

Effect of skimmed milk and vegetable powders on shelf stability of millet-based composite flour

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Abstract

BACKGROUND: Millet porridge is a major complementary food used in Uganda but it is limited in protein and micronutrients such as zinc and beta-carotene. Addition of milk and vegetable powders are known to greatly improve the nutrient content of millet flour. However, there was limited information on the shelf stability of the resultant composite flour. This study aimed at assessing the effect of milk and vegetable powders on the shelf stability of millet-based composite flour.

RESULTS: There was a general increase in the moisture content, peroxide value (PV), free fatty acids (FFA), thiobuturic acid (TBA) and total plate count (TPC) of both composite and millet flours over the eight weeks storage period. However, higher moisture content, PV, FFA, TBA and TPC values were recorded in the composite flour compared to millet flour (control) at each sampling interval. Sensory evaluation results revealed that panelists preferred porridges prepared from millet only compared to those from composite flour. The degree of liking of porridges from both composite and millet flours generally decreased over the storage period. However, both porridges were deemed as acceptable by the end of the storage period. The TPC also remained below 10^5 cfu g⁻¹ which is the maximum limit recommended by the Uganda National Bureau of Standards (UNBS).

CONCLUSION: The study findings indicated that the addition of milk and vegetable powders negatively affected the stability of the composite flour. We recommend further studies to stabilize the product during storage.

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Keywords: porridge; sensory analysis; free fatty acids; peroxide value

INTRODUCTION

In most parts of Uganda especially western and central regions, millet-based porridges are the main complementary foods for children aged 6 to 59 months.¹ The millet-based porridges used in infant feeding are deficient in proteins, vitamin A and minerals especially zinc and potassium.² This situation denies the infants and children a balanced diet which is necessary for the maintenance of good health.³ The implication of feeding children with such food is the incidence of acute malnutrition, a major cause of high infant morbidity and mortality.⁴ About 11% of children under five years in Uganda are under weight, 29% are short while 4% are thin as a result of nutritional inadequacy which prevents growth to their genetic potentials.⁵ Mothers and caregivers combine different plant-based foods especially soy with other cereals or cereal porridges with cow's milk.⁶ This is because they understand that dietary diversity is an effective approach to improve the quality of plant-based diets.⁷ Combining cereals and legume flours in the ratio of 70:30 has been shown to have nutrient potentials that complement one another in general and have an amino acid pattern that can compare with that of animal protein.⁸ However, the digestibility of protein in plant-based foods is low due to anti-nutrients like phytates which bind the proteins.

Addition of skimmed milk powder to cereal-based complementary foods provides a better option because it has a higher protein content (34–37%)⁹ compared to millet flour (9–10%).¹⁰ Milk powder also has good protein quality that is highly digestible¹¹ compared to the protein obtained from plant foods. The vegetables have high levels of vitamins A and C; minerals like zinc, sodium and potassium. Cowpea leaves (2249 μ g 100 g⁻¹) and carrots (33 954 μ g 100 g⁻¹)¹² are rich sources of beta-carotene that is converted into vitamin A in the body. Pumpkin seeds are rich sources of zinc (39.9 mg kg⁻¹), sodium (4.80 mg kg⁻¹) and potassium (20 mg kg⁻¹).¹³ A combination of millet, milk powder and

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vegetables therefore has potential to generate a composite flour with a nutrient profile that meets the dietary requirements of children 6–59 months.⁷ However, adding vegetables and skimmed milk powder could affect the sensory properties of millet-based composite flour and also compromise its shelf stability. The objective of this study was to evaluate the effect of adding vegetables and skimmed milk powder on the shelf stability of composite flour made from millet, skimmed milk powder and vegetable powders.

MATERIALS AND METHODS

Experimental design

Two flours namely: (i) composite flour containing germinated millet flour, skimmed milk powder and vegetable (cowpeas, carrots and pumpkin seeds); (ii) millet flour from ungerminated grains (control) were used in the study. The control was millet flour from ungerminated millet grains because it is in the form in which most mothers and caregivers use to prepare porridge for the children.¹ Both flours were separately packaged in aluminium-laminated polyethylene (ALP) packaging materials and stored at room temperature ($25 \pm 5^\circ\text{C}$). Aluminium lining was used because of its excellent chemical resistance, good water vapour barrier properties and great impact resistance.¹⁴ The packaged samples were then coded and dated to facilitate easy identification and retrieval. The packages were sampled for analysis at fortnight intervals for a period of eight weeks. The eight weeks storage period was informed by the common storage practices for composite flours by mothers/caregivers in Uganda which does not usually exceed eight weeks. The packages opened for analysis at each sampling interval were discarded. The shelf stability of the samples was assessed by monitoring moisture content, peroxide value (PV), free fatty acid (FFA), thiobarbituric acid (TBA) tests, total plate count (TPC), total coliform, yeast and mould as well as sensory acceptability.

