Proximate, anti-nutritional, microbial and sensory acceptability of bread formulated from Wheat (*Triticum aestivum)* and Amaranth (*Amaranthus Caudatus*)

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ABSTRACT

Background: Breads are made throughout the world. Bread can be prepared from cereal like wheat, maize, rice. Nowadays gluten-intolerance, requirement of healthy and nutritious products has increased and interest's towards underutilized crops has also been increasing with the aim of improving global food security and to ease an adverse effect of climate changes. Amaranth is one of nutritionally balanced and naturally grown underutilized crop but it is mainly considered as weed in Africa including Ethiopia.

Method: The aim of the study is to develop bread from wheat and amaranthus and to evaluate proximate composition, anti-nutritional, microbial and sensory acceptability of bread. The experiment contained 100% wheat as control and four blending proportion (90% wheat and 10% amaranthus, 80% wheat and 20% amaranthus, 70% wheat and 30% amaranthus, 60% wheat and 40% amaranth). Complete randomized design is used for proximate composition, anti-nutritional and microbial data analysis whereas Randomized complete block design with three replications was applied for sensory acceptability. SAS for windows version 9 were used for data analysis.

Result: the study revealed that moisture, protein, fat, fiber and anti-nutritional content were increased as amaranthus concentration is increased from 10% to 40%. However, carbohydrate, microbial load and sensory acceptability were decreased. But the gross energy is constant.

Conclusion: from the study it can be concluded that beside the good nutritional profile of amaranthus, it has anti-nutritional content which needs to limit the concentration of amaranthus in blending with other grain during product development.

Keyword: Proximate, anti-nutreint, microbial, sensory, bread

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INTRODUCTION

Bread is popular in worldwide and it can be prepared from cereal like wheat, maize, rice. Nowadays, need's for nutritious products is increasing (Crockett, 2009). Similarly, interest's towards underutilized crops has also been increasing with the aim of improving global food and nutrition security. Amaranth is one of nutritionally balanced and naturally grown underutilized crop (Kauffman and Weber, 1990), but it is mainly considered as weed in Africa including Ethiopia. Thus engaging in Amaranth cultivation and appreciation for consumption could be valuable for reducing existed both food and nutrition insecurity in developing countries like Ethiopia.

The consumption of bread from wheat is popular. But, the limited nutritional profile of wheat is an alarm to think for other cereal which is good in nutritional profile so as to compliment it with wheat in bread production (Gopalan *et al.*, 2007). According to (Ikram *et al*., 2010) amaranthus has carbohydrate (48–69%), protein (12–

18%) and fat (5–8%). It has also high concentration of limiting amino acids like lysine (0.747g) and tryptophan (0.181g) (Teutonico and Knorr, 1985) with numerous benefits. Beside all this, it is also relatively good in sulfur-containing amino acids which are limited in the pulse crops at the normal circumstance high amount of iron, zinc and calcium (Bressani *et al*., 1987).

Amaranthus is known in Ethiopia specifically in south and south west part but limited concern has been given to the crop. However, in some area it is using to prepare local beverage known as 'Chaqa', porridge, pancake-like bread (injera), bread, borde, *kitta* (unleavened bread) and atmit. Though, bread from wheat and amaranthus could good for good nutritional profile of bread as it provide energy, vitamins and minerals.

Material and Methods

Sources and preparation of materials

The raw materials for preparation of bread were wheat and amaranthus. Wheat was obtained from Hawassa local market and Amaranthus was obtained from Gamo zone (Arbaminch). The grains were sorted and extraneous material was removed then washed, cleaned and sun dried. Both the dried whole wheat and amaranthus was later milled using cyclone mill and sieved in to fine flour of uniform particle size by passing them through 0.5mm mesh screen.

Preparation of wheat flour

The extraneous matter was removed from wheat and then the grain was washed, cleaned using tap water, drained, sundried and milled using a cyclone mill to pass through a 0.5mm mesh screen so as to get the flour. The milled grain was then packed by polyethylene bag and finally stored at room temperature.

