Physiochemical, Nutritional and Sensory Quality of Bread Produced From Wheat, Croaker and Tilapia Fish Composite Flour

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Abstract

The main objective of this work was to evaluate the physiochemical, nutritional and sensory properties of bread produced from wheat, croaker and tilapia fish composite flours. Croaker and tilapia fish were dried and milled into flour. Wheat-croaker and wheat-tilapia fish flour were respectively blended in the ratio of 100:0, 95:5, 90:10, 85:15 and 80:20, and labelled sample 95:5CF, 90:10CF, 85:15CF and 80:20CF for wheat: croaker blends and samples 95:5TF, 90:10TF, 85:15TF and 80:20TF for wheat:tilapia blends. !00% wheat flour (100WF) was used as control. Bread was baked with corresponding samples and labelled accordingly. Bulk density of the composite flour increased (0.87 – 0.92g/cm^3), while swelling power and oil absorption capacity decreased from 10.60 – 8.67 and 2.74 – 1.47, respectively with increased substitution of fish flour. The Ash, fat, protein and crude fiber content of bread produced from these composite flours also increased with increased substitution of fish flour. Energy value increased, with higher energy values of 277.66 kcal/100g and 274.54 kcal/100g, respectively, seen in bread substituted with 20 % tilapia and croaker fish flour. Loaf volume and specific volume decreased with substitution of fish flour in the composite blend. However, loaf weight increased significantly from 167.31 – 187.09g. Sample 80:20TF gave significantly higher calcium and phosphorus values of 63.21 and 4.49ng/100g, respectively. Percentage invitro protein digestibility of the composite bread was significantly high when substitution with 10, 15 and 20 % tilapia fish flour, given values of 19.17, 18.84 and 19.41kcal/100g, respectively. There was no significant difference in the taste, aroma and texture scores of the wheat and composite flour bread. Bread produced from the composite flour all received equal acceptability with 100 % wheat flour bread.

Keywords: *Physicochemical, Nutritional, Sensory, Croaker, Tilapia Fish, Bread.*

1. Introduction

Bread is a staple food prepared from a dough of flour and water usually by baking. It is a good source of good nutrient such as macronutrients (carbohydrates and protein) and micronutrients (minerals and vitamins) that are essential for human health. Its origin dates to the Neolithic era and is still one of the consumed and acceptable staple food products in all parts of the world.

The word bread is used to describe the whole range of different varieties of bread which may vary in weight, shape, crust hardness, crumb cell structure, softness, colour and eating quality. There are many combination and proportion of types of flour and other ingredients and different traditional recipe and modes of preparation of bread. Bread may leaven by different processes ranging from the use of naturally occurring microbes (sourdough recipe) to high pressure artificial aeration method during preparation or baking. However, some products are left unleavened either for preference or for traditional or religions reasons.

Bread is highly nutritious eaten in one form or another by nearly every person on earth. An excellent source at vitamins, protein and carbohydrates. bread has been an essential element of human diets for centuries in all regions (Ryan 2005). Bread is a solid foam; a typical bread has the crust with characteristic golden-brown color and white crumbs. Bread has a short life due to its chemical composition and moisture content compared to other baked products.

Nutritionally bread contains high percentage of carbohydrate and fat both of which are needed for energy production and source of calories. Other nutrients like vitamins, mineral and protein are relatively in small proportion. The problems of malnutrition in Nigeria although different in magnitude and severity among different areas are due to protein, vitamins iron and other mineral deficiency (Adebooye 1996), Many Nigeria consume bread without any nutrient supplement like butter, geisha etc suggesting the problem or malnutrition. Therefore, there is need to enrich or fortify bread in order to improve its nutritional value. Food fortification is the addition of one or more essential nutrients to food weathers or not it is normally contained in the food for the purpose of preventing or correcting a demonstrated deficiency with one or more nutrient in the population or specific population group (Brekkan, 1995)

A nutrient or substance is considered an appropriate fortifier when and only the nutrient is stable in the food under normal conditions at storage, distribution and use. Fortification has become a means of ensuring nutritional adequacy of the diet. Examples of appropriate food fortifiers include soy flour, fish protein concentrate which are rich in protein. In Nigeria, bread has become the second most widely consumed non-indigenous food products after rice. It is consumed extensively in most homes, restaurants and hotels. It has been hitherto produced from wheat as a major raw material. In Nigeria, wheat production is limited, and wheat flour is imported to meet the local flour needs for baking products. Thus, huge amount of foreign exchange is used every year for the importation of wheat. Effort has been made to promote the use of composite flour in which flour from locally grown gaps replace a portion of wheat flour for use in bread, thereby decreasing the demand for imported wheat and helping in producing bread.

