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Nutritional, Microbial and Sensory Properties of Flat-bread (*kitta*) Prepared from Blends of Maize (*Zea mays* L.) and Orange-fleshed Sweet Potato (*Ipomoea batatas* L.) Flours

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Abstract Vitamin-A deficiency is a major public health problem in developing countries including Ethiopia. Promoting consumption of locally available vitamin -A rich foods that can be grown in home gardens can reduce the problem of vitamin-A deficiency. The main objective of this study was to determine nutritional, microbial and sensory properties of flat-bread prepared from maize (*Zea mays* L.) and orange-fleshed sweet potato (*Ipomoea batatas* L.) flour blends. Flat-bread was developed with different proportion of orange-fleshed sweet potato to maize flour: 25%:75%, 30%:70%, 35%:65% and 0%:100% (control). The proximate analysis of flat-bread and flour was done. The total bacterial and mold/yeast count was conducted in Hawassa University and the sensory acceptability of flat-bread was carried out by mothers and children pairs using five point hedonic scales and preference test was done by children. Based on proximate analysis result the orange-fleshed sweet potato incorporated flat-bread samples were found to be rich in β- carotene and fulfill 61.63 to 86% of the recommended dietary allowance of pre-school children. The total coliform, mold and yeast counts showed that all of the orange-fleshed sweet potato incorporated flat-bread samples were microbiologically acceptable. The sensory acceptability result showed that orange-fleshed sweet potato incorporated maize flat-bread were liked/accepted in all sensory attributes by mothers/caregivers of pre-school children.

Keywords Pre-school children, Vitamin-A, Maize-flat bread, Proximate, Microbial load, Sensory acceptance

1. Introduction

Vitamin A deficiency is a major public health problem in developing countries: with children and pregnant/lactating women being the most vulnerable. Vitamin-A is essential for a well-functioning immune system and vision, and its deficiency significantly increases the risk of mortality in children [1]. There are different strategies to tackle vitamin-A deficiency. Traditional interventions consist of administration of vitamin-A capsules. Although a single dose can be given every six months or every year, these medical interventions are costly [2]. Promoting consumption of locally available vitamin -A rich foods that can be grown in home gardens can reduce the problem of vitamin A deficiency, due to its technical feasibility and cost-effectiveness. From the locally available foods orange-fleshed sweet potato (OFSP) is one which can be very suitable to overcome vitamin A deficiency in East Africa [3]. OFSP is beta-carotene rich and an excellent source of pro-vitamin A.

In many developing countries, sweet potatoes are secondary staple food and play roles in controlling vitamin-A deficiency.

Food fortification is another approach used to reduce vitamin A deficiency that works by adding vitamin-A to food commodities like sugar and vegetable oils. An alternative approach is bio-fortification, which consists of breeding staple crops to increase their content of vitamins and/or minerals. Compared to the other strategies, bio-fortification is considered a more sustainable approach because it has the potential to provide vitamins or minerals throughout the year. For the same level of impact on public health, the cost of bio-fortification is estimated to be half of vitamin-A supplementation [2]. Moreover, rural and low-income communities, who have been shown to be more at risk, can be reached by this approach and it also creates opportunities for income generation from production and marketing of these crops.

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The objective of the current study was to improve the pro-vitamin A and sensory properties of traditional flat–bread, also called *kitta* [4], prepared from maize *(Zea mays L.)* by blending it with flours of orange-fleshed sweet potato *(Ipomoea batatas L.).* The flat bread was selected for it is a staple food of large proportion of Ethiopian population, which is also being used for children 5 years of age. The study was targeting to reducing the prevalence of vitamin A deficiency in the study area through improving maize-based staple food.

2. Materials and Methods

2.1. Preparation of Study Materials

The OFSP samples were prepared using the procedures employed by [5], with minor modifications. Matured, undamaged and average sized roots were selected, washed in clean water, manually, drained and sliced to 2 mm thick slices. The slices were then soaked in 1% salt solution for 30 minutes as a pretreatment for better retention of beta-carotene and other nutrients [6]. The samples were then solar dried, milled and sieved through 1 mm (Axel Kistner, London, England). The procedure employed by [7], were followed in preparation of the maize flour. The grain was sun dried, milled and sieved through 1 mm (Axel Kistner, London, England) the flours were packed into high density polyethylene bags until they were required for the preparation of *kitta*.