Source of raw materials and laboratory reagents

Millet, pumpkin seeds, carrots and cowpea leaves were purchased from Kisenyi Market, Kampala, Uganda while skimmed milk powder was purchased from Pearl Dairies in Mbarara district, Uganda. All the materials were delivered to the laboratory at the School of Food Technology, Nutrition and Bioengineering, Makerere University for further processing. Laboratory reagents were purchased from Neo Faraday Laboratory Supply, Kampala, Uganda.

Preprocessing of millet grains

Millet grains were sorted, cleaned and soaked in portable water for 12 h. The soaked grains were drained by spreading on a wire mesh. Drained millet was placed on trays lined with a wet towel and then covered with another wet towel. The millet on the trays was stored in a dark room at $25 \pm 5^\circ\text{C}$ and allowed to germinate for 48 h. The germinated grains were washed thrice in portable water and dried in the sun for three days. The germinated millet grains were milled into fine flour using a locally fabricated hammer mill.

Preparation of vegetables

Cowpea leaves and carrots were washed with running tap water to remove surface soil. The cow pea leaves and carrots were blanched in a water bath (Grants Instrument Ltd, Shepreth, UK) maintained at 80°C for 10 min and 95°C for 5 min, respectively. After blanching, carrots were shredded using a hand grater

with holes of diameter 0.6 cm. The shredded carrots and cowpea leaves were dried for 6 h in an electric cabinet dryer (B.MASTER, Milan, Italy) set at 55°C . Dried carrots and cowpea leaves were then milled into fine powders using a locally fabricated hammer mill. Pumpkin seeds were cleaned and roasted at 140°C for 20 min using an infrared food oven (GU-6, Wellington, New Zealand). Dry pumpkin seeds were blended into fine powder using an electric blender (Lilaram Manomal and Sons, Vadodara, India).

Formulation of the composite flour

Nutri-survey software, 2007 was used to estimate the amounts of skimmed milk and vegetable powders needed to add to millet flour in order to meet the protein (13 g day^{-1}) and energy (1046 and 902 kcal day^{-1} for boys and girls, respectively) requirements of children aged 6–59 months.¹⁵ The details of the various combinations used to reach the optimum levels of millet flour, skimmed milk and vegetable powders are indicated in Table 1.

Microbial analysis of millet-based composite flour

The TPC, total coliform, yeast and mould in millet and millet-based composite flours were determined using the international-ISO 4833:2013,¹⁶ 4832:2006¹⁷ and 21527-2:2008.¹⁸ Plates with colonies ranging from 30 to 300 were considered for counting and results expressed as colony-forming unit per gram (cfu g^{-1}).

Determination of lipid oxidation in the millet-based composite flour

The degree of lipid oxidation was determined using the FFA test, PV test and TBA test. FFA and PV tests measure the primary oxidation products¹⁹ while TBA number measures the secondary oxidation products.²⁰

Determination of FFA

FFA was determined according to the method described by Zhang *et al.*²¹ The FFA content was calculated using Eqn (1).

$$\text{FFA}\% = \frac{(v - b) \times N \times 28.2}{w} \quad (1)$$

where v represents the volume in millilitres of titration solution, b is the volume in millilitres of the blank, N the normality of the titration solution, w the weight of the sample in grams and 28.2 is the molecular weight of oleic acid divided by ten.

Determination of PV

PV was determined following methods described by Pomeranz and Meloan.²² The PV was calculated using Eqn (2).

$$\text{Peroxide value} = \frac{(S - B) \times \text{Molarity of thiosulphate} \times 100}{\text{Sample weight}} \quad (2)$$

where S is the titre of the sample and B is the titre of the blank.