Composite flour is a mixture of wheat with other materials replacing substantially a portion of wheat flour to form suitable flour for baking purposes. The idea of composite flour began by Food and Agricultural organization (FAO) in 1960s to reduce the important dependency of developing countries. (Onyeku *et al;* 2008, Sibel, 2006; Owuamanam, 2007). There is now a substantial quantity of composite bread in the market, such bread requires at least 70% wheat flour to be able to rise because wheat contains gluten.

Wheat in conventional flour, rich in gluten, which makes a better preference for bread baking. It is however expensive because it is not grown in Nigeria due to the unfavourable climatic condition and a huge foreign exchange (Igbabu Biana Dooshima, 2014). The unbridled importation of food by developing countries is detrimental to their local economy and threatens food security. Many developing countries spend large proportion of their foreign exchange earnings on food especially wheat. It is therefore, of economic importance if wheat importation is reduced by the substitution with other locally available raw materials (Onyeku *et al*; 2008) such as cassava, maize, potato and other carbohydrate flour. The objective of this work was thus to produce and evaluate the physicochemical, nutritional and sensory characteristics of bread from wheat, croaker and tilapia fish composite flour.

2. Materials and Methods

Wheat flour, Croaker fish and Tilapia fish were purchased from Mile 1 market Port Harcourt, Rivers State. The analytical grade reagents used for analysis were products of the British Drug Houses (BDH) England and were of analytical grade.

2.1 Preparation of Croaker and Tilapia Fish flour

The fish were sorted and graded, washed thoroughly with clean water to remove all extraneous matters. Fish fillet was removed with a sharp knife, then sliced into 0.5 cm difference to decrease drying period. Then dried at 50^{0}C for 3 days with a conventional air oven, using the procedure of (Zebib *et al.*, 2020**)**. The dried sample was grounded to pass through 60 mesh screen (0.25 mm) and were stored in an air-tight polyethylene plastic bag for further use as shown in the figure 1 wheat flour was sieved through 60 mesh screen (0.25 mm) and packed.

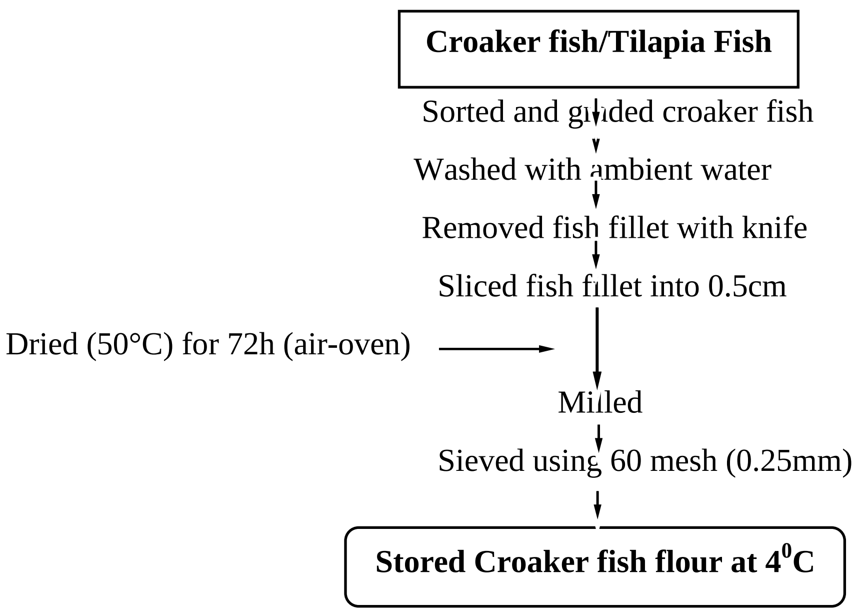

Figure 1 Flow chart for the production of Croaker and Tilapia fish flour (Zebib *et al.*, 2020)

2.2 Sample formulation

The wheat and fish flour blends are shown in Table 1.