Preparation of Amaranthus flour

The amaranthus grain was cleaned from extraneous matter and soaked in steam water for 12hrs with 1:3w/v concentration to ensure effective removal of anti-nutrient (Lorenz, 1984). The initial temperature of steam was around 70^{o}C held for 10minutes and the water was changed at six hours interval. The amaranthus grains was sundried and milled using cyclone mill (Tecator AB, Haganas, Sweden) to pass through a 0.5mm mesh screen and filled in polyethylene bags. After getting the flours of amaranthus and wheat, it was mixed according to the formulation (Table-1).

Table3-1: Formulation of wheat and amaranthus flour.

Composite flour	C	C1	C2	C3	C4
wheat flour	100%	90%	80%	70%	60%
Amaranth flour	_	10%	20%	30%	40%

Where C is control (100% wheat), C1 is 90% wheat and 10% amaranthus, C2 is 80% wheat and 20% amaranthus, C3 is 70% wheat and 30% amaranthus, C4 is 60% wheat and 40% amaranthsu

Preparation of bread

Preparation of dough for bread was done by mixing 1% iodized salt, composite flour, yeast and water. After mixing all ingredients composite flour was kneaded until it become soft, smooth, stiff and stayed for two and half hour for rising (fermentation). Pre-heated local clay griddle (*Mitad*) was used for baking and leaf of enset *(Ensete ventircosum)* was used for wrapping the dough to be baked. The baking was continued until a brown color developed (which will take about 25 minutes at 150^{o}c). The bread which was prepared was stayed at room temperature to cool down, wrapped using polyethylene bags.

Chemical composition analysis

The proximate composition of bread was determined according to (AOAC, 1998). The moisture content was determined using official method 934.01; ash content was determined using official method 923.03, crude fat content was determined using official method of 920.39; crude protein was determined using official method of 981.10, crude fiber was determined by AOAC, 2000 and total carbohydrate was determined by difference method. Condensed Tannin and phytate contents were determined by using the method used by (Norhaizan &

Faizadatul, 2009). The phytate content was calculated by dividing the measured value of phytic acid by molecular weight (240) of phytic acid.

Microbial Analysis of bread

Total mold, yeast and bacteria count were carried out on bread samples after 2 day room temperature storage using the procedure of (AOAC, 1984). Bread samples was taken aseptically and homogenized in 99 ml sterile peptone water 0.1% in a blender for about 2 minutes and serial dilutions were made. Dilution of 0.1 ml was spread plated in sterile Petri dishes, the stomacher dilution represent 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilution were prepared by using 9 ml peptone water tubes and plate count agar (PCA) with chloroanphinicol addition and incubated at 25 °C for 5 days for mold and yeast count and molten 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.

Sensory Evaluation of Breads

The bread samples were coded with three digit numbers and randomly the samples were given to randomly presented panelists in random order. The sensory evaluation was carried out using a five point hedonic scale (1= dislike very much, 2= dislike, 3= neither like nor dislike, 4= like, 5= like very much) in terms of color, taste, aroma, texture and overall acceptability with 20 panelists in triplicate.

Experimental design

Treatments with blending at different proportions of wheat and amaranth (90:10, 80:20 and 70:30, 60:40) and 100% wheat (control) were used to asses' chemical composition, microbial load and sensory acceptability. Complete randomized design was used for chemical composition and microbial load analysis whereas Randomized complete block design (RCBD) was used for sensory acceptability analysis.

Data Analysis

One way analysis of variance using SAS software version 9 was used for data analysis. The means separation was done using Tukey's HSD test at p<0.05.

