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The effect of herbal *Lippia javanica* extracts on the bioactive content, functional properties, and sensorial profile of biofortified-orange maize based fermented maheu

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ABSTRACT

Lippia javanica is commonly used to treat respiratory ailments because of its rich phytochemical profile, however its importance as a food additive in maheu production is not known. The study was aimed at determining the effect of herbal *Lippia javanica* extract (L.J.E) on the bioactive content, functional properties, and sensorial profile of biofortified-orange maize based fermented maheu. The prepared orange maize gruels were mixed with 5 %, 10 %, and 15 % v/w L.J.E and fermented spontaneously for 24 h. Total polyphenols (TP), antioxidant activity (AOA), β-carotene, vitamin A and vitamin C content were analysed using Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, ABTS (2,2′-azinobis-3-ethyl-benzothiazoline-6-sulphonate), HPLC, retinol activity equivalent (RAE) conversion, and dichlorophenolindophenol titration method respectively. Physiochemical properties, mineral content and product acceptability were analysed using standard AOAC methods. The β-carotene, vitamin A, and vitamin C range was 0.8–1.1 μg/g, 3.55–3.90 μREA/100g and 6.3–11.1 mg/100g respectively. TP, AOA, total antioxidant activity range was 8.2–19.4 mg GAE/g, 28.1–41 EC_{50}mg/L and 31.3–48.3AE/100g respectively. The pH, total soluble sugars, and titratable acidity was 3.6–3.9, 4–4.5 % and 0.08–0.16 %. Minerals present in maheu were zinc (0.8–1.1mg), iron (2.6–3.1mg), magnesium (93.2–97.1mg), calcium (80.1–89.4mg), and potassium (72.5–82mg). Maheu with 15 % v/w L.J.E had better improved bioactive content, functional properties and sensorial profile.

1. Introduction

The use of the medicinal plant in food processing to enhance food quality and human health is expanding in many parts of the world (Chawafambira, 2021). *Lippia javanica*, an underutilized indigenous medicinal plant, has gained popularity as a tisane in Sub-Saharan Africa (SSA) (Maroyi, 2017). The herb is referred to as umsuzwane or Zumbani in Zimbabwe and has a long history of traditional uses as an indigenous herbal tea, a food additive, a health drink, and a plant that promotes health when ingested (Bhebhe et al., 2016). Carvone, caryophyllene, ipsdienone, ipsenone, limonene, linalool, myrcenone, myrcene, ocimenone, piperitenone, p-cymene, tagetenone, and sabinene are the most abundant volatile oils in *L. javanica* (Chagonda & Chalchat, 2015). This herbal plant is rich in polyphenols, a group of phytochemical molecules which act as antioxidants (Mfengu et al., 2021). The presence of both volatile and non-volatile secondary metabolites such alkaloids, flavonoids, amino acids, triterpenes, iridoids, and minerals has been reported (Maroyi, 2017). The herb becomes an important ingredient in food synergies especially with cereals which lack these nutrients.

Maheu is a traditionally fermented non-alcoholic gruel beverage of pH 3.6 to 4.0 made mainly from white maize meal and lactic acid bacteria (*Weissella species, Pediococcus pentosaceus, Lactococcus lactis, Leuconostoc lactis*, and *Lactococcus species*) in Southern African (Pswarayi & Ganzle, 2019). Many bacteria from the yeast species have been reported to cause end fermentation in mahewu production (Solange et al., 2014). Maize (*Zea mays L.*) is the staple meal in large swaths of Sub-Saharan Africa, with daily intake of up to 450 grams per person (Ekpa et al., 2019). Maize also contains phenolics, carotenoids (orange maize), anthocyanins (blue maize), phlobaphenes (red maize), insoluble and soluble dietary fiber, and polar and nonpolar lipids, all of which are proven to improve health and prevent disease (Serna-Saldivar, 2016; ŽIlić et al., 2012). Orange maize produced from conventional breeding by

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Table 1
Experimental design for the maheu formulations.

Formulations	Orange maize meal	L.J.E	Finger millet	Sugar
Control	100g	0g	2g	2g
Treatment 1	100g	5g	2g	2g
Treatment 2	100g	10g	2g	2g
Treatment 3	100g	15g	2g	2g

HarvestPlus Program mainly for food and nutritional security in Africa (Akinsola et al., 2021) can be used to produce traditional maheu. Nutritionally, orange maize contains carotenoids; lutein (2.6–9.6 μg/g), zeaxanthin (2.1–9.7 μg/g), β-cryptoxanthin (0.8–2.9 μg/g), β-carotene (1.4–4.1 μg/g), total xanthophyll's (5.4–17.1 μg/g), provitamin A carotenoids (1.4–4.1 μg/g), tannins (2.1–7.3 mg/g), phytate (0.4–7.1 %) (Elemosho et al., 2020). This study used orange maize because of its better provitamin A carotenoid content as compared to yellow and white maize (Muzhingi et al., 2008) and better vitamin C, calcium and carbohydrate compared to yellow maize (USDA & NIH, 2020). Further, orange maize contains a starch, amylose, amylopectin and free sugar content range of 40.1–88.9 %, 15–34.1 %, 65.9–85.0 %, and 1.09–6.5 %, respectively (Elemosho et al., 2020).

The fermented mahewu product is popular in most Southern African countries with many names such as *amaheu* (in South Africa and Swaziland) (Fernandesa et al., 2021; Simatende et al., 2015), and *maxau* (in Namibia) (Misihairabgwi & Cheikhyoussef, 2017). In its production, cooked and gelatinised starches from maize meal are cooled to room temperature, inoculated with grain malt (2–4 %) and naturally fermented using lactic acid for up to 72hrs (Kayitesi et al., 2017). Additionally, sorghum, and grain malts (made from sorghum and millet) are normally used to make maheu (Salvador et al., 2016). In industrial production of mahewu, *Lactobacillus bulgaricus var delbrueckii* and *Lactobacillus brevis* cultures are used (Nyanzi et al., 2010). Traditional fermented foods have recently gained popularity among consumers due to their cultural, healthy, and excellent culinary and preservation benefits (Banwo et al., 2021, 2022). This is because of COVID-19 pandemic that caused a significant negative impact on the global food and nutrition security, particularly in Southern Africa (SADC, 2020). Therefore, the utilisation of herbal plants as food sources in food formulations can possibly improve the nutrient profile of fermented maheu.