Table 1 **Formulation Table for Wheat/Tilapia Fish Flour Blend**

Sample	Wheat flour (%)	Tilapia flour (%)	Croaker Fish Flour (%)
100 WF	100	0	

95:5 TF	95	5	
90:10 TF	90	10	
85:15TF	85	15	
80:20TF	80	20	
95:5CF	95		5
90:10 CF	90		10
85:15 CF	85		15
80:20 CF	80		20

2.3 Production of Bread

The bread was prepared using the method described by Mudau, M. *et al*., (2021). All ingredients were weighed; white wheat flour + fish flour (100g), sugar 6g, salt (2g), yeast (2g), fat (4g) and water (50g) using analytical balance. The ingredients were mixed thoroughly, kneaded, shaped and dropped into baking pans and allowed to proof for 2h at 35 °C, baked in an electric oven at 180°C for about 20 min.

2.4 Proximate Composition

Percentage moisture, Ash, fat, protein and crude fiber were determined using AOAC (2012) standard method, while Carbohydrate content was determined by difference; % available carbohydrate = 100 – (%moisture + % Ash + % Fat + % crude protein + % crude fibre). Energy value (kcal per 100 g) was estimated using the Atwater conversion factor (Oyet and Chibor, 2020). Energy (kcal per 100 g) = [9 × Lipids% + 4 × Proteins% + 4 × Carbohydrates%].

2.5 Functional Properties

Functional properties of the wheat/fish composite flour; bulk density, swelling index, water absorption capacity and oil absorption capacity were determined according to the standard procedures;

2.5.1 Bulk Density

The method of Akpapunam and Markakis (1981) was used. A 10 ml-graduated cylinder was gently filled to mark with the sample. The filled cylinder was gently tapped on a laboratory bench about 10 times until there was no further diminution of the sample level after filling to the 10 ml mark. The procedure was adopted for each of the sample and the bulk density was calculated using the formula:

$$\text{Bulk density (g/ml)} = \frac{\text{packed weight of sample}}{\text{volume of material after tapping}}$$

2.5.2 Swelling Index

Method described by Kusumayanti (2015) was used. 3g (dry basis) of each flour were transferred into clean, dry graduated (50ml) cylinders. Flour samples were gently levelled into it and the volumes noted. Distilled water (30ml) was added to each sample; the cylinder was swirled and allowed to stand for 60 minutes while the change in volume (swelling) was recorded every 15 minutes. The samples were centrifuged at 1600 rpm for 15 minutes. The precipitated part was weighed. The swelling power of each flour sample was calculated as

$$\text{Solubility (\%)} = \frac{\text{Weight of soluble matter x 100}}{\text{Sample weight}}$$

Swelling power (%) = $\frac{\text{Weight of swelling sediment x 100}}{\text{Sample weight}}$

2.5.3 Water/Oil Absorption Capacity

The method described by Elkhalifa *et al*. (2005) was used to determine the water/oil absorption capacity of the flour samples. Five millilitres of water/oil were added to 1.0 g of the sample in a centrifuge tube. The mixture was sonicated for 1 min to disperse the sample and the suspension was allowed to stand for 30 min. The suspension was then centrifuged after standing at 3500 rpm for 30 min and the water absorbed was calculated using the formula:

Water/oil absorbed (ml/g) = $\frac{a-b}{a}$

where a = initial volume of water
b = final volume of water

2.6 Mineral Content

Mineral analysis was done by dry ashing according to procedure 14.013 of AOAC (2012). Muffle furnace (Model SKL, China) at temperature of 550 °C was used for ashing. After sample preparation, total mineral determination was done using Atomic Absorption spectrophotometer (AAS) (Hitachi Z-5300, polarized Zeaman, Hitachi Ltd; Japan). The light source was Hollow cathode lamp of each element, using acetylene and air combinations, with air pressure of 0.3 Mpa, and air flow rate of 6.5 L/min, acetylene pressure of 0.09 Mpa and a flow rate of 1.7 L/min was used. Other operating conditions such as wavelength and lamp current are given for each element as follows: Ca = 422.7 nm and 2 mA, Fe = 248.3 nm and 2 mA. Phosphorous was determined by molybdenum blue method and the absorbance read at 700 nm using a spectrophotometer UV-visible (CELiL model CE2021 U.K)