Result and Discussion

Table 4-1: Effect of blending ratio on proximate composition of wheat-amaranths bread

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate	Energy
C	7.18±0.02^{b}	8.17±0.13^{b}	3.95±0.01^{c}	1.36±0.00^{d}	1.86±0.01^{c}	77.48±0.14^{a}	378.15±0.38^{a}
C1	7.25±0.03^{b}	8.23±0.35^{b}	4.12±0.29^{b}	1.35±0.01^{d}	1.95±0.01^{c}	77.10±0.24^{a}	378.46±0.29^{a}
C2	7.67±0.14^{a}	9.34±0.34^{ab}	4.42±0.08^{ab}	1.46±0.27^{c}	2.16±0.27^{b}	74.95±0.18^{b}	376.94±0.51^{a}
C3	7.86±0.11^{a}	9.83±0.13^{a}	4.66±0.01^{ab}	1.78±0.28^{b}	2.78±0.28^{a}	73.09±0.28^{c}	376.62±0.42^{a}
C4	7.71±0.02^{a}	9.96±0.12^{a}	4.94±0.02^{a}	1.99±0.02^{a}	2.99±0.02^{a}	73.41±0.15^{c}	377.94±0.35^{a}

Where C is control (100% wheat), C1 is 90% wheat and 10% amaranthus, C2 is 80% wheat and 20% amaranthus, C3 is 70% wheat and 30% amaranthus, C4 is 60% wheat and 40% amaranthsu. Means followed by different superscript letter across the column indicate significant difference at (P < 0.05).

Proximate compositions

The moisture content of bread is varied from 7.18 to 7.71. Bread made from 20%, 30% and 40% of amaranthus had higher (p<0.05) moisture content as compared to control and 10% amaranthus blended bread. The study revealed that the moisture content was increased as amaranthus concentration is increased. The higher in moisture content of bread made from higher amaranthus concentration is might be due to high water absorption capacity of amaranthus as reported by (Ferreira, 1999).

The protein content of bread is varied from 8.17 to 9.96. Bread made from 30% and 40% amaranthus had higher (p<0.05) protein content as compared to bread made from 20%, 10% amaranthus and control (100% wheat). The higher protein content is from bread made from 40% of amaranthus and as amaranthus concentration increases the protein content is increased. The higher in protein content is might be due to amaranthus has high protein content as compared to wheat (Nascimento *et al.,* 2014).

The fat content is varied from 3.95 to 4.94. Bread made from 10%, 20%, 30% and 40% amaranthus had higher ($p<0.05$) fat content as compared to control (10% wheat). The study showed, as amaranthus concentration increases from 10% to 40% the fat content was increased. The higher in fat content as amarnthus increased is because amaratnhus has higher nutritional profile as compared to wheat and other cereal as reported by (Bassore & Desalegn, 2017).

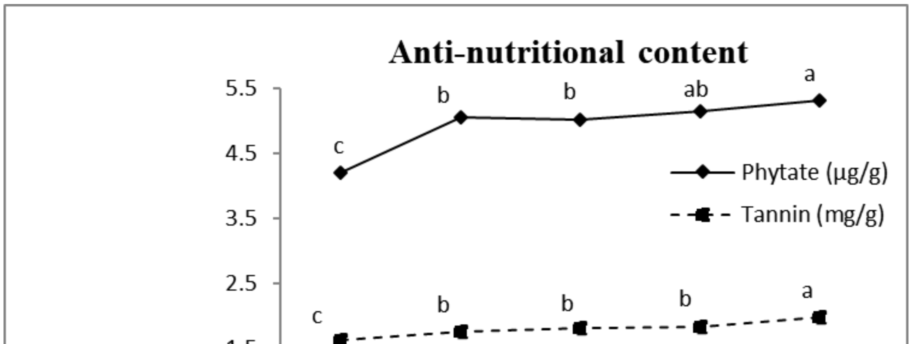
The ash content of bread is varied from 1.36 to 1.99. Bread made from 20%, 30% and 40% of amaranthus had higher ($p<0.05$) ash content as compared to bread made from 10% amaranthus and control (100% wheat). The higher in ash content is might be due to amaranthus has higher minerals content than wheat.

The fiber content is varied from 1.86 to 2.99. The fiber content is slightly increased as amaranthus concentration is increased from 10% to 40%. However, Bread made from 30% and 40% amaranthus had significantly higher ($p<0.05$) fiber content as compared to control (100% wheat), 10% and 20%. The higher in fiber content as amarnthus increased is because amaratnhus has good nutritional profile and higher fiber content as it is very fine cereal as compared to wheat (Nascimento *et al.,* 2014).