Maheu has been produced from cassava and the addition of *moringa oleifera leaf* powder (Olusanya et al., 2020), *aloe barbadensis* powder (Mashau et al., 2020), *beta vulgaris L* (Boyiako et al., 2020), and provitamin A-biofortified maize (Awobusuyi et al., 2016). However, production of maheu using these food materials is low and limited hence this study utilised orange maize in the production of traditional maheu with added herbal L.J.E to increase maheu consumption. Further, the benefits of utilisation of herbal L.J.E as an ingredient incorporated in food synergies is not available. Herbal L.J.E has the potential to be used as a food additive in the development of novel food products such as fermented beverages because its good source of phytochemicals, vitamins and minerals. This study was aimed at determining the effect of herbal L.J.E on the bioactive content, functional properties, and sensorial profile of biofortified-orange maize based fermented maheu.

2. Materials and methods

2.1. Sample collection

Orange maize variety (ZS242) was obtained from the International Maize and Wheat Improvement Center (CIMMYT) in Zimbabwe. Finger millet flour and *L. javanica* leaves were purchased from a local market in Chinhoyi.

2.2. Preparation of L. javanica extracts

The extraction was done using the water extraction technique described by Bhebhe et al. (2016) with modifications. Sun-dried *L. javanica* leaves were separated, cleaned and then milled into powder using a laboratory mill (Model RLA, 201–80014, Hammer mill, UK). The ground powder was then mixed with boiled deionised water. The mixture was then stirred with a magnetic stirrer for 15 min and then steeped for 35 min. The mixture was strained using a fine mesh tea strainer and then filtered through a Whatman No. 1 filter paper under vacuum to obtain extract. The collected extract was then stored under refrigeration temperatures and used in the formulations.

2.3. Experimental design

The experimental design was adopted from Maakelo et al. (2021). In this study finger millet was used as an inoculum and L.J.E was added in this experimental design.

2.4. Processing and fermentation

The recipe of a typical traditional fermented maheu consumed by most people in rural areas of Zimbabwe was standardised with the help of three experienced women in the laboratory. The final recipe used in the experimental design is shown in Table 1.The orange maize grains were cleaned, milled using a laboratory mill (Model RLA, 201–80014, Hammer mill, UK) and sieved through a size 800 μm (Model. HL 20, Gallenkamp and Co Ltd) sieve to obtain maize flour. The production of fermented and control maize maheu samples was carried out using a method described by Maakelo et al. (2021) with few modifications in processing temperatures as represented in Figs 1 and 2.In this study the cooking, cooling, fermentation and pasteurisation temperatures were 98 °C, 25 °C, 45 °C and 95 °C respectively. Using the modified method, the orange maize meal was reconstituted by mixing with water (1:9, w/w, 98 °C) and boiled in a pot to make a smooth slurry with occasional stirring to avoid formation of lumps. After mixing well, the heat was reduced, and the gel was left to simmer for 30 min at 80 °C with occasional stirring until cooked. The cooked smooth porridge was then cooled to about 25 °C and transferred to a fermentation container and 2 % of finger millet flour was added as an inoculum. The L.J.E were then added at 5 %, 10 %, and 15 % v/w. The mixture was placed in an incubator (Panasonic Healthcare Co., Ltd, Japan) set at 45 °C to ferment for 24 h. After fermentation, 2 % sugar was added, and the maheu samples were pasteurized at 95 °C for 1 minute and packaged in a clean glass bottle with a lid. Physicochemical, bioactive compounds and sensory evaluation analysis were done on the maheu samples.

2.5. Moisture content, pH, Titratable acidity, total soluble sugars and minerals

The Association of Official Analytical Chemists (AOAC) (AOAC method 925.45), dry ashing (AOAC method 938.08), and Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent 5100, Agilent Technologies, Santa Clara, California, USA) were used to determine the moisture content, ash content, and mineral content, respectively (Dhlakama & Chawafambira, 2022). Total soluble sugars were determined sing a digital refractometer (MA871, North Carolina, Milwaukee Instruments, USA) at 20 °C in (°Brix). A digital pH meter (BT-675, BOECO, Hamburg, Germany) that was calibrated with pH 4.0 and 7.0 in accordance with the method described by Dhlakama and Chawafambira (2022), was used to measure pH. Vitamin C content was determined using the Dichlorophenolindophenol (DCPIP) titration test. Maheu samples for mineral analysis were first digested using concentrated HNO_3 and H_2SO_4, followed by the addition of ultrapure H_2O_2 to complete digestion. The mineral content was then determined using a method adopted from the Standards Association of Zimbabwe Test