Determination of TBA value

The TBA value was determined using the Association of Official Agricultural Chemists (AOAC) official method.²³ The TBA value was calculated using Eqn (3).

$$\text{TBA value} = \frac{50 \times (A_s - A_b)}{M} \quad (3)$$

where A_s is the absorbance of the sample; A_b the absorbance of the blank; M mass of sample; 50 is a factor based on the volume from a 25 mL volumetric flask and a cuvette path length of 1 cm.

Table 1. Proportions of millet flour, skimmed milk and vegetable powders used during product formulation to obtain the nutritional requirements (per 100 g) for children 6–59 months

Millet flour (g)	Skimmed milk powder (g)	Carrot powder (g)	Cowpea powder (g)	Pumpkin seed powder (g)	Energy (kcal)	Protein (g)
60	15	15	5	5	290	12.6
60	25	5	5	5	325	16.1
55	30	5	5	5	326	17.3
65	20	5	5	5	323	14.8^a
50	35	5	5	5	328	18.6

^a The formulation in bold typeface was chosen as optimal and used in this study because it provided 113.9% and 35.8% of daily protein and energy requirements respectively for children aged 6–59 months.

Determination of moisture content

Moisture content was determined using the AOAC official method.²⁴ The percentage moisture content was calculated as shown in Eqn (4).

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (4)$$

where W_1 is the weight of the empty dish; W_2 is the weight of the wet sample and dish; W_3 is the weight of the dry sample and dish.

Sensory evaluation of millet and millet-based composite porridges

Porridge preparation

Porridges were prepared by adding separately 100 g of millet and millet-based composite flours in 200 mL of cold water. The resulting paste was added to 500 mL of boiling water and cooked for 15 min with constant stirring. The prepared porridge was kept in coded thermos vacuum flasks to keep it hot.

Acceptability test

Sensory acceptability of the millet-based porridges was evaluated by a panel of 30 regular consumers (15 males and 15 females aged 18–40 years) selected from students and staff in the School of Food Technology, Nutrition and Bio-Engineering, Makerere University. Each panelist was provided with a separate sensory booth with adequate lighting conditions. Hot porridge samples were provided in plastic disposable cups marked with three-digit random codes. Drinking water was also provided to rinse the palate before and between two tasting sessions. The sensory attributes of porridges that were evaluated included; colour, taste, aroma and overall acceptability. These attributes were selected because they are prone to the changes associated with chemical reactions that take place in the product during storage. The panelists rated the sensory attributes of the porridges on a nine-point hedonic scale where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely. Prior to assessing the samples, the panelists were briefed on the nine-point hedonic scale and its application in sensory analysis.

Statistical analysis

All determinations were conducted in duplicates. Microbial count (in cfu g⁻¹) data were normalized by conversion to log cfu g⁻¹.

All data were subjected to statistical analysis of variance (ANOVA) and correlation analysis using XLSTAT software version 2017 to determine variation between means and statistical correlations between samples and variables respectively. Significance variation was accepted at $P < 0.05$.

RESULTS

Physicochemical changes in the millet flours during storage

The physicochemical properties of the millet based composite flour and control (millet flour only) monitored during the eight weeks storage period are summarized in Fig. 1. The results indicated that there was a general increase in moisture content, PV, FFA and TBA value of millet-based composite flour and the control during the eight week storage period (Fig. 1). The results indicated that there was a significant ($P < 0.05$) increase in moisture content of both flours over the eight weeks storage period. The moisture content of the millet-based composite flour increased from 6.10% to 7.32% while that of millet flour increased from 7.20% to 7.97% during the storage period. Despite millet flour having a higher final moisture content at the end of the study period, the results indicated that overall, the composite flour registered a higher percentage (1.2%) increase in moisture content compared to millet flour (0.77%).

The results further showed that during the first two weeks of the study period, there was a gradual increase in the PV (0.30–0.40 meq kg⁻¹) and FFA value (1.49–1.54%) in millet-based composite flour. This was followed by a significant ($P < 0.05$) rapid increase in PV (0.50–0.90 meq kg⁻¹) and FFA value (1.70–2.50%). The TBA value of the millet-based composite flour increased from 4.19 to 9.00 over the eight weeks storage period. A similar trend for PV, FFA and TBA was observed in millet flour. However, at all sampling intervals during storage, millet-based composite flour recorded higher values for PV, FFA and TBA than that of millet flour (control).