2.2. Development of OFSP Flour Enriched Flat-Breads

Blending OFSP flour with that of maize flour was done at 25, 30 and 33%. Dough was prepared by mixing 1% iodized salt, the blended flour and water. The dough was kneaded until it became soft, smooth and non-sticky. Pre-heated local clay griddle (*Mitad*) was used for baking and the leaf of enset *(Ensete ventircosum)* was used for wrapping the dough being baked. The baking continued until a brown color developed (which took 10 minutes at 140 °C). The prepared flat-bread was cooled down at room temperature and wrapped in thin black plastic bags. The samples were stored in dry, cool and dark place until it was required for the other experiments.

2.3. Nutrient Analysis of OFSP Flour Enriched Flat-Breads

Nutrient compositions such as moisture content, crude protein, crude fat, crude fiber and ash of the flat-breads as well as flours of OFSP and maize were determined according to [8] standard method at the laboratories of Ethiopian Public Health Institute (EPHI). Moisture content was determined by drying the samples at 105 °C for 3 hours. Protein content (% N x 6.25) was determined by the Kjedahl method. Crude fat test was carried out based on Soxhlet extraction method utilizing diethyl ether. The ash content was determined by dry ashing in muffle furnace at 550 °C. Dietary fiber was determined by digesting defatted samples with diluted (1.25%) sulphuric acid (H_2SO_4) solution for 30 minutes at boiling point followed by digestion with 1.25% sodium hydroxide (NaOH) solution for the same duration. All the analyses were carried out in triplicates and in dry weight basis.

Fibre free carbohydrate content were determined by difference method [9]. The gross energy content was determined by multiplying percentages of crude fat by Atwaters conversion factors, 9 and that of crude protein as well as carbohydrates by 4 [10].

The β-carotene content was analyzed using open column chromatography for the extraction and UV visible spectrophotometer for reading the absorbance, a method described by [11] and [12]. The pigment was extracted by using petroleum and the absorbance was read at a wave length of 440 nm. The β-carotene values were used to calculate vitamin A contributed to the diet, expressed as retinol equivalents (RAE) [13], where 12 μg β-carotene equated to 1 μg RAE.

2.4. Microbial Analysis of the Flat-bread

The total mold, yeast and bacteria count was carried out on the flat- bread samples during 2 day room temperature storage using the procedure of [14]. Flat-bread samples were taken aseptically and homogenized in 99 ml sterile peptone water 0.1% in a blender for about 2 minutes and serial dilutions were made. One milliliter of each dilution were pour plated in sterile Petri dishes, the stomacher dilution represent the 10^{-1} dilution, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilution prepared by using 9 ml peptone water tubes and plate count agar (PCA) with chloroanphinicol was added and incubated at 25 °C for 5 days for mold and yeast count and the melted plate count agar (PCA) was used and incubated for 48 hours at 35 °C for total bacterial count .Counts of visible colonies by using colony counter was made and expressed as log CFU/g of the original sample.

2.5. Sensory Evaluation of Flat-Breads

The flat-bread samples were coded with three digit numbers and randomly presented to the panellists (mothers/caregivers and children) in random order. In sensory evaluation by mothers/caregivers, a five point hedonic scale (1= dislike very much, 2= dislike, 3= neither like nor dislike, 4= like, 5= like very much) was used to evaluate the samples in terms of color, appearance, flavor, taste, texture and overall acceptability. For the evaluation by the pre-school children, a preference test was used, where the children were asked to pick sample of their preferences. Panellists were children and their mothers /care givers in the study area. The samples were served to each panellist in triplicates and average scores were reported.

2.6. Experimental Design and Data Analysis

The nutritional analysis of samples was done in duplicates and the microbial and the sensory evaluation were undertaken in triplicates. Completely Randomized design

(CRD) (for the microbial and nutritional data) as well as randomized complete block design (RCBD) (for sensory scores) experimental designs were used. Analysis for the nutritional and microbial data was carried out using one way analysis of variance (ANOVA) using SAS software version 9 for windows. The sensory data was analyzed using a two way ANOVA, where the variability emanating from the individual panellists was taken care of using the blocking principle. Means separation was done using the Fischer's least significant differences (LSD) at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition of OFSP and Maize Flours

The moisture content of OFSP flour was found to be 8.63% (Table 1) which was lower than that maize flour (11.04%). [15] reported a similar finding in sweet potato and wheat flour (8.49%). The level of crude protein in OFSP flour was (4.60%); lower than that of maize flour (9.01%) [16]. A crude fiber level (4.92%) of OFSP flour was analyzed to be greater than that of maize flour (2.10%). Both ash and fiber levels had similar pattern of increment with the OFSP levels. Similar trends were reported by [17], for blending taro (*Colocasia esculenta*) flour with that of wheat. Flour derived from cereals is perfect for producing a wide range of baked products [18]. However, cereals are poor in β-carotene content.