The carbohydrate is varied from 77.48 to 73.41. The carbohydrate is slightly decreased as amaranthus concentration is increased from 10% to 40%. The lower in carbohydrate as amarnthus increased is because the higher in moisture, protein, fat, ash and fiber.

The gross energy is varied from 378.15 to 377.94. The energy is insignificantly decreased ($p>0.05$). The lower in gross energy is might be due the lower in carbohydrate as amaranthus concentration is increased.

Anti-nutritional content of wheat- amaranths based bread



	C	C1	C2	C3	C4
Phytate (µg/g)	4.19	5.05	5.02	5.14	5.31
Tannin (mg/g)	1.63	1.76	1.82	1.84	1.98

Figure 1: Effect of blending ratio on anti-nutritional content of wheat- amaranths based bread

Anti-Nutritional content

The anti-nutritional (phytate and tannin) content of bread is varied from 4.19 to 5.31 and 1.63 to 1.98 respectively. Bread made from 30% and 40% of amaranthus had similar ($p>0.05$) phytate content and similarly bread made from 10%, 20% and 30% of amaranthus had also similar ($p>0.05$) phytate content but, they had higher ($p<0.05$) phytate content as compared to control (100% wheat). Bread made from 10%, 20% and 30% of amaranthus had similar ($p>0.05$) tannin content, but they had higher ($p<0.05$) tannin content compared to control (100% wheat) and lower ($p<0.05$) tannin content as compared to bread made from 40% of amaranthus.

In general the study revealed that the anti-nutritional content of bread is increased as amaranthus content is increased from 10% to 40%. The higher in phytate and tannin content as amaranthus concentration increased is because as reported by (Bassore & Desalegn, 2017) amaranthus had high anti-nutritional content.

Total plate count, mold and yeast of wheat –amaranths based bread

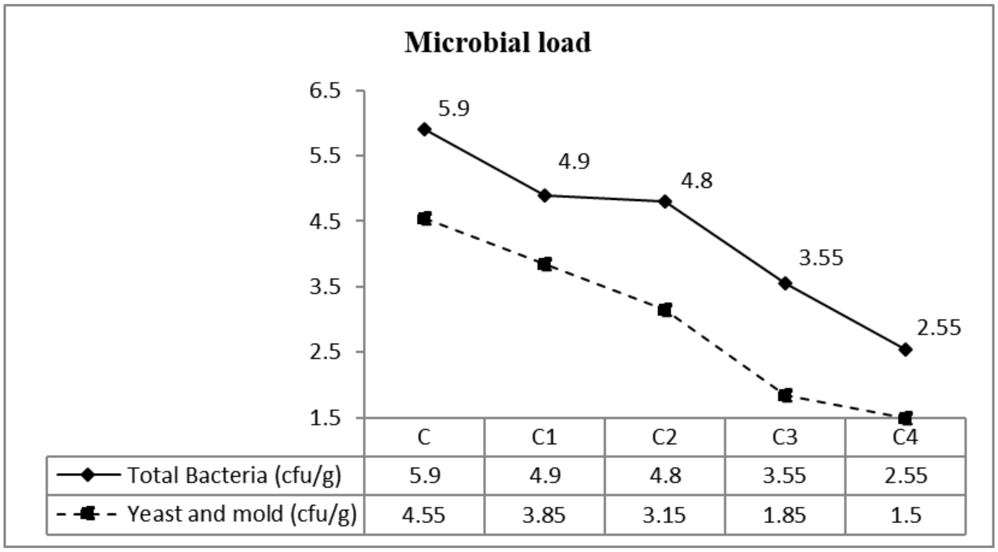


Figure 2: Effect of blending ratio on microbial load (total plate count and mold, yeast) of wheat – amaranths bread

Microbial

The microbial load of bread was shown in (Figure 2). Total bacteria of bread were varied from 5.9cfu/g to 2.55. Bread made from 40% of amaranthus and 60% of wheat had lower total bacteria as compared to control (100% wheat) and the study indicates that as amaranthus concentration increase the total bacteria count was decreased significantly ($p<0.05$). The lower in total bacteria as increasing amaranthus is might be due to amarnthus is soaked in steam water for 24hrs, this indicate there were lower microbial growth for steam water products as steam keeps down the microbial growth. Generally, when comparing with microbiological standards of blended foods, total bacteria count has 10^3 to 10^5 cfu/g was still within acceptable value. It was known that total plate count values for cereal and legume based products exceeding 10^6 CFU/g are considered microbiologically unsafe (Olaoye et al., 2006). From this investigation none of the treatment was exceed over 10^6 CFU/g.