Microbial changes in the millet flours during storage

Figure 2 shows results from microbial enumeration of both millet-based composite flour and control. Findings from the study showed that there was a significant ($P < 0.05$) increase in the total plate counts from 2 to 3.6 log cfu g⁻¹ in millet-based composite flour over the storage period. Total coliforms in the composite flour also increased from 0 to 0.6 log cfu g⁻¹ while yeasts and moulds increased from 0 to 0.5 log cfu g⁻¹. The increase in TPC, coliforms, yeasts and moulds was higher in millet-based composite flour than in millet flour.

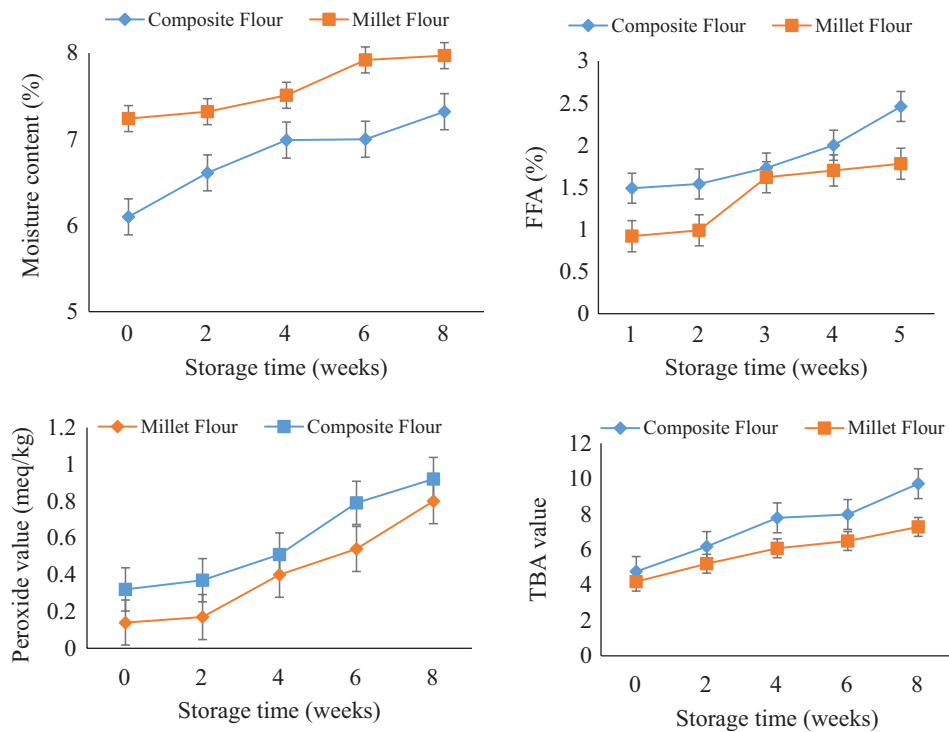


Figure 1. Changes in the moisture content, peroxide value (PV), free fatty acid (FFA) and thiobarbituric acid (TBA) values of millet flours during storage.