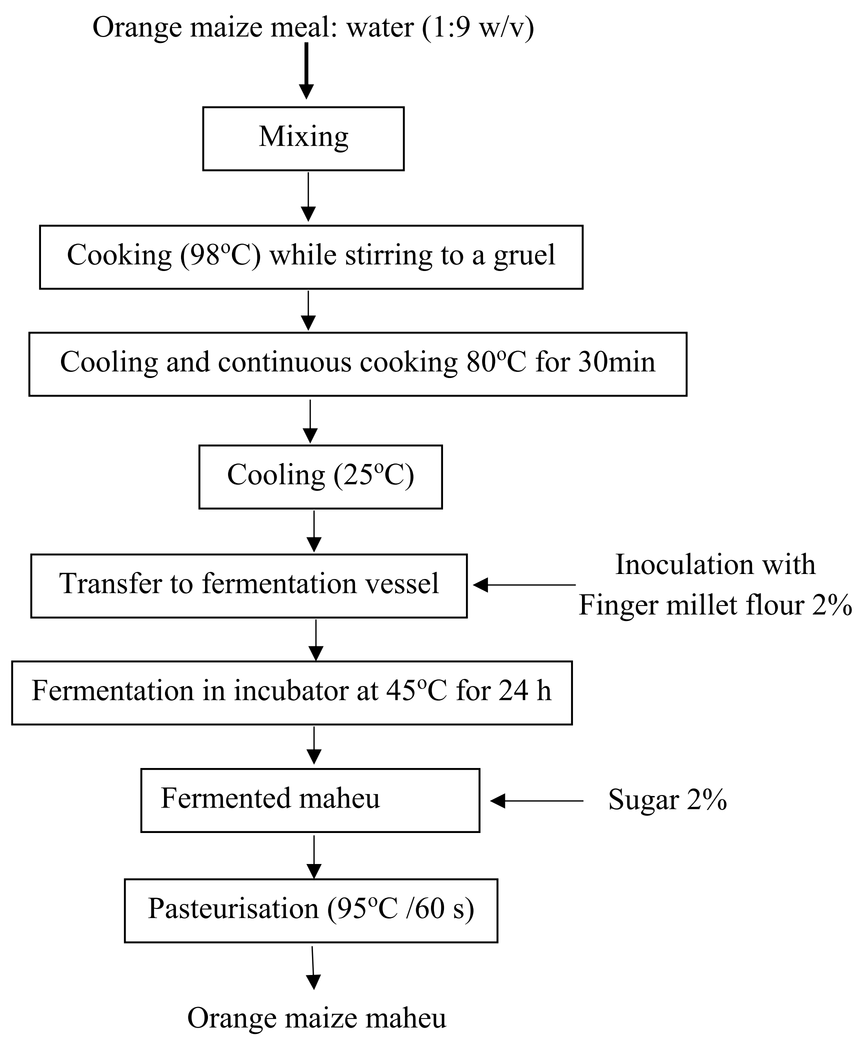

Fig. 1. Processing flowchart for control sample of fermented maize maheu.

Method CF-TM-054 using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (ICAP 6500 Radial, ICP - 20104501, England, United Kingdom). The standard procedure by AOAC was used to determine titratable acidity (TA) (AOAC, 2005).

2.6. *Total polyphenols*

Total polyphenols were determined using a method described by Dhlakama and Chawafambira (2022). Using a standard equation y=0.5742x+0.1193, R^2=0.9985, results were reported as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g).

2.7. *Antioxidant radical scavenging and total antioxidant activity assay*

The antioxidant activity was carried out using a modified 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity technique described by Dhlakama and Chawafambira (2022). The results were reported as mg of Trolox equivalent per 100 g sample.

The total antioxidant activity was calculated using the ABTS (2,2′-azinobis-3-ethyl-benzothiazoline-6-sulphonate) technique reported by Chawafambira et al. (2022). Ascorbic acid was used as a standard and results represented as μmol AE /100 g of the sample.

2.8. *β-carotene and Vitamin A content*

Extraction of carotenoids (β-carotene) was conducted using a modified method first reported by Rodriguez-Amaya and Kimura (2004), and then by Dhlakama and Chawafambira (2022). Individual carotenoids were measured using a formula adapted from Chawafambira et al. (2021) as follows:

$$C\left(\frac{\mu g}{g}\right) = \frac{A_X \times C_S \times V(ml)}{A_S \times P_g}$$

where: A_x = Carotenoid peak area; C_s = Standard concentration; A_s = Standard area; V = Total extract volume and P = Sample weight. In vitamin A determination, an FDA guideline with the relationship 1 μg RAE = 12 μg beta-carotene was used. The result was presented as μg RAE (Chawafambira et al. 2021).

2.9. *Sensory evaluation*

Ethical consideration were applied in the selection of volunteer panellists (n = 85). Ethical clearance for the sensory evaluation process was granted and the panelist were first asked to prepare written consent letters prior to their inclusion in the sensory evaluation process. The prepared maheu samples were coded using a unique three digit code to remove bias. After recruitment and selection of panelist, a sensory evaluation score card, a pen and a bottle of water to rinse their mouth

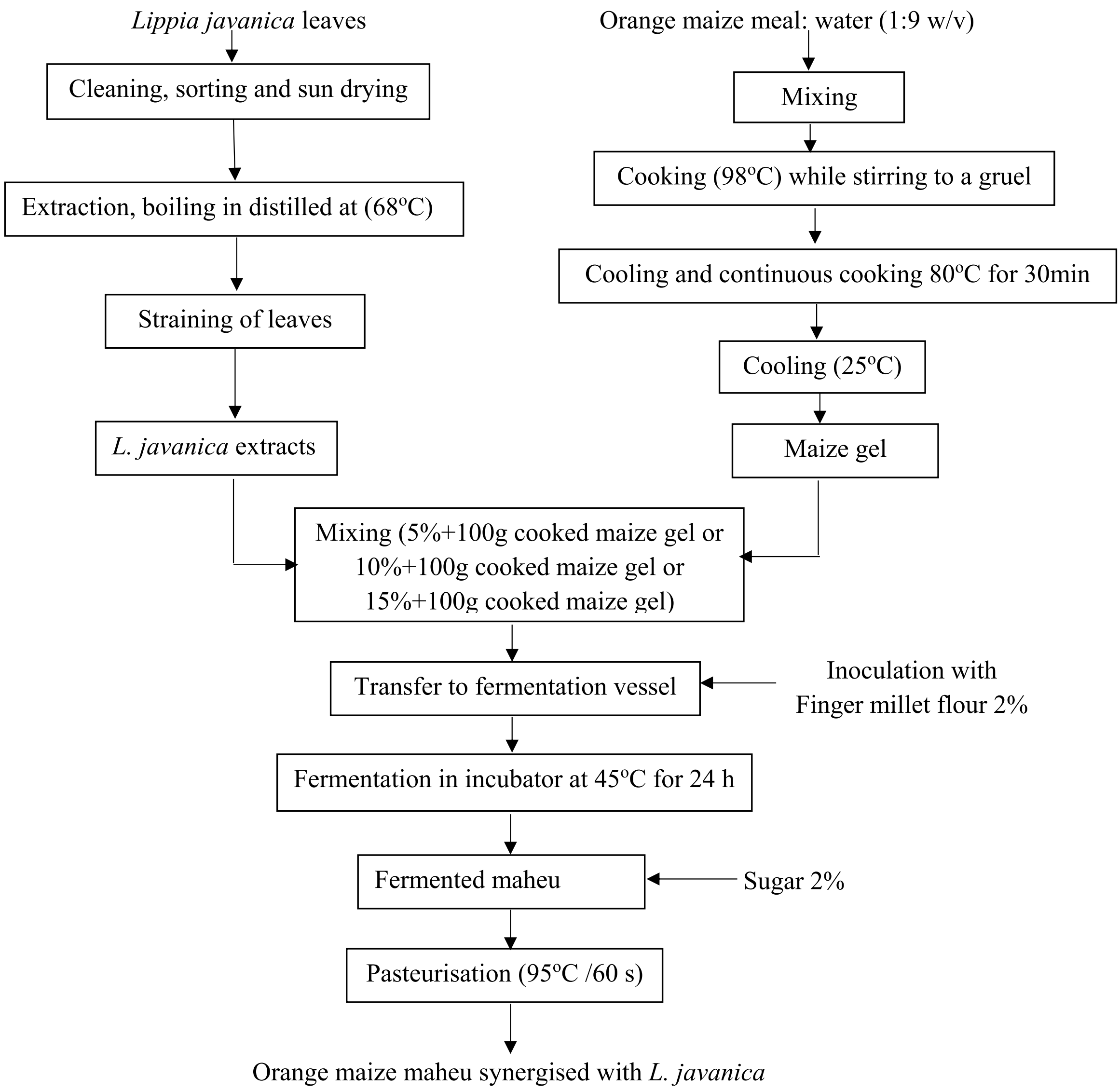

Fig. 2. Processing flowchart for orange maize maheu with added L.J.E.