Both yeasts and mold count was shown in (Figure 2). The mean value for yeast and mold of bread was varied from 4.55cfu/g to 1.55cfu/g. Bread made from 40% of amaranthus and 60% of wheat had lower yeast and mold as compared to control (100% wheat, 90%:10, 80%:20, 70%:30) and the study indicated as amaranthus concentration increase mold and yeast count was decreased significantly ($p<0.05$). The lower in mold and yeast as increasing amaranthus is might also be due to amarnthus is soaked in steam water for 24hrs, this indicate there were lower microbial growth for steam water products as steam keeps down the microbial growth.

Table 4-4: Effect of blending ratio on sensory acceptability of wheat–amaranths bread

Treatment	Color	Taste	Aroma	Texture	Overall acceptability
C	4.64 ± 0.48^{a}	4.50 ± 0.55^{a}	4.45 ± 0.50^{a}	4.52 ± 0.50^{a}	4.61 ± 0.49^{a}
C1	4.11 ± 0.59^{b}	4.07 ± 0.46^{b}	3.88 ± 0.70^{b}	3.83 ± 0.85^{b}	4.07 ± 0.60^{b}
C2	3.66 ± 0.52^{c}	3.45 ± 0.50^{c}	3.61 ± 0.62^{bc}	2.92 ± 0.51^{c}	3.78 ± 0.56^{c}
C3	3.19 ± 0.55^{d}	3.35 ± 0.48^{cd}	3.48 ± 0.62^{c}	2.71 ± 0.55^{cd}	3.40 ± 0.54^{d}
C4	3.16 ± 0.53^{d}	3.14 ± 0.35^{d}	3.28 ± 0.70^{d}	2.68 ± 0.47^{cd}	3.40 ± 0.49^{d}

Where C is control (100% wheat), C1 is 90% wheat and 10% amaranthus, C2 is 80% wheat and 20% amaranthus, C3 is 70% wheat and 30% amaranthus, C4 is 60% wheat and 40% amaranthsu. Means followed by different superscript letter across the column indicate significant difference at ($P < 0.05$).

The color, taste, aroma, texture and overall acceptability of bread were varied from 3.16 to 4.64, 3.14 to 4.5, 3.28 to 4.45, 2.68 to 4.52 and 3.4 to 4.61 respectively. Bread made from 100% wheat had higher sensory acceptability as compared to bread made from wheat substituted with (10%, 20%, 30% and 40% amaranthus). The study showed, the color, taste, aroma, texture and overall acceptability was decreased significantly ($p<0.05$) as amaranthus concentration is increased. The lower in sensory acceptability is might be due to amaranthus is dark red in color and not well adapted by consumer as people are adapted white color of bread. According to Lorenz (1981), amaranth supplemented product has got nutty flavor which is not acceptable by the panel in terms of taste and aroma as the concentration increased. The lower in texture is might be due to the water absorption capacity of amaranth. According to (Martha and Shimelis, 2012) the water absorption can be increased as amaranthus concentration is increased in injera production with *teff*.

Conclusion

In conclusion proximate composition (moisture, protein, fat, ash, fiber) and anti-nutritional (phytate and tannin content) of bread were become high toward the increment of amaranthus. However, carbohydrate, gross energy and microbial load (total bacteria, mold and yeast count) were lowered as amaranths concentration increased and treatments were within acceptable range of microbial load below 10^6 CFU/g. From the study in all treatment as the amaranths concentration increase the color, taste, aroma, texture and overall acceptance were decreased. Considering the result obtained the amaranths substitution up to 40% of soaking had lower acceptability. However, it is evident that all treatment is within acceptable sensory characteristics.

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