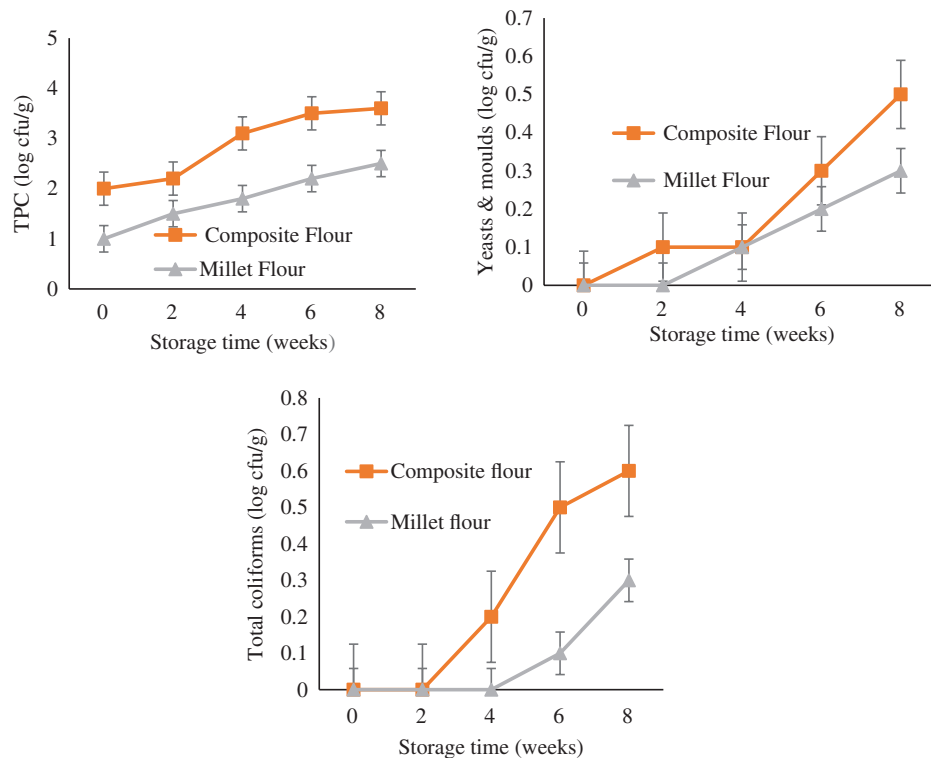


Figure 2. Microbial changes in millet flours during storage.

Changes in the sensory properties of porridge from millet flours during storage

The mean hedonic scores of sensory attributes for porridge from millet-based composite flour and control are presented in Fig. 3. The results indicate that throughout the storage period, porridges

prepared using millet-based composite flour were rated lower than that from millet flour for all the parameters assessed. The rating for colour of the porridges from the composite flour was initially 5.1 (average of neither like nor dislike) while that from millet was rated at 7.1 (like moderately). The colour rating of