Table 2
Proximate composition, pH, total soluble sugars, titratable acidity, and vitamin C contents (wet basis) of orange maize maheu mixed with L.J.E.

Treatment	M (%)	β-carotene (μg/g w.b)	Ash (%)	pH	TA (%)	TSS (%)	Vitamin C (mg/100g w.b)
Control	84.4 ± 1.3a	0.8 ± 0.00a	0.8 ± 0.05a	3.9 ± 0.01a	0.08 ± 0.01a	4.0 ± 0.1a	6.3 ± 0.05a
T1	87.3 ± 0.1b	0.9 ± 0.01a	0.9 ± 0.01a	3.8 ± 0.00a	0.18 ± 0.00ab	4.2 ± 0.1ab	7.8 ± 0.01a
T2	86.1 ± 0.3ab	1.0 ± 0.01a	1.0 ± 0.02ab	3.7 ± 0.01ab	0.2 ± 0.02b	4.3 ± 0.01b	9.2 ± 0.02ab
T3	88.8 ± 2.1c	1.1 ± 0.00b	1.2 ± 0.01cb	3.6 ± 0.01b	0.36 ± 0.01b	4.5 ± 0.0c	11.1 ± 0.05b
Range	84.4–88.8	0.8–1.1	0.8–1.2	3.6–3.9	0.08–0.36	4.0–4.5	6.3–11.1

Where M = moisture, TA = titratable acidity, TSS = total soluble sugar content, T1 = 100 g cooked orange maize gel + 5 % L.J.E, T2 = 100 g cooked orange maize gel + 10 % L.J.E and T3 = 100g g cooked orange maize gel +15 % L.J.E. Mean values in a column followed by different letters are significantly different at $p < 0.05$.

after each analysis were given. A 5–point hedonic scale (1=Very bad, 2=Bad, 3=Average, 4=Good, 5=Very good) was designed according to Maakelo et al., (2021) and then translated into local language "Shona" for easy understanding of the sensory process. The panelists were given the prepared maheu samples in coded cups and asked to evaluate the taste, colour, texture, aroma, appearance, overall acceptance and preference (paired comparison) in individual testing booths. In addition, Focus group discussion were conducted and panelists were asked to indicate their mostly consumed plant food sources and consumption patterns of other orange maize based products other than maheu using a translated food frequency questionnaire. Panelists were not allowed to discuss their responses during the sensory evaluation exercise.

2.10. Statistical analysis

The data was expressed as mean ± standard deviation (SD) and analysed using One-way Analysis of Variance (ANOVA) by IBM Corporation (2017) SPSS® Statistics Version 25 (USA). The Friedman's test on

Table 3
Total polyphenols, AOA, Total antioxidant activity and vitamin A contents of orange maize maheu mixed with L.J.E.

Treatment	Total polyphenols (mg GAE/g)	AOA (EC_{50}mg/L)	Total Antioxidant activity (μmol AE/100g)	Vitamin A (μREA/100g w.b)
Control	8.2 ± 0.7a	28.1 ± 2.2a	31.3 ± 2.1a	3.90 ± 0.01a
T1	13.4 ± 1.1b	33.4 ± 1.3b	38.1 ± 1.1a	3.81 ± 0.01a
T2	18.7 ± 0.6c	38.1 ± 3.1c	40.1 ± 3.1b	3.60 ± 0.00a
T3	19.4 ± 1.4c	41.0 ± 2.1c	48.3 ± 1.4bc	3.55 ± 0.02b
Range	8.2–19.4	28.1–41	31.3–48.3	3.55–3.90

Where AOA = antioxidant activity, T1 = 100 g cooked orange maize gel + 5 % L.J.E, T2 = 100 g cooked orange maize gel + 10 % L.J.E and T3 = 100g cooked orange maize gel +15 % L.J.E. Mean values in a column followed by different letters are significantly different at $p < 0.05$.

mean sensory attributes scores and Pearson chi-square test was used to analyse the paired preference at 5 % level of significance.

3. Results and discussion

3.1. Moisture content

The prepared orange maize maheu samples had moisture content that varied from 84.4 to 88.8 % (Table 2). Similar moisture content results were observed by Fadahunsi and Soremekun (2017), in maheu with added different concentrations of *moringa oleifera leaf* powder (Idowu et al., 2016). Foods with a high moisture content have a shorter shelf life because of the high water activity (>80 %) which promotes microbial growth and chemical reactions among the components of maheu (Maakelo et al., 2021).

3.2. Ash content

The maheu samples had an ash content that ranged from 0.8–1.2 %. A significant increase ($p < 0.05$) in the ash concentration was observed as the L.J.E concentration increased from 5 % to 15 % in the samples. Ash content increased by 50 % from the control maheu with the addition of 15 % L.J.E. The outcome suggests that L.J.E might have supplied some minerals in the fermented maheu because previous studies have reported an ash content of 6.1 ± 1.3 % in *L. javanica* leaves (Chawafambira, 2021). Similar trends in increase in ash content were observed by Olusanya et al. (2020) in maheu fortified with 2, 4, and 6 % Moringa oleifera leaf powder. But the ash content found in this study was less than the ash contents (1.34–1.50 %) and (1.66–2.27 %) reported for maize maheu by Idowu et al. (2016). This was ascribed to the use of milled whole grain maize as an ingredient in the formulations, while in this study, refined orange maize meal was used in the recipe, and the refining process during milling had removed the bran and most minerals (Maakelo et al., 2021). The process of catalysing phytic acid by the fermenting microorganisms' phytase enzymes has also been reported to cause an increase in the ash content and the bioavailability of minerals during the fermentation process (Maakelo et al. 2021; Idowu et al., 2016).