This study showed that maize flour has no β-carotene but OFSP flour has the highest β-carotene level equivalent to 1987 RAE. The results of the current study showed higher β-carotene levels for OFSP than the reports of [19], which might be due to differences in the drying methods and investigated varieties.

Table 1. Proximate and vitamin-A Composition of OFSP and Maize Flours

Nutrients	Ingredients	
	OFSP flour	Maize flour
Moisture (%)	8.63±0.02	11.04±0.02
Protein (%)	4.60±0.01	9.01±0.03
Fat (%)	1.42±0.03	4.61±0.01
Ash (%)	5.37±0.04	1.20±0.06
Fiber (%)	4.92±0.06	2.10±0.02
Vitamin-A content (μg RAE)	1987±0.05	0.00±0.00

Values are averages of duplicate readings (mean ± standard deviation). OFSP: Orange- fleshed Sweet Potato flour.

3.2. Proximate Composition of Flat-Bread

As the sweet potato flour increased in the blended flat-bread the moisture contents were also found increasing (Table.2), and the reason could probably be the high water binding capacity of the starch in the sweet potato flour. The result of the current study is in line with that reported by [20]. Moreover study reported by [19] on OFSP and wheat blended bread and [21] on sorghum and OFSP blended flat-bread were also found that the substitution level of OFSP were reported to be associated with increased level of moisture content. The protein content has been observed to decrease with an increase in the amount of the OFSP blends from 8.94% for the control flat-bread to 7.57% for that with 35% OFSP blended. Similar trend was reported by [22], for cakes from sweet potato and wheat flour blends. [23, 24] concluded that the increase of the sweet potato flour decreased the protein and ash contents of baked foods made from sweet potato–wheat flour blends. As the amounts of OFSP flour in the formulation increases, the amount of fat in the flat-bread decrease. The reason may be due to the presence of high fat in maize flour (4.61%) than in OFSP flour (1.42%) as indicated by [24] for madiga (local bread in Nigeria) produced from composite flour of wheat and sweet potato. The crude fiber content of the flat-bread has increased with an increase in the level of OFSP flour from 2.08% for 100% maize and 3.51% for the 35% OFSP incorporated flat-bread. The finding of the current research is in agreement with the result reported by [19]. [22, 25] and [26] concluded that the increased sweet potato level resulted in increasing crude fiber contents of breads made of sweet potato–wheat blends. The fiber contents of food are important from nutritional point of view that facilitates the digestion and absorption process in human body systems [27]. It was observed that with an increase in the proportion of OFSP flour, the ash content of the flat-bread increased (2.05% for the control to 3.5% for the 35% OFSP incorporated flat-bread). The result is in agreement with that reported by [28]. The reason for the observed ash content with the increasing OFSP level is likely due to the fact that OFSP flour was found to be higher in ash content (5.37%) than maize flour (1.20%). This implies that incorporation of OFSP flour into the cereal flours used for flat-bread making could enhance the mineral content, as ash is indicative of the amount of minerals contained in any food sample [14]. The total carbohydrate was found to be 61.35% for the control and 55.99% for 35% OFSP added flat-breads. The result of this research is in line with that reported by [29]. It was reported that the increased level of sweet potato flour resulted in decreasing carbohydrate contents of sweet potato–wheat blended breads [19, 22, 26]. A decrease in the gross energy level was observed with an increase in the proportion of OFSP flour. The highest energy level was observed for control (343 kcal/100 g) and the lowest was for 35% OFSP flour blended flat-bread (289.65kcal/100g). Similar trend was reported by [30] where increasing the amount of sweet potato in the blends of sweet potato-soy bean in cookies resulted in decreased energy value of the final product.