2.7 In Vitro Protein Digestibility

The in-vitro protein digestibility of cookie samples was determined using the procedure of Mertz *et al*. (1984) and modified by Monsour and Yusuf (2002). Sample was homogenized and 200mg of cookie was weighed into a flask and suspended in 15ml of 0.1M HCl containing 1mg of porcein pepsin and incubated at 37oC for 3 hr. The pepsin hydrolyzed suspension was then neutralized with 0.5M NaOH and incubated with 6mg of pancreatin in 7.5ml of phosphate buffer (pH 8.0) for 24hr at 37^{o}C. After the incubation, the sample was treated with 15ml of 10% TCA. The mixture was filtered through Whatman No 1 filter paper. The TCA soluble fraction was assayed for nitrogen estimation using micro Kjedahl method. A blank sample was also determined. The protein digestibility was calculated by the following formula:

$$\text{Protein Digestibility \%} = \frac{\text{Nitrogen in Supernatant} - \text{Nitrogen in Blank}}{\text{Nitrogen in Sample}} \times 100$$

2.8 Physical characteristics of the bread samples

loaf volume was determined using Rape seed displacement method (AACC, 2000, Standard 10-05), done by loading millet grains into an empty calibrated box to the marked level and unloading. The bread sample was then placed in the box and the measured millet loaded again. The volume of the leftover grains from the box was taken, using a measuring cylinder, and recorded as the loaf volume in cm^3. Loaf weight was measured with the laboratory weighing balance. Specific volume was calculated by dividing the loaf volume measured by the loaf weight of the bread sample in grams (cm3/g).

2.9 Sensory Evaluation

Sensory evaluation was performed on the bread using the method of Iwe (2007). The bread samples were evaluated by 20 selected Semi-trained panellists from the Department of Food Science and Technology, Rivers State University, Port Harcourt. A 9point Hedonic scale was used. Evaluation was on most preferred quality attribute for each treatment levels with respect to colour, taste, aroma, texture and overall acceptability.

2.10. Statistical Analysis

All the analyses were carried out in triplicate. Data obtained were subjected to Analysis of variance (ANOVA), differences between means were evaluated using Tukey's multiple comparison test. Significance accepted at p< 0.05 level. The statistical package in MINITAB 20 computer program was used.

3. Results and Discussion

3.1 Functional Properties of Wheat, Tilapia and Croaker Fish Composite Flour

Bulk density was seen (Table 2) ranging from 0.87 - 0.92 g/cm^3 with higher value shown in samples 80:20CF, 85:15CF and 85:15TF. Bulk density is a measure of the heaviness of a flour sample (Oladele and Aina, 2007). The bulk density of flour used to determine its packaging requirements. It is depending on the particle size and moisture content of flours. Bulk density of composite flour increased with an increase in the incorporation of different flours with wheat flour (Hasmadi *et al*., 2022). Bulk density of the composite flour samples increased as the percentage substitution with fish flour increased. Flour substitution with croaker flour gave higher bulk density. Bulk density is a reflection of the load the sample can carry if allowed to rest directly on one another. Bulk density of flour substituted with 20 % Croaker fish flour was significantly (p<0.05) higher with value of 0.92, followed by samples 85:15CF, 90:10CF and 85:15TF, with values of 0.91, 0.90 and 0.90, respectively. The lower the bulk density value, the higher the amount of four particles that can stay together and thus increasing the energy content that could be derivable from such diets (Ikpeme-Emmanuel *et al*., 2009).
Swelling power of the wheat and composite flour samples ranged from 8.67 – 10.87. Swelling power of flour substituted with 5 % Croaker fish flour was higher. While lower swelling power was seen in sample 80:20TF. The swollen power decreased with increasing level of walnut flour substitution. Adegunwa *et al*, (2014) reported that swollen power of flour granules is an indication of the extent of associative forces inside the granule. The swollen capacity of flour depends on size of particles, types, variety and preparation methods (Igbadul *et al*, 2014). The variation in the swollen capacity of flour is a sign of the extend of associated forces within the granule, this indicates the degree of exposure of the internal structure of the starch present in the flour due to surplus of moisture (Suresh *et al*, 2015). The result agrees with the study carried out by Adegunwa *et al* (2014) who stated that swollen capacity can be related to the water absorption index of the starch-based flour during heating. However, the swelling power of the wheat/fish flour samples were not significantly different (p>0.05) from that of the control (100% wheat flour). There was no significant difference in the percentage solubility of the flour samples.

