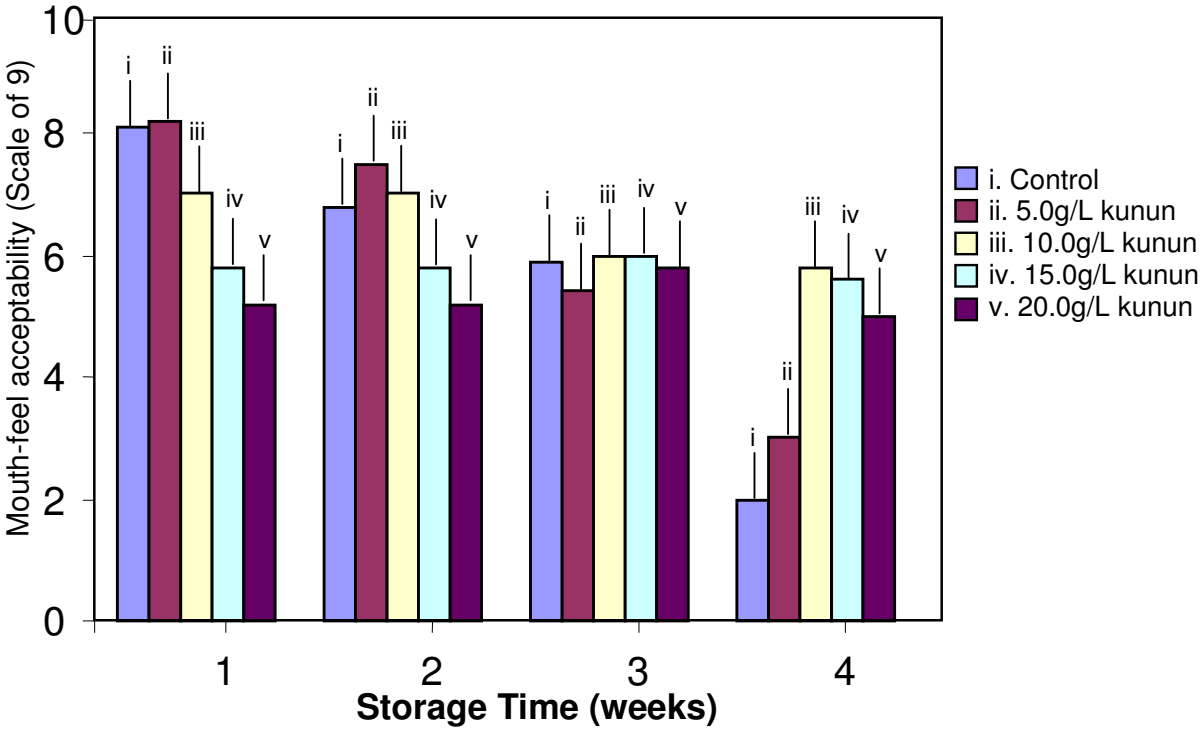

Fig.4: Mean mouth-feel scores of kunun zaki beverage incorporated with Piper guineense extract as organic preservative

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Table 2: Bacterial and fungi contents of kunu zaki preserved with organic piper guineense extract, under ambient storage (4 weeks)

Physical / Microbiological Parameter	Week 1					Week 2					Week 3					Week 4				
	TPC (x10^1 cfu/mL)	LAB (MPN/mL)	SCMPN/mL	GSMPN/mL	CFMPN	TPC (x10^1 cfu/mL)	LAB (MPN/mL)	SC MPN/mL	GS MPN/mL	CF MPN	TPC (x10^1 cfu/mL)	LAB (MPN/mL)	SC MPN/mL	GS MPN/mL	CF MPN	TPC (x10^1 cfu/mL)	LAB (MPN/mL)	SC MPN/mL	GS MPN/mL	CF MPN
Kunu sample AAA Control (0.0g extract/L	5.0	4.1	3.6	0.2	NIL	11.0	7.6	1.2	0.4	0	16.7	13.2	2.5	0.6	0	22.0	17.0	8.8	1.0	0
BBB (5.0g extract/L)	5.8	3.6	2.2	0.2	0	4.2	2.8	2.2	0.4	0	6.0	5.4	4.0	0.6	0	15.2	14.8	9.0	0.8	0
CCC (10.0g extract/L)	4.4	3.2	1.6	0.3	0	3.6	2.2	1.4	0.5	0	3.4	2.8	2.4	0.7	0	9.8	8.0	4.8	1.2	0
DDD (15.0g extract/L)	4.2	2.8	2.0	0.4	0	2.6	1.8	1.4	0.6	0	3.0	2.6	1.8	0.8	0	8.6	6.8	4.2	1.2	0
EEE (20.0g extract/L)	4.8	3.0	2.2	0.2	0	2.5	2.1	2.4	0.3	0	3.2	2.6	2.7	0.4	0	8.0	7.2	4.6	0.8	0

Values represent means, n = 2

TPC – Total Plate Count.

LAB – Lactic Acid Bacteria.

SC – Saccharomyces cerevisiae.

GS – Geotricum species.

CF – Coliform.

CFU – Colony forming units.

MPN – Most probable number.

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