Table 2. Proximate composition, gross energy and of developed Flat-bread

Maize:OFSP	Moisture	Protein	Fat	Ash	Fiber	CHO	Energy (kcal)
100:00	20.56±0.01^{d}	8.94±0.01^{a}	6.95±0.04^{a}	2.05±0.07^{d}	2.08±0.09^{c}	61.35±0.07^{a}	343±0.79^{a}
75:25	25.85±0.22^{c}	7.97±0.00^{b}	5.17±0.06^{b}	3.21±0.00^{c}	3.20±0.00^{b}	57.59±0.00^{b}	308.78±0.62^{b}
70:30	27.47±0.62^{b}	7.86±0.01^{c}	4.42±0.01^{c}	3.41±0.01^{b}	3.37±0.00^{a}	57.27±0.03^{c}	300.17±0.25^{c}
65:35	28.98±0.06^{a}	7.56±0.04^{d}	3.87±0.05^{d}	3.53±0.01^{a}	3.51±0.01^{a}	55.99±0.00^{d}	289.65±0.50^{d}
CV	1.29	0.28	0.90	1.20	1.25	0.07	0.18

Values are averages of duplicate readings (mean ± standard deviation) Where, CV= coefficient of variance, CHO=carbohydrate. Means followed by different superscript letter within the column indicate significant difference at ($P < 0.05$).

Table 3. Beta-carotene content of OFSP flour enriched flat-breads per 100 g in comparison to Pre- School children's Daily Requirement of VA content/day

Maize: OFSP	Beta-carotene (µg/100 g)	VA content (µg RAE)	% contribution to RDA	
			2-3 yrs	4-5 yrs
100:00	0.00±0.00^{d}	0.00±0.00^{d}	0.00	0.00
75:25	3283.00±28.28^{c}	277.58±3.30^{c}	69.34	61.68
70:30	3737.0±14.14^{b}	314.22±1.14^{b}	78.55	69.83
65:35	4132.0±11.31^{a}	344.33±0.94^{a}	86.08	76.52
CV	0.60	0.77		

Values are averages of triplicate measurements (mean ± standard deviation) Where, CV= coefficient of variance. Means followed by different superscript letter within the column indicate significant difference at ($P < 0.05$): 1 µg RAE=12 β-carotene [13].

3.3. Vitamin -A Contribution of the flat-bread to RDA of Pre-school Children

An increase in the vitamin A level was observed with an increase in the proportion of OFSP flour (Table.3). The vitamin A content ranged from 277.58 -344.33 µg/100 g for OFSP incorporated maize flat-bread. According to [31] the vitamin-A RDA for under-five children of 2 to 3 years of age was reported to be 400 µg/day. The vitamin-A RDA for children of 4-5 years of age was reported to be 450 µg/day. If 2-3 years old children eat the 25, 30 and 35% OFSP incorporated maize flat- bread daily, they can get 69.34, 78.55 and 86.08% of the daily RDA of vitamin-A respectively. If 4-5 years old children can eat the same flat-bread daily they can get 61.63, 69.83 and 76.52% of their daily RDA of VA respectively (Table 3). The current study was in line with the reports made by [19].

Compared to other strategies such as, supplementation and fortification, OFSP incorporation into staple foods is sustainable and affordable food based approach in rural communities in Ethiopia. Supplementation and fortification demands higher costs, good infrastructure and utilization of industrially processed products. Therefore, growing OFSP as a home garden crop is practical food based approach to alleviate VAD in the community. This is because children like OFSP incorporated foods due to the added sweetness.

3.4. Microbial Safety of the Flat-Bread

There were no statistically significant differences ($p<0.05$) in microbial load of flat-bread due to different blending proportions of maize and OFSP (Table 4). Total plate count ranged from 0.60 to 0.65 (log10 CFU/g) whereas mold and yeast count ranged from 1.03 to 1.11 (log10 CFU/g). These values are in agreement with that reported by [32]. The lower value of the finding might be due to the fact that the soaking in salt solution as a pre- treatment technique during raw material preparation which might have inhibited the microbial load as reported by [33]. It is also possible that baking killed some of the micro-organisms in the production of flat-bread [34]. The International Commission for Microbiological Specification for Foods (ICMSF) [35], suggests that ready-to-eat foods with plate count of coliforms between 0-10^{3} CFU/g are acceptable. Ready-to-eat foods with microbial loads between 10^{4} and 10^{5} CFU/g are tolerable and those with 10^{6} CFU/g and above are unacceptable. It is known that aerobic plate count values for cereal and cereal based products exceeding 10^{6} CFU/g are considered microbiologically unsafe [36]. For bread the standards in force are formed just from the number of yeasts and fungi that must not exceed 10^{2} CFU/g [37].