Water and oil absorption capacity ranged from 0.77 -0.79 and 1.47 - 1.98, respectively. Water Absorption Capacity (WAC) is the ability of flour to absorb water and swell for improved consistency in food. Niba *et al*. (2001) described water absorption capacity as an important processing parameter that has an implication for viscosity. Furthermore, water absorption capacity is important in bulking and consistency of products (Akusu *et al*., 2019a). WAC is desirable in food systems to improve yield and stability and give body to food (Offia-Olua, 2014). There was no significant difference (p>0.05) in the water absorption capacity of the wheat and composite flour samples. This showed that all the samples have a degree of WAC which is important in bulking and consistency of products as well as in baking applications (Giami, 1993, Iwe *et al* 2016).

Oil absorption capacity of 100 % wheat flour (sample WF) was significantly (p<0.05) higher, with value of 2.74. Oil absorption capacity is the taste absorbent capacity of flour which is very vital in food formulations as oil improves the taste and mouth feel of foods (Doke and Guha, 2015; Odoemelam, 2003). It is important because it contribute to the organoleptic properties (aroma, appearance, smoothness, creaminess, taste) of food products. Oil absorption capacity has been attributed to the physical entrapment of oil. It is an indication of the rate at which the protein binds to fat in food formulations (Hasmadi *et al*., 2020). Oil absorption capability required in most food applications, such as in bakery products, wherein required in flavour retention and improvement of palatability (Abu *et al*., 2005). Food ingredients with high oil absorption capacities are therefore suitable as functional properties in food products such as cookies, doughnut etcetera (Borja *et al*, 2013). Flours with lower oil absorption capacity have higher flavour retaining abilities (Ajatta, Akintola and Osundahunsi 2016). The ability of protein in flour to bind with oil makes it useful in food system where optimum oil absorption is desired. Oil absorption properties could be used as functional ingredient in food such as pastry, bread and cakes (Chandra and Samsher, 2013)

Table 2. Functional Properties of Wheat, Croaker and Tilapia Fish Flour Blends

Sample	Bulk Density	Swelling Power	% Solubility	Water Abs.	Oil Abs.
100 WF	$0.87^{f} \pm 0.01$	$10.60^{ab} \pm 0.21$	$17.55^{a} \pm 0.09$	$0.78^{a} \pm 0.00$	$2.74^{a} \pm 0.05$
95:5CF	$0.87^{ef} \pm 0.00$	$10.87^{a} \pm 0.07$	$20.73^{a} \pm 0.25$	$0.78^{a} \pm 0.00$	$1.99^{b} \pm 0.00$
90:10 CF	$0.90^{bcd} \pm 0.00$	$9.89^{ab} \pm 0.47$	$19.64^{a} \pm 0.28$	$0.77^{a} \pm 0.00$	$1.99^{b} \pm 0.00$
85:15 CF	$0.91^{ab} \pm 0.01$	$9.32^{ab} \pm 0.05$	$21.55^{a} \pm 2.35$	$0.78^{a} \pm 0.02$	$1.59^{c} \pm 0.01$
80:20 CF	$0.92^{a} \pm 0.00$	$9.48^{ab} \pm 0.67$	$20.72^{a} \pm 0.96$	$0.79^{a} \pm 0.00$	$1.59^{c} \pm 0.00$
95:5 TF	$0.88^{def} \pm 0.00$	$10.36^{ab} \pm 0.33$	$19.03^{a} \pm 1.03$	$0.78^{a} \pm 0.00$	$1.98^{b} \pm 0.00$
90.10 TF	$0.89^{cde} \pm 0.01$	$9.84^{ab} \pm 0.58$	$20.12^{a} \pm 1.32$	$77.00^{a} \pm 0.04$	$1.98^{b} \pm 0.02$
85:15TF	$0.90^{abc} \pm 0.00$	$9.88^{ab} \pm 0.93$	$21.65^{a} \pm 0.14$	$0.78^{a} \pm 0.01$	$1.55^{c} \pm 0.07$
80:20TF	$0.89^{cd} \pm 0.00$	$8.67^{b} \pm 0.40$	$20.26^{a} \pm 1.26$	$0.79^{a} \pm 0.00$	$1.47^{c} \pm 0.00$