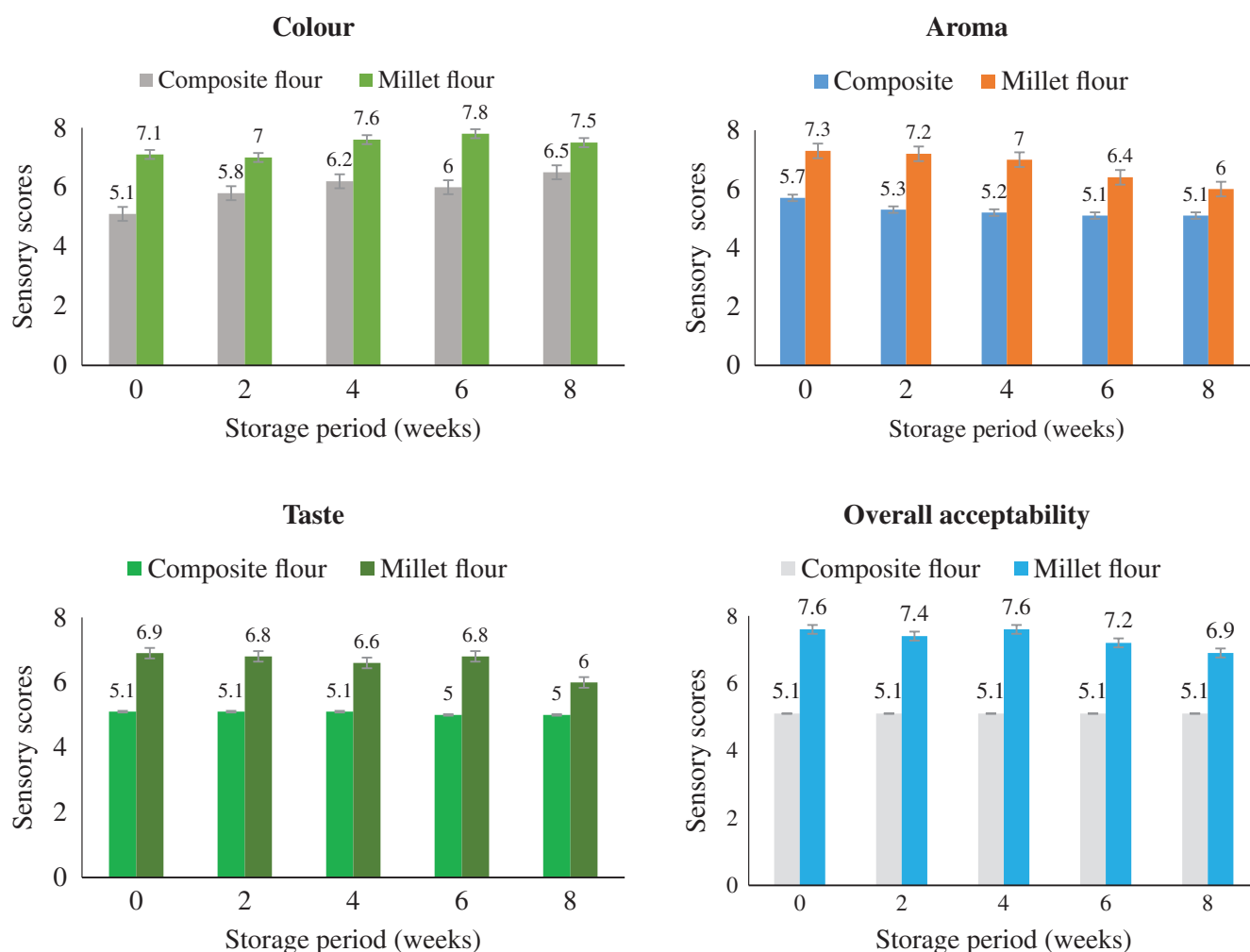


Figure 3. Sensory evaluation scores of porridges from millet flours over the eight weeks storage period.

both porridges increased with storage time but porridges from composite flour continued to record lower ratings (5.8–6.5) compared to porridges from control (7.0–7.5) from the second to eighth week, respectively. The degree of liking for taste and aroma decreased over the storage period (Fig. 3). However, the porridges prepared from millet flour recorded the largest decrease (0.9) in the taste rating compared to porridges from composite flour (0.1). The degree of liking for the aroma from porridges prepared from composite flour decreased from 5.7 to 5.1 while that of porridges from millet flour decreased from 7.3 to 6.0 over the storage period. The results further indicated that the overall acceptability (5.1) was lower compared to that of porridges from millet flour (6.9).

Table 2 provides a summary of the relationship between physicochemical properties of the flours and the sensory attributes of the resultant porridges. The results indicate that FFA ($r = -0.761$), PV ($r = -0.673$) and TBA ($r = -0.713$) were negatively and significantly ($P < 0.05$) correlated with aroma while PV and TBA ($r = 0.913$) had a positive and significant relationship. A negative and significant ($P < 0.05$) relationship was also observed between FFA and taste ($r = -0.638$). The results of factor analysis confirmed the suspicion that oxidation products had a negative effect on the sensory properties of the porridges.

DISCUSSION

The stability of a food product is influenced by both intrinsic and extrinsic factors. The intrinsic factors include the composition of the raw materials used to make the product, the water activity, microbial contamination and the extent of processing.²⁵ The extrinsic factors include environmental conditions like light, humidity, oxygen and temperature of the surroundings where the product is stored as well as the period of storage.²⁶ A combination of these factors determine the rate at which deleterious reactions proceed and the growth and multiplication of microorganisms.

One of the major causes of deterioration in low moisture foods such as flours is lipid oxidation which involves a series of complex reactions involving the food components like lipids, transition metals, water, oxygen and enzymes. In this study, it was observed that the addition of skimmed milk powder and vegetable flours was associated with higher PV, FFA and TBA values over the storage period. The results from an earlier study by Tumwine *et al.*²⁷ indicated that the millet-based composite flour had a higher fat content (3.7%) compared to the control (1.6%). The high fat content of the composite flour was due to the addition of pumpkin seed flour since the powdered milk used was skimmed and had a lower fat content not exceeding 0.5%. The higher PV, FFA and TBA values observed in millet-based composite flour was therefore as a result of auto-oxidation of the associated lipids.

Table 2. Relationship between physicochemical properties of flours and sensory attributes of porridges

Variables	FFA								
PV	0.932 ^a	PV							
TBA	0.923 ^a	0.913 ^a	TBA						
Colour	-0.140	0.021	-0.035	Colour					
Aroma	-0.761 ^a	-0.573 ^a	-0.713 ^a	0.648 ^a	Aroma				
Taste	-0.638 ^a	-0.517	-0.571	0.817 ^a	0.949 ^a	Taste			
Overall acceptability	-0.577	-0.433	-0.515	0.857 ^a	0.941 ^a	0.980 ^a	0.960 ^a	Overall acceptability	

^a Correlation coefficient (*r*) is significantly different from zero with a significance level alpha = 0.95.
Note: FFA, free fatty acid value; PV, peroxide value; TBA, thiobarbituric acid value.