3.3. pH and TA content

The pH of the maheu samples ranged between 3.6–3.9 and there was a significant difference ($p < 0.05$) in the pH among the control sample and maheu samples with 10 % and 15 % L.J.E ($p < 0.05$). The observed pH values in this experiment were lower as compared to other pH values on maheu obtained in previous studies (Fadahunsi & Soremekun, 2017; Olusanya, 2018, Chawafambira & Mkungunugwa, 2021; Mashau et al.,

Table 4
Mineral content and recommended dietary allowance (RDA) of orange maize maheu mixed with L.J.E.

Treatment	Iron (mg / 100g w.b)	Calcium (mg / 100g w.b)	Zinc (mg / 100g w.b)	Potassium (mg / 100g w.b)	Magnesium (mg / 100g w.b)	Sodium (mg / 100g w.b)
Control	2.6 ± 0.01a	80.1 ± 0.01a	0.8 ± 0.01a	72.5 ± 2.2a	93.2 ± 2.1a	2.6 ± 0.5a
T1	2.7 ± 0.02ba	86.2 ± 0.01b	0.9 ± 0.01a	74.1 ± 1.4a	94.2 ± 1.2a	2.8 ± 0.3a
T2	2.8 ± 0.01ab	88.2 ± 0.01b	1.0 ± 0.05ab	80.2 ± 0.8b	95.2 ± 2.1a	2.9 ± 0.1a
T3	3.1 ± 0.02c	89.4 ± 0.01b	1.1 ± 0.02b	82.0 ± 1.2b	97.1 ± 2.6b	3.1 ± 0.4b
Range	2.6–3.1	80.1–89.4	0.8–1.1	72.5–82	93.2–97.1	2.6–3.1
Where T1 = 100 g cooked orange maize gel + 5 % L.J.E, T2 = 100 g cooked orange maize gel + 10 % L.J.E and T3 = 100g cooked orange maize gel +15 % L.J.E. Mean values in a column followed by different letters are significantly different at p < 0.05						
RDAs for minerals in children (mg/day)						
RDA children (1–9yrs)	7	100	5	3800	130	120
% contribution of maheu	37–44	8–9	16–22	1.9–2.2	72–75	0.2–0.3

Table 5
Major plants consumed as Food Resources by the panellists.

Local Name	Botanical Name	Family	Use	Rank	Consumption (%)
Crops Cereals:					
Maize/corn (Eng); chibage (Sh);umumbu (Nd)	*Zea mays*	Gramineae	Food and animal feed	1	100
Sorghum (Eng); mhunga (Sh), amabele (Nd)	*Pisum sativum L.*	Poaceae	Food, fodder, brewing	2	82
Finger millet (Eng); zviyo/ rukweza (Sh); uphoko (Nd).	*Eleusine coracana*	Poaceae/ Gramineae	Food, brewing, baking, malt flour	3	76
Legumes:					
Groundnuts (Eng);nzungu (Sh); amazambane(Nd)	*Arachis hypogaea*	Fabaceae	Food	4	68
Roundnuts (Eng); nyimo (Sh); indlubu (Nd).	*Vigna subterranea*	Fabaceae	Food, a relish	5	60
Wild fruits					
Wildloquat,(Eng);mushuku/muzhanje (Sh); umhobohobo (Nd)	*Uapaca kirkiana*	Euphorbiaceae	Fruit eaten raw.	1	76
Chocolate berry (Eng); mutsubvu, (Sh); umtshwankela (Nd)	*Vitex payos*	Lamiaceae	Fruit pulp raw	2	60
Monkey orange(Eng); mutamba (Sh); umkhemeswane(Nd)	*Strychnos cocculoides*	Strychnaceae	Fruit eaten raw	3	53
Bird plum (Eng); munyii (Sh); umnyiyi (Nd)	*Berchemia discolor*	Rhamnaceae	Fruit pulp eaten raw.	4	51
Bakota plum (Eng); munhunguru (Sh), umqokolo (Nd)	*Flacourtia indica*	Salicaceae	Fruit eaten raw	5	48

Key: Eng = English, Sh = Shona, Nd = Ndebele.

Table 6
Knowledge and consumption of orange maize products other than maheu.

	Panellists Group 1 (n = 23)	Group 2 (n = 30)	Group 3 (n = 27)	Group 4 (n = 20)	Group 5 (n = 25)
Having knowledge of orange maize (%)					
Varieties	11.1	14.2	10.3	11.4	8.2
Production	1.0	1.4	1.1	1.0	<1
Consumption	82.5	78.4	71.6	64.5	55.6
Nutritional data	1.0	1.2	0.0	2.1	0.0
Access to nutritional education	3.7	6.1	8.1	11.2	4.5
Orange maize products consumed at least once a day throughout the year (%)					
Maize meal (*sadza*)	37.2	14.1	36.2	20.2	42.3
Maize porridge	62.2	44.1	64.5	58.1	31.0
Green maize (roasted)	36.2	33.3	28.6	34.1	41.2
Green maize (boiled)	33.2	54.6	34.2	54.2.	41.2
*Other maize products	35.1	39.7	40.1	34.8	45.6

*Other products excluding fermented maize based foods include: roasted dried maize grains (*maputi*); maize grains are pounded and boiled (*manhuchu*); dry maize grain are boiled (*mangai*); and boiling of maize grains, peanuts, and cowpeas mixture (*mutakura*).

Table 7
Number and percentages of panellists who gave the different ratings for the sensory attributes evaluated ($n = 85$).