Values are mean ± standard deviation of triplicate samples

Mean values bearing different superscripts in the same column differ significantly (p<0.05)

3.2 Proximate Composition of Bread Produced from Wheat, Tilapia and Croaker Fish Composite Flour

Result for proximate composition of bread produced from wheat, croaker and tilapia fish composite flour (Table 3), showed percentage moisture ranging from 31.02 - 38.07 %. The moisture range was lower than 42.98 – 48.29 % reported by Cercel *et al*. (2016) for bread produced from wheat-fish protein concentrate composite flour, but corroborated with 36.06 – 42.79 % moisture reported by Monteiro *et al*. (2018) for bread produced from wheat-tilapia fish composite flour. The relatively high moisture content observed for composite bread samples might be due to the incorporation of croaker and tilapia fish flour. Fish is high in water content; equally, protein has high water holding capacity. Lower moisture of 9.22 -10.16 %, 11.91% and 25 – 29.00 % were also reported by Zebib *et al*. (2020) for wheat -labeobarbus fish flour, Mirjane *et al*. (2014) for wheat-fish flour bread, and Adeleka and Odedeji (2021) for wheat tilapia bread, respectively. Idolo (2011) reported a higher moisture of 28.00% for bread produced with 100% wheat flour. Different in moisture content is probably due to different in baking temperature and time (Kiin-Kabari *et al*., 2020). Moisture content of bread produced from wheat flour substituted with 10 % tilapia fish flour was higher, this value however was not significantly ($p>0.05$) difference from the moisture content of bread from samples 85:15CF, 85:15TF and 80:20TF. Significantly lower moisture content was seen in bread produced with 100 % wheat flour and samples 95:5TF with values of 31.02, 31.52 and 31.50 % respectively. Low moisture content is a better indicator of product potential to have longer shelf life (Adelakun *et al* 2018). The higher the moisture contents of food the lower the shelf life stability (Ajatta *et al* 2016).

Ash content ranged from 1.15 - 2.29 %. These values corroborated with results of Cercel et al. (2016), Zebib et al. (2020) and Monteiro et al. (2018) for wheat-fish composite bread, but lower than 3.52 % reported by Adeleka and Odedeji (2010). The ash content of bread produced from 85:15 wheat/ croaker fish flour (sample 85:15CF) was higher, with value of 2.29 %. This value was however not significantly different ($p>0.05$) from those of samples 90:10CF, 95:5TF and 90:10TF. There was no significant difference in ash content of the control (bread baked with 100% wheat flour) and those of other flour blends except sample 80:20TF, which was significantly lower ($p<0.05$) with value of 1.15 %.

Fat content of the bread samples ranged from 2.36 - 8.54 %. Fat content of breed substituted with 20 % tilapia and croaker fish flour (samples 80:20TF and 80:20CF) were significantly higher with values of 8.54 and 7.41%, respectively. Fat content of the composite bread was shown to increase as the percentage substitution of croaker and tilapia fish flour increased. This trend corroborated with the reports of earlier researchers (Adeleka and Odedeji, 2010; Zebib *et al*., 2020; Monteiro *et al*., 2018). There was no significant difference ($p>0.05$) in the fat content of the control (100% wheat flour bread) and those of samples 95:5CF, 90:10CF, 85:15CF, 95:5TF and 85:15TF. Substitution with fish powder enhanced the fat content of the bread samples. Fish oil are reported to be rich in omega-3 and omega-6 fatty acids (Meht *et al*., 2015). Research has shown that omega-3-riched fish powder can reduce the oxidation of lipids while exposed to high temperatures, such as during baking (Serna-Saldivar and Abril, 2011).

