Enhancement of Nutritional Value of Kocho Using Fish Protein Powder

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Abstract

This work was aimed to improve the nutritional value of a traditional flat bread from Kocho through incorporation of fish protein powder (FPP) made from small Barbus. Four types of Kocho bread were prepared with inclusion of 0, 5, 10 and 15% FPP in Kocho flour and standard methods were used to evaluate the nutritional and sensorial quality of the resulting flat bread. From the results obtained in this study, it was concluded that up to 5% FPP can be incorporated in Kocho to obtain nutritionally enhanced Kocho flat bread without significant change in the sensorial attributes. At 5% FPP incorporation level, protein, lipid and ash content of the breads could be enhanced by 95%, 21% and 95%, respectively. However, further research should be carried out to understand the nutritional benefit to be obtained and on the shelf life of the breads. Study on the potential use of FPP from different species should also be explored.

Introduction

Ensetventricosum is the edible species of the separate genus of the banana family, thus named ‘false banana’. Variation within the species to altitude, soil and climate has allowed widespread cultivation in the mid- to highlands of western Arsi-Bale, the Southern Peoples Nations Nationalities Regional State (SPNNRS), and western Oromia including West Shewa, Jima, Ilubabor and Welega. Three *Enset* derived foods are popular but the most common one is Kocho. The plant is cut before flowering, the pseudo stem and leaf midribs are scraped, the pulp is fermented for 10-15 days and finally steam-baked flatbread is prepared.

Enset is a simple basic starch crop, quite low in protein, not very adaptable to varying food dishes but very high in productivity. As many as 15 million people consume the low-protein *Enset* products as staple or co-staple foods. Sometimes solely with Vitamin A foods but commonly without the needed protein supplement (Harrison J. *et al*., 2014).

The implications of heavy dependence on these "poor nutrition" crops may have serious implications on the physical and mental health of the people of the "*Enset* Culture" (Grantham-McGregor, S. 1995).Therefore, the need for investigating ways of increasing the protein content of *Enset*based foodneeds empirical investigation.

FPP, extracted from Small *Barbus,* is one of the most vital sources of animal protein and it has been widely accepted as a source of quality protein and other nutrients for the maintenance of healthy body (Ravichandran et al., 2012). They are rich in essential amino acids, are a good source of complex B vitamins, and contain a wide variety of minerals including phosphorus, magnesium, iron, zinc, and even iodine in marine fish (Arino et al., 2013).

Nutritional value studies have shown the remarkable beneficial effect of adding FPP to the diet. Twelve grams, less than 15 ml, of FPP a day will supply the needed protein to a child; a small FPP plant processing 50 tons of raw fish a day could provide enough FPP for three quarters of a million children (Windsor, 2001). However, in some areas of the world where large sections of the population suffer from malnutrition, it may still be more logical to develop orthodox fish industries to meet shortage of protein, and to confine FPP to emergency use. The FPP is a valuable protein supplement to improve the protein quality and quantity of indigenous diets, particularly the diets of pre-school children and other vulnerable groups. It is used to help increase the weight and height of children (Frokjaer1994; Owusu-Amoako2001; Sen (2005). Therefore, this research was aimed to investigate the suitability of incorporating FPP made from small Barbusin *kocho*to enhance nutritional value of *kocho.*

Materials and Methods

Material preparation

Seventy-two live Small *Barbus*species (*BarbusPaludinosus*) with mean 10.8g weight and 10.0cm length (Figure 1a) were collected from Lake Ziwayand transported to NFALRC laboratory. Then, the fish were gutted, washed thoroughly and stored in deep freezer until extraction of FPP.*Kocho* was purchased from open market in Sebeta town and was stored in a laboratory until use.

Extraction of FPP

FPP was extracted according to Sikorski and Naczk (1981) (Figures 1b and 1c). The whole fish was ground and then the protein was extracted using isopropanol. After grinding, the supernatant was collected and extracted three times. The first extraction was carried out at 25°C for 50 min in isopropanol. The second extraction was carried out at 85°C for 90 min with isopropanol. The third extraction was carried out at 80°C for 70 min with azeotropicisopropanol. Then the final supernatantfraction was collected, dried, milled and screened to separate out bone particles.

Small*Barbus*species fish FPP Extraction process FPP

Figure 1. The small Barbusspecies and FPP extraction process

Preparation of *Kocho (*flat bread)

The *Kocho* (flat bread) was prepared following the traditional preparation technique described inYirmaga (2013). *Kocho* flour was replaced by FPP flour at 0%, 5%, 10% and 15% and the blending was conducted efficiently to ensure uniform distribution of FPP flour in all the treatments (Figure 2). Then the baking process was carried out following the traditional *Kocho*backing process in a uniformly heated stove.