Treatment	Rating	Taste	Aroma	Texture	Colour	Overall Acceptance
Control	Very bad	0[a] (0.0)[b]	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)
	Bad	5 (5.8)	7 (8.2)	5 (5.8)	4 (4.7)	2 (2.3)
	Average	18 (21.1)	23 (27.0)	22 (25.8)	21 (24.7)	25 (29.4)
	Good	44 (51.7)	46 (54.1)	41 (48.2)	48 (56.4)	47 (55.2)
	Very good	18 (21.1)	10 (11.7)	16 (18.8)	12 (14.1)	11 (12.9)
T1	Very bad	0 (0.0)	1 (1.1)	1 (1.1)	2 (2.3)	0 (0.0)
	Bad	3 (3.5)	4 (4.7)	5 (5.8)	9 (10.5)	3 (3.5)
	Average	23 (27.0)	19 (22.3)	16 (18.8)	23 (27.0)	21 (24.7)
	Good	45 (52.9)	47 (55.2)	44 (51.7)	39 (45.8)	49 (57.6)
	Very good	14 (16.4)	14 (16.4)	16 (18.8)	12 (14.1)	12 (14.1)
T2	Very bad	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)
	Bad	2 (2.3)	1 (1.1)	0 (0.0)	1 (1.1)	2 (2.3)
	Average	21 (24.7)	23 (27.0)	24 (28.2)	27 (31.7)	22 (25.8)
	Good	46 (54.1)	47 (55.2)	49 (57.6)	45 (52.9)	50 (58.8)
	Very good	15 (17.6)	14 (16.4)	11 (12.9)	12 (14.1)	11 (12.9)
T3	Very bad	1 (1.1)	0 (0.0)	1 (1.1)	4 (4.7)	0 (0.0)
	Bad	3 (3.5)	5 (5.8)	4 (4.7)	3 (3.5)	2 (2.3)
	Average	22 (25.8)	24 (28.2)	20 (23.5)	23 (27.0)	24 (28.2)
	Good	42 (49.4)	44 (51.7)	46 (54.1)	40 (47.0)	48 (56.4)
	Very good	17 (20.0)	12 (14.1)	14 (19.2)	15 (17.6)	11 (12.9)

[a] Number of subjects.
[b] Percentage of total number of panellists; Acceptability rating 1–5: 1 = very bad; 5 = very good.

2020). The TA ranged between 0.08–0.36 % in maheu samples. The TA is a measurement of the lactic acid produced as the sugar is broken down during fermentation. The addition of L.J.E might have provided the

Table 8
Differences in paired preference with gender ($n = 85$).

Gender	*Treatments* Control	T1	T2	T3
Women	36[a] (62.0)[b]	22 (37.9)	38 (65.5)	20 (34.4)
Males	15 (55.5)	12 (44.4)	8 (29.6)	19 (70.3)
Total number of panellists	51 (60)[c]	34 (40)	46 (54)	39 (46)

[a] Number of panellists.
[b] Percentage (%) of the sample within a gender group.
[c] Percentage (%) of the total number of panellists.

Table 9
Preference of maheu samples across age groups.

Age group	n	*Treatments* Control	T1	T2	T3
20–34	16	4[a] (25)[b]	5 (31.2)	9 (56.2)	7 (43.7)
35–44	18	5 (27.7)	6 (33.3)	11 (61.1)	9 (50)
45–54	32	20 (62.5)	14 (43.7)	17 (53.1)	23 (71.8)
55+	19	10 (52.6)	9 (47.3)	9 (47.3)	12 (63.1)
Total participants	85	39 (45.8)	34 (40)	46 (54.1)	51 (60)

[a] Number of participants.
[b] Percentage (%) preference of the sample within an age group.

sugars as a substrate. This can be supported by results observed on total sugars (1.1 ± 0.03 g) in L.J.E in previous studies (Chawafambira, 2021). The TA increased as the pH was decreasing and was comparable with other previous studies. For example, a TA of 0.30 % for 36 h fermentation, 0.2–0.6 % for 72 h fermentation and 0.23–1.50 % for 24–36h fermentation were reported by Chawafambira and Mkungunugwa (2021), Mashau et al. (2020) and Idowu et al. (2016) respectively. The pH (<4.0) and TA (0.1 %) were reported to inhibit the growth and survival of *Bacillus cereus*, a prevalent pathogen in cereal products (Maakelo et al. 2021; Byaruhanga et al., 1999). The observed decrease in pH and increase in acidity in this study suggests the production of lactic acid (Maakelo et al. 2021) and other secondary products during fermentation at 45°C for 24 h. The secondary products of lactic acid fermentation include acetic, butyric, propionic acids and can result in the reduction of the pH and acidification of almost 0.6 % (Chawafambira and Mkungunugwa, 2021). The fermentation temperature of 45 °C used in this study was beyond the optimum temperature (30–32 °C) for lactic acid (Sharma et al., 2020). This is because at a temperature and pH combination of 45–65 °C and pH 5.0–7.0 which is above the said optimum temperatures from literature, most lactic acid bacteria phytases enzyme activity increases and results in improved breakdown of phytic acid present in the orange maize, mineral bioavailability and protein digestibility (Maakelo et al. 2021; Idowu et al., 2016). Kayitesi et al. (2017) reported an adaptation temperature of 51 °C for pure cultures of *Lactobacillus acidophilus, L. bulgaricus*, and *Streptococcus lactis* on maize meal substrate used in mageu production.

3.4. Total Soluble Sugars (TSS) content

The TSS content for the maheu samples ranged from 4.0–4.5 % and showed significant difference ($p > 0.05$) among treatment samples. The TSS indicates the soluble sugars content. The relative high %TSS observed in this study is attributed to the total soluble sugar in the L.J.E and 2g of table sugar added after fermentation. A previous study on L.J.E reported a 4.1 ± 1.01 % total soluble sugar content (Chawafambira, 2021). Also, Maakelo et al. (2021) reported an increase in TSS in maheu due to the addition of table sugar in maheu. This might support our observed results. In other researches on the addition of 10g of Aloe vera powder in maheu (Maakelo et al. 2021), the use of provitamin A-biofortified maize grains in maheu, and the use of probiotics resulted in a TSS range of 4.7–5.4 % (Chawafambira and Mkungunugwa, 2021), 1.9–4.0 % (Awobusuyi et al., 2016) and 4.8 °Brix (Chawafambira and

Table 10
Mean scores for the sensory evaluation of fermented maheu samples with L.J.E ($n = 85$).

Treatment	Taste	Colour	Texture	Aroma	Appearance	Overall Acceptability
Control	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.2	3.6 ± 0.1	3.9 ± 0.2	3.9 ± 0.1
T1	3.9 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.9 ± 0.2	3.9 ± 0.1	3.9 ± 0.1
T2	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	4.0 ± 0.2	4.1 ± 0.1
T3	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.2	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
p–value[a]	<0.05	<0.05	ns	<0.05	ns	<0.05

Mean ± standard deviations are reported.
[a] Friedman's test, ns = not significant.