The progression of lipid oxidation in the millet-based composite flour could have been further catalysed by the rich iron content in the product arising from addition of cowpea leaves that are rich sources of iron.²⁸ Endogenous iron has been reported to enhance lipid oxidation in low moisture food products made from rice flour and its effect has been reported to become more pronounced in the presence of moisture and oxygen.²⁹ The findings of this study are in agreement with the study conducted by Ravikumar and Narayanan³⁰ that reported an increase in FFA from 0.071 to 0.82 g 100 g⁻¹ in bio-fortified pearl millet flours over a 60 days storage period.

Lipid oxidation results in the formation of undesirable flavours and aromas which reduces food acceptability.³¹ In this study, porridges prepared from composite flour were given lower scores for both taste and aroma compared to the porridges prepared from millet flour only. Factor analysis revealed a negative and significant ($P < 0.05$) association between secondary products of oxidation and aroma. Secondary products of lipid oxidation are volatile and produce off-aromas that are detectable by humans at very low concentrations.²⁵ The lower aroma and taste scores for the porridges prepared from composite flour were attributed to the accumulation of secondary products of oxidation. Overall, porridges prepared from the composite flour were less liked by the panelists. In addition to the effects associated with lipid oxidation, the green colour of the composite flour arising from addition of cowpea leaves as well as the grainy texture imparted by the addition of pumpkin seeds that have substantial amounts of fibre had a negative impact on panelists' perception with respect to the product. Given the negative effect of vegetable powders on the sensory attributes of the porridges, adoption of such porridges as a weaning food will require robust advocacy campaigns to sensitize mothers and caregivers on the rich nutrient content of the composite flour in comparison to the millet flour which they are used to.

Environmental conditions like temperature and relative humidity of the storage environment also play an important role in the stability of the product.³² Even though the product was kept in an ALP package to ensure minimal interaction of the product and the environment, the results of the study indicated that there was an increase in moisture content between the fourth and eighth week with a simultaneous increase in TPC. The increase in moisture content could have been due to absorption of moisture from the environment while the growth of microorganisms could be attributed to suitable room temperature (25 ± 5 °C) where the flours were stored and the high nutrient content of the product. This observation is in agreement with the results reported in composite flours (wheat flour/horse gram/pearl millet) in which TPC increased from 0 to 4.1×10^3 cfu g⁻¹.³³ At every sampling interval, the composite

flour recorded a higher TPC compared to the control. This could be attributed to the vegetable powders added to the millet flour and implies that the pre-processing treatments of blanching (carrots and cowpeas) and roasting (pumpkin seeds) did not completely inactivate all the vegetative microbial cells and spores associated with the vegetables.³⁴ Despite the increase in the microbial population over the storage period, TPC, yeast and mould counts remained within the recommended Uganda National Bureau of Standards (UNBS) limits of 5 and 4 log cfu g⁻¹, respectively, for millet-based flours.³⁵ Therefore, the composite flour was deemed safe for consumption during the period of eight weeks.

The sensory evaluation of the porridges was carried out using regular millet porridge consumers instead of the target group of children 6–59 months. This approach has limitations because much as the panelists were regular millet porridge consumers and could easily evaluate the suitability of the presented samples as a weaning food, the information obtained only provided their tastes and preferences. Studies that recruited mothers as sensory panelists have also mentioned similar challenges.³⁶ The best approach would have been to serve the porridges to the target children and measure the quantities consumed. This is however challenged by the costs involved in implementing such a study and the restrictions associated with subjecting children to food that is still under experimentation.

CONCLUSION

The study findings indicated that the addition of skimmed milk and vegetables powders negatively affected the shelf stability of the composite flour and the sensory attributes of the resultant porridge. However, the TPC, coliforms, yeast and mould counts remained within the recommended UNBS limits and the resultant porridge was moderately acceptable. We recommend further studies to stabilize the product and improve its acceptability. Stability can be improved by defatting the pumpkin seeds to reduce the level of polyunsaturated lipids in the composite flour and the use of both natural and synthetic antioxidants. The acceptability of the product can be improved by substituting cowpea leaves with other iron and protein rich plant foods such as amaranth grains that may not compromise the colour of porridge. Alternatively, the proportions of cowpea leaves in the formulation can be reduced to minimize the effect of cowpeas on the colour of the porridge.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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