Figure 2 Blended FPP and Kocho flours and dough formed for baking flat bread

Determination of proximate composition

Proximate composition analysis for homogenized samples wascarried out in triplicate for protein, moisture, lipid and ash contents.Moisture content of the sample was measured using oven drying method as described in AOAC (2000). According to AOAC (2005), Crude protein was determined by the Kjeldahl method and to obtain the crude protein content the result was multiplied with the conversion factor 6.25.

Fat content was determined after extracting the total lipid by diethyl ether solvent system as stated in Folch et al., 1957. 5 g of the sample was weighed extracted into a 250-ml round-bottom flask with 50 ml diethyl ether for about four hours at 110°C in the soxhlet extractor. Then after, the organic solvent was removed at 40-60°C under reduced pressure using a rotary evaporator. Finally, the weight of fat was calculated by:

$$Weight\ of\ fat = (weight\ of\ container + weight\ of\ extracted\ fat) - (weight\ of\ the\ container)$$

$$Fat\ content\ (\%) = \frac{mass\ of\ fat\ extracted\ (g)}{weight\ of\ original\ sample\ (g)} \times 100\%$$

Ash content was determined through after ashing the samples in a muffle furnace at 550 °C for one hour as described in AOAC (2000).

Sensory evaluation

The four formulated *Kocho*were evaluated for their sensory acceptability (color, flavor, taste, texture and overall acceptability) using five point hedonic scale where 1=dislike extremely, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, 5= like extremely. Twenty untrained panelists were used for the evaluation.

Determination of functional properties and quality criteria

Water / Oil absorption capacity

One gram of sample was weighed into a clean conical graduated centrifuge tube and was mixed thoroughly with 10 ml distilled water/oil using a warring mixer for 30 seconds. The sample was then allowed to stand for 30 minutes at room temperature, after which it was centrifuged at 5000 rpm for 30 minutes. After centrifugation, the volume of the free water (supernatant) or oil was read directly from the graduated centrifuge tube. The absorbed water was converted to weight (in grams) by multiplying by the density of oil (0.894 g/ml) and water (1 g/ml). The oil and water absorption capacities were expressed in grams of oil/water absorbed per gram of flour sample Carcea and Benecini (1986).

$$Asorbed\ water/oil = Total\ water/oil - Free\ water/oil$$

Bulk density

The gravimetric method was used. A weighed sample (10 g) was put in a calibrated 10 ml measuring cylinder. Then the bottom of the cylinder was tapped repeatedly onto a firm pad on a laboratory bench until a constant volume was observed. The packed volume was recorded. The bulk density was calculated as the ratio of the sample weight to the volume occupied by the sample after tapping Onwuka (2005).

$$Bulk\ density\ (^{g}/_{ml}) = \frac{Weight\ of\ sample\ (g)}{Volume\ of\ sample\ (ml)})$$

Swelling index

This was determined as the ratio of the swollen volume to the ordinary volume of a unit weight of the flour. The method followed by Abbey and Ibeh (1988) was used. One gram of the sample was weighed into a clean dry measuring cylinder. The volume occupied by the sample was recorded before 5ml of distilled water was added to the sample. This was left to stand undisturbed for an hour, after which the volume was observed and recorded again. The index of swelling ability of the sample was given by the formula below

$$Swelling\ index = \frac{Volume\ occupied\ by\ sample\ after\ swelling}{Volume\ occupied\ by\ sample\ before\ swelling}$$

Determination of total volatile basic-nitrogen (TVB-N)

Fish extracts for determination of Total Volatile Bases Nitrogen (TVB-N) was prepared by homogenizing 100 g of fish sample with 200ml ml of 7.5% (w/v) aqueous Trichloroacetic Acid (TCA) solution in a laboratory homogenizer for 1 min at high speed. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant liquid will be then filtered through what man No. 1 filter paper. Then, TVB-N was measuredby steam-distillation of the TCA-fish extract using the method of Malle and Tao (1987). Then, 25ml of the filtrate was added to a Kjeldahl-type distillation tube, followed by 5 ml of 10% (w/v) aqueous NaOH solution. Then steam-distillation was performed using a vertical steam distillation unit and the distillate was received into a beaker containing 15 ml of 4% (w/v) aqueous boric acid and 0.04 ml of methyl red and bromocresol green indicator solution up to a final volume of 50 ml. The titration was allowed to run against aqueous 0.05 M sulfuric acid solution.

$$\text{TVB-N mg/100g} = \frac{4mg/mol \times A \times B \times 300}{25ml}$$

Where; A = ml of sulphuric acid and B = normality of sulphuric acid.

Water activity (a_w)

Water activity was measured by portable water activity meter. The sensor head was calibrated for 3 hours by putting special paper on sample cup and moistened with the saturated barium chloride solution. After11/2 hours calibration, it was adjusted to 0.90. Then the sample was put for 3 hours in a disposable cup, completely covering the bottom of the cup. After 3 hours, the result was recorded (Bassal *et al*., 1992)

Statistical analysis

Statistical data wasanalyzed using one-way analysis of variance (ANOVA) and the significant differences between means weredetermined by post hoc Duncan’s multiple range test. Differences were considered to be significant when $p < 0.05$.