Mkungunugwa, 2021) respectively. The starch from the orange maize meal was hydrolysed into simple sugars and also other sugars from L.J.E might have not been completely utilized by the natural fermenting microorganisms. These sugars might have also contributed to the results on TSS. The sugars acted as a substrate during fermentation and resulted in the production of organic acids. The TSS has a very significant positive correlation with the observed TA values in the maheu samples (Table 2).

3.5. Vitamin C content

Vitamin C (L-ascorbic acid) is a potent antioxidant and free radical scavenger, improves the absorption of inorganic iron, reduces the risk of sepsis, and inhibits the production of nitrosamines in the stomach (Timoshnikov et al., 2020). The vitamin C content was 6.3 to11.1 mg/100g in maheu samples. Maheu samples with 15 % L.J.E had a high vitamin C content. Vitamin C content increased by 76 % from the control maheu with the addition of 15 % L.J.E. This can be attributed to the observed vitamin C (17.2mg/100g w.b) content in L.J.E (Chawafambira, 2021). Further, the process of anerobic fermentation could have resulted in the production of metabolic acids such as ascorbic acid at varying concentrations during fermentation (Wang et al., 2021). This can be used to explain the slight increases in vitamin C content in the maheu. Conversely, the limited degradation of vitamin C can be attributed to the reduced activity of the enzyme L-ascorbate oxidase found present in cereals. During fermentation, the enzyme might have been produced by the fermentation microorganism but its activity was reduced due to the inhibiting conditions created during fermentation. The high cooking temperatures (98°C) employed in the study and pH of 3.5 in maheu inhibits the activity of L-ascorbate oxidase. The optimum conditions of the enzyme L-ascorbate oxidase is pH 5.6 and 30°C (EFSA, 2019).

The observed vitamin C contents in the fermented maheu samples could be attributed to the action of lactic acid bacterial and some yeast fermentations (Maakelo et al., 2021; Nkhata et al., 2018). Possibly the L.J.E also could have provided the available simple sugar (1.1 ± 0.03g of total sugars) (Sharma et al., 2020) during gelatinisation process and the glucose might have acted as a precursor for the biosynthesis of L-ascorbic acid during fermentation by fermenting microorganisms in the maheu samples (Nkhata et al., 2018). That fermentation resulted in a slight increase in the vitamin C content is in line with previous researchers' findings. Vitamin C content of okra seeds, citrus peels and white cabbage increased with fermentation (Adetuyi et al., 2014; Oboh et al., 2011; Kusznierewicz et al., 2008). In contrast, other authors have found noticeable reduction in vitamin C content of *Lupinus albus* during fermentation (Frias et al., 2005)

3.6. Total polyphenols and AOA

The total polyphenols of the maheu samples ranged between 8.2–19.4 mg GAE/g and there was a significant difference ($p < 0.05$) among maheu samples (Table 3). The total polyphenols increased by 63 % from the control maheu and more than 100 % with addition of 10 % L.J.E and 15 % L.J.E in the maheu samples. The increase in total polyphenols could be ascribed to the presence of phenolic compounds in the L.J.E. The presence of phenolic compounds and their derivatives in L.J.E such as 3,4-dihydroxy-β-phenylethoxy-O-(6"-β-caffeoyl-α-rhamnopyranosyl-(1"',3")-O-β-glucpyranoside) commonly known as isoverbascoside 3 and coumarin 1, 3,4-dihydroxy-β-phenylethoxy-O-(4"-β-caffeoyl-α-rhamnopyranosyl-(1"',3")-O-β-glucopyranoside), commonly known as verbascoside 2 have been reported by Olivier et al. (2010). Further, the different flavanones such as 6-methoxyluteolin 3', 4', 7-trimethyl ether 10, 6 methoxyluteolin 4'-methyl ether 9, cirsimaritin 8, and apigenin 7 have also been reported in L.J.E (Mujovo et al., 2008).

The antioxidant activity of the fermented maheu samples was 28–41 EC_{50}mg/L. There was significant increase in AOA with an increase L.J.E addition in maheu samples. This could be explained by the presence of dietary phytonutrients such as phenolics and vitamins in the L.J.E. A wide range of flavonoids and phenolic glycosides that include chrysoeriol 14, crassifolioside 11, luteolin 12, tricin 15, isothymusin 16, diosmetin 13, eupatorin 17, 5-dimethyl noboletin 18, salvigenin 20, genkwanin 19, and an alkaloid xanthine 22 were identified in *L. javanica* by Madzimure et al. (2011) and their presence might explain the reported AOA in maheu samples in this study. Olivier et al. (2010) reported high levels of verbascoside 2 (1.5 mg/g dry weight) and Shikanga et al. (2010) observed an AOA value of 358 EC_{50}μg/mL and a total phenolic content of 14.8 mg GEA /mL of dry weight in L.J.E. Further, Muchuweti et al. (2006) reported a 74.4 % inhibition of the DPPH radical by methanolic L.J.E and this characteristic can also explain the observed AOA of the fermented maheu samples.

3.7. β-carotene and Vitamin A content

The β-carotene and Vitamin A content in the fermented maheu range was 0.8-1.1 μg/g and 3.55–3.90 μREA/100g w.b respectively. The provitamin A carotenoid, beta-carotene found in orange maize is converted into vitamin A in the intestines once consumed in a diet. This study showed a relatively low β-carotene content in the fermented maheu samples and this could be attributed to the effect of processing (heating and fermentation) and genetic characteristics of the orange maize that determine the carotenoids in the grains. Chawafambira et al. (2021) reported the presence of β-Cryptoxanthin (1.21 ± 0.01 μg /g DW), 13-cis-β-carotene (0.02 ± 0.02 μg /g DW), α-Carotene (0.82 ± 0.01 μg /g DW), 9-cis-β-carotene (0.80 ± 0.01 μg /g DW), All-trans-β-carotene (0.85 ± 0.01 μg /g DW), lutein (7.56 ± 0.60 μg /g DW) and zeaxanthin (1.52 ± 0.01 μg /g DW) in orange maize. Further, the total carotene content was 12.78 ± 0.5μg/g DW in orange maize (Chawafambira et al. 2021). The orange maize variety used in this study. Carotenoids could have been degraded during cooking. This is because the heat treatment process has been reported to cause the isomerization of trans-carotenoids to their cis-isomers and affects their biological activity (Chawafambira et al., 2022, 2021). This could have contributed to the observed relatively low vitamin A content observed in this study.