Table 4. Microbial Load on Flat-bread (log10 CFU/g)

Maize: OFSP	Total plate count	Mold and Yeast count
100: 00	0.65±0.05^{a}	1.11±0.00^{a}
75: 25	0.65±0.06^{a}	1.07±0.10^{a}
70: 30	0.65±0.06^{a}	1.03±0.05^{a}
65: 35	0.60±0.00^{a}	1.11±0.04^{a}
CV	9.29	5.83

Values are means of triplicates ± standard deviation, Where, CV= coefficient of variance. The means with the same letter across the column are not significantly different at 95% Confidence Interval ($P< 0.05$). All values are average of duplicates.

Table 5. Sensory Acceptability Test in the Community Mothers/Caregivers (N=30)

Maize: OFSP	Color	Appearance	Flavor	Taste	Texture	Overall acceptability
100:00	4.61 ± 0.49^a	4.63 ± 0.48^a	4.79 ± 0.41^a	4.62 ± 0.49^a	4.78 ± 0.41^a	4.65 ± 0.48^a
75: 25	4.48 ± 0.56^a	4.50 ± 0.50^a	4.71 ± 0.46^a	4.49 ± 0.56^a	4.70 ± 0.46^a	4.51 ± 0.56^a
70: 30	4.43 ± 0.62^a	4.60 ± 0.49^a	4.77 ± 0.42^a	4.44 ± 0.62^a	4.76 ± 0.42^a	4.46 ± 0.56^a
65: 35	4.60 ± 0.52^a	4.50 ± 0.62^a	4.64 ± 0.51^a	4.61 ± 0.52^a	4.63 ± 0.51^a	4.63 ± 0.51^a
Mn	4.53	4.55	4.72	4.54	4.71	4.56
CV	12.43	11.19	9.98	12.44	9.68	12.11

Values are means of triplicates ± standard deviation, Where, Mn= grand mean and CV= coefficient of variance. The means with the same letter are not significantly different at 95% Confidence Interval (P>0.05). All values are average of duplicate measures.

3.5. Sensory Acceptance of the Flat-Bread

Mothers/caregivers of children under 5 years of age and the children themselves have participated. The average age of the mothers/caregivers was 32 and that of the children was 3.3 years. The result of sensory evaluation by the mothers/caregivers showed that all OFSP incorporated flat-breads had scores less than that of the control flat- bread for all sensory attributes though the difference is not statistically significant p>0.05 (Table 5). This may be due to the fact that people had innate (natural) behaviour that they are familiar with the common products and take it as a reference. In sensory evaluation by mothers /caregivers of pre-school children all sensory attributes of OFSP incorporated flat-bread had scores greater than four, which meant the products are moderately liked. All pre-school children preferred the given OFSP incorporated flat-bread to the control. The degree of preference increased with the substitution level of OFSP flour in the formulation (Table 6). This might be due to the fact that the sweetness increased and children might have liked. The colour of the samples was yellow and the brightness increased with the increase in the proportion of OFSP flour, which could be a reason for its preference by children.

Table 6. Sensory Acceptability Test in the Community Mothers/ Caregivers (N=30)

Maize: OFSP	Number	Percent
100: 00	4	13.33
75: 25	6	20
70: 30	9	30
65: 35	11	36.67

Getting acceptance of OFSP flour enriched flat-breads by the most vulnerable groups will be the best methods to alleviate vitamin A deficiency in the study area. These results of the current study agreed with the report of [3], regarding its acceptability and preference to adults and children in the eastern African region. [38] also reported that sweet potato incorporated products were generally well accepted by young children and increase serum retinol level in Sub-Saharan-Africa. [28] also reported that a substitution of 30% of wheat flour by sweet potato flour was feasible and acceptable for baked products. The result of the current study is also in agreement with [28], which reported the acceptability of cookies with the substitution of wheat by sweet potato flour up to 40%.

4. Conclusions

The flat-bread samples developed from OFSP incorporated maize flours in the different blending proportions were acceptable and were liked by consumers. The flat-bread samples were also found to contribute 61.63 to 86.08% of the RDA. of pre-school children The microbial loads of the OFSP blended flat-bread were with-in the limit of microbiologically acceptable products.

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