Results and Discussion

As Table 1 illustrates *Kocho* had highmoisture content (51.52%) because it is a pest (dough), while the moisture content of the FPPwas 3.07 %. The protein content of the FPPwas 72.38% and that of *Kocho* was 1.97%. On the other hand, FPP had higher ash value (24.37%) than *Kocho* (0.87%) while the lipid content of *Kocho* and FPP was 1.59% and 1.9%, respectively. As expected the protein content of *Kocho*was too low and fortification with FPP may enhance the flat bread.The protein content of FPP in this study was comparable to other fish species (Shaviklo, 2015). Yimerga (2013) found a higher protein content of Kocho than the present study. This might be due to the use of different varieties of *Enset* in the two studies.

Table 1. Proximate composition of *Kocho* and FPP (%)

Parameter	*Kocho*	FPP
Moisture content	51.52 ± 0.24	3.071 ±0.06
Crude ash	0.868 ± 0.18	24.375 ± 0.14
Crude protein	1.968 ± 0.74	72.38 ± 0.85
Crude Lipid	1.59 ± 0.09	1.9 ± 0.14

The values of functional properties and quality criteria of FPP are depicted in Table 2 and all of them were within acceptable range of FAO.

Table 2: Functional properties and quality criteria of FPP

Parameter	Values
Water holding capacity(ml/gm)	3.34
Oil holding capacity (ml/gm)	1.43
Bulk density (gm/ml)	0.54
Swelling index	1.56
TVB-N (mg/100gm)	19.5
Water Activity (a_w)	0.55
Extract yield (%)	18.7

Effect of FPP inclusion on *Kocho* based flat bread proximate composition is depicted in Table 3. Except at the 15% FPP incorporation, the moisture content of the flat bread did not much vary with the bread from 100 % *Kocho* (check). As anticipated the protein, lipid and ash contents of the flat breads apparently increased with inclusion FPP and the three parameters showed linearly increasing trend with increasing FPP incorporation level. Similar findings showed that a 3 % inclusion of FPP in wheat flour increased its protein content from 10.4% to 12.4% (Venugopal, 2006). Compared to the bread from 100% Kocho, inclusion of 5% FPP increased the protein, lipid and ash content of the resulting breads by 95%, 21% and 95%, respectively. This may indicate that, a small amount of FPP supplement could enhance the nutritional value of *Kocho* and showed the potential for addressing malnutrition.

Table 3: Proximate composition of the composite flat breads

Treatment	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
100% kocho (Check)	62.97±0.04	3.22 ± 1.31	1.04± 0.47	0.75 ± 0.09
5% Substitution	62.90±0.50	6.28 ± 0.81	1.26 ± 1.10	1.46± 0.09
10% Substitution	62.90± 0.07	8.83 ± 0.62	1.32 ± 0.76	2.05 ± 0.49
15% Substitution	59.20 ± 0.66	9.356 ± 0.71	1.42 ± 0.60	2.55 ± 0.08

Results of the sensorial analysis done on the breads are showed in Table 4 and Figure 1.

Table 4: Sensory analysis of treatments

Treatment	Color	Texture	Flavor	Taste	Overall acceptability
100% kocho (Check)	3.50±1.35	3.48±1.19	3.74±1.17	3.10±0.69	3.76±1.08
5% Substitution	3.45±1.06	3.19±1.09	3.36±1.27	3.05±0.49	3.71±0.84
10% Substitution	2.90±1.19	2.98±1.20	2.93±1.18	2.90±0.73	3.14±1.07
15% Substitution	2.57±0.97	2.67±1.00	2.52±0.97	2.14±0.75	2.79±0.95

The 15% inclusion were found to be within acceptable level. Inclusion of 5% FPP gave flat bread with apparently closer sensorial quality to 100% Kocho flat bread than higher doses of FBB. Color, taste and overall acceptability of the 5% FPP incorporated flat breads were closer to the check. However, with increasing FPP levels (to 10% and 15%) the sensorial acceptability of the breads tended to decrease visibly.Similarly 5 % inclusion of FPP was the best level of fortification of biscuits in Nigeria (Ibrahim, 2009).

Conclusion and Recommendation

Although no previous research proved the use of FPP in enhancing the nutritional value of *Kocho,* this activity managed to evaluate the potential of FPP for such purpose. From the results obtained in this study, it was concluded that acceptable FPP incorporated *Kocho* flat bread could be made at 5% FPP inclusion. At this incorporation level, protein, lipid and ash content of the breads could be enhanced by 95%, 21% and 95%, respectively. However, further research should be carried out to understand the nutritional benefit to be obtained and on the shelf life of the breads. Study on the potential use of FPP from different species should also be explored.

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