3.8. Mineral content

Maheu samples are a good source of healthy beneficial minerals needed for the proper functioning of the body as shown in Table 4. There was an increase in mineral content in maheu samples due to addition of

L.J.E when compared to the control sample. This could be attributed to the good mineral content in L.J.E. A previous study by Chawafambira (2021) reported an iron, magnesium, calcium, zinc, potassium and sodium content of 77.3, 267, 485, 3.8, 1631, and 1 mg/100g in L.J.E respectively. Also, the addition of finger millet as an inoculum might have contributed to the increase in mineral content. Finger millet is a good source of zinc (2.3 mg), iron (3.3–14 mg), phosphorus (130–250 mg), calcium (398 mg), potassium (430-490 mg), and sodium 49 mg (Ramashia et al. 2018). The calculations on the estimated contribution of maheu on the RDA for minerals in children (1–9years) showed that maheu samples with 15 % L.J.E could potentially contribute about 72–75 % of the RDA for magnesium (130 mg / day), 1.9–2.2 % of the RDA for potassium (3800 mg / day), 37–44 % of the RDA for iron (7 mg / day), 22–26 % of the RDA for sodium (120 mg / day), and 16–22 % of the RDA for zinc (5 mg / day) in children (1–9 years) when consumed as part of the diet. Our RDA results were higher as compared to a study by Buzigi (2020) who reported a contribution 19 % towards meeting the RDA for iron and zinc in children 2 years old in a homemade complementary food produced from a blend of bean and pumpkin. Further, in this study, maheu samples were prepared mimicking the traditional process as compared to the international process prescribed by the Codex Alimentarius on fermented foods for children up to 2 years. This might have resulted in the variations observed in the nutritional content (Buzigi, 2020) in our study.

3.9. Consumption and Utilisation patterns of orange maize based products

The major plants used as food resources by the panellists are represented in Table 5. Maize was widely consumed and used to sustain their livelihoods. This is because it is the local staple food in Zimbabwe. In addition, different wild fruits were reported as important to supplement diets in times of droughts and food shortages. Similar results were observed by Mudzengi et al. (2012). A wild fruit/traditional fruits is defined as fruits that grow naturally from wild plants, are culturally recognized as local food, and have been consumed for generations (Nemapare et al., 2023). Wild loquat fruits were most consumed wild fruits. The high consumption level of wild fruits can be explained by their rich nutrients content (Nachvak et al., 2020) and as a hunger-resisting survival method by the locals. In contrast to herbal plants, most indigenous fruits are either consumed fresh or processed/preserved for subsequent use. The knowledge and consumption of orange maize products by panellists other than maheu is shown in Table 6. Food group discussions showed that participants had some knowledge on the traditional fermented foods including maheu. This study observed that there is low information among the participants on orange maize varieties, nutritional data and access to nutritional education. This can be attributed to the level of education among the participants and limited information on the characteristics and benefits of orange maize in the society. It is therefore important for policy makers in government to support the production initiatives and increase awareness on the utilisation of orange maize in the country. These findings can explain the possibility for high uptake on the utilisation of orange maize in preparation of maheu synergised with L.J.E.

3.10. Sensory analysis

Table 7 indicate the percentages of panellists who gave the different ratings for the sensory attributes of the fermented maheu samples. Most of the participants rated taste, texture, and colour of maheu samples as "good" compared to the control samples. Deferent test results showed that women had a high percentage score than males for the fermented maheu samples (Table 8). The percentage of the sample within a gender group explained the number of panelist that scored "good and/above" and preferred the maheu sample as compared to another product on the market. Chowdhury et al. (2011) reported that customer acceptance is negatively affected by introducing an unfamiliar food product. This could suggest the difference results observed in this study.

Govender et al. (2014) reported an undesirable colour and poor acceptability of maheu samples due to the presence of carotenoids (Govender et al., 2014). The process of bio-fortifying maize can affect the grain colour as well as the flavour and aroma (Awobusuyi et al. 2016). Nevertheless, the presence of carotenoids pigments in orange maize did not affect the ratings on colour of the prepared maheu in this study. In this study, maheu fermented from orange maize with addition of L.J.E was well accepted by the panellists. The age of the panellist might have effected their ratings on preferences and acceptability in this study (Table 9). Other authors have reported the effect of age of consumers on that preference of foods prepared using yellow maize (Pillay et al., 2011).

The mean scores for the sensory attributes of the maheu with L.J.E are presented in Table 10. The results observed in this study were encouraging because the sensory attributes of the maheu samples were rated as good by most of the panellists. The ratings of taste and aroma could be attributed presence of volatile compounds that are produced during fermentation. Spontaneous fermentation process by *Leuconostoc spp., Lb. delbrueckii, L. lactis, S. lactis* (Franz et al., 2014) and *Pediococcus pentosaceus* (Gadaga et al., 1999) could have produced the perceived flavour and aroma compounds. These volatile compounds include acids, ketones, esters, aldehydes, alcohols, and hydrocarbons (Chaves-López et al., 2020). Malted millet used as an ingredient in the production of maheu is a possible source of fermentation microorganisms (Pswarayi & Ganzle, 2019). Uzogara et al. (1990) reported an improvement in textural characteristics of maize-based products due to fermentation. This could explain the favourable ratings in texture of the maheu samples in this study.

The overall acceptance of maheu samples with L.J.E was good when compared control sample. However, it is important to provide nutritional education and health benefits of maheu produced from orange maize with addition of L.J.E.

4. Conclusion

Traditionally fermented maheu was successfully produced from orange maize mixed with different concentrations of L.J.E. The addition of L.J.E in orange maize based fermented maheu showed positive effects on ash, vitamin and mineral content. Also, the addition of 15 % L.J.E improved the total polyphenols, AOA, and total antioxidant activity in maheu. RDAs for minerals in children, especially iron, magnesium and zinc improved in maheu samples with 15 % L.J.E. Further, the utilisation of 15 % L.J.E in maheu improved the sensory attributes (appearance, colour, texture) and overall acceptability of maheu. The study recommends the potential to utilising herbal L.J.E in the production of novel fermented food products with improved bioactive compounds, functionality, and sensory characteristics.

CRediT authorship contribution statement

Armistice Chawafambira: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Talknice Zvamaziva Jombo:** Data curation, Methodology, Software, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Ethical Statement

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