Protein content ranged from 8.63 - 14.85 % with samples 80:20CF and 80:20TF given significantly ($p<0.05$) higher values of 14.85 and 14.35 %, respectively. Protein content of the bread samples increased as the percentage substitution of fish flour increased. Protein range in this study corroborated with reports of earlier researchers (Adeleka and Odedeji, 2010; Zebib *et al*., 2020; Monteiro *et al*., 2018). Fish is an essential source of high-quality protein (Ohen and

Abang, 2007), providing about 16% of animal protein consumed by the world's population (Adegoke *et al.*, 2020).

Similar protein range (10.20–14.20%) and lipid values (2.90–5.30%) was reported by Bastos *et al*. (2014) for bread from wheat-fish residue flour. From earlier study, the protein content of bread samples with the addition of chicken meat and chicken meat powder were increased from 7.60% to 18.44% and 18.70% for white bread, and from 8.85% to 14.23% and 16.49% for whole wheat bread, respectively (Cakmak *et al.*, 2013). Proteins serves as antibodies, they serve as primary sources of amino acids, the building block of cellular protein. There are several beneficial health effects of fish protein such as reduced obesity, decreased oxidative stress of adipose tissue, decreased tumor necrosis factor, controlled type 2 diabetes, improved resolution of inflammation and lowering the cardiovascular risk (Tilami and Sampels, 2018). The high protein content of fish powder may also have an effect in reducing the glycemic index of foods and this in turn may have a potential beneficial health effect with regard to weight control and obesity (Berrios *et al*., 2017). Low glycemic index foods can be achieved through the utilization of protein and lipid rich ingredients combined with cereal gains in products such as bread (Desai *et al*., 2018).

No significant difference ($p<0.05$) was seen in the crude fiber content of the bread sample. Crude fiber through its water absorption capacity aid peristalsis movement of food through the digestive tract. Fiber is known to be essential for effective gastro-intestine functions and in the treatment and prevention of many diseases and gastro intestinal disorder (Nkama, *et al*., 2000).

Carbohydrate content of the wheat and composite bread samples ranged from 35.85-52.52 %, with 100% wheat bread given significantly ($p<0.05$) higher value of 52.52 %. Carbohydrate content decreased with increase substitution of fish flour. This trend was also presented by Zebib *et al*. (2020), who reported decrease in total carbohydrate of bread from 72.02 % to 64.40 % on substitution of wheat flour with fish flour. This might be due to the effect of starch dilution through the incorporation of the fish flour.

Table 3 Proximate Composition of Bread Produced from Wheat, Tilapia and Croaker Fish Composite Flour

Sample	% Mc	% Ash	% Fat	% C. Protein	% C. Fiber	% CHO
100 WF	$31.02^{d} \pm 0.02$	$1.93^{b} \pm 0.05$	$4.52^{b} \pm 0.02$	$8.63^{f} \pm 0.00$	$1.76^{a} \pm 0.00$	$52.52^{a} \pm 0.60$
95:5CF	$31.52^{d} \pm 0.13$	$1.89^{b} \pm 0.06$	$4.78^{b} \pm 1.04$	$9.30^{e} \pm 0.06$	$2.27^{a} \pm 0.35$	$50.26^{ab} \pm 1.19$
90:10 CF	$34.29^{c} \pm 0.04$	$2.04^{ab} \pm 0.00$	$4.44^{b} \pm 0.55$	$11.30^{c} \pm 0.00$	$3.05^{f} \pm 0.39$	$44.88^{c} \pm 0.19$
85:15 CF	$36.18^{abc} \pm 0.01$	$2.29^{ab} \pm 0.14$	$3.29^{bc} \pm 0.25$	$13.62^{b} \pm 0.00$	$2.06^{a} \pm 0.81$	$42.57^{cd} \pm 0.42$
80:20 CF	$35.33^{bc} \pm 0.10$	$2.03^{b} \pm 0.02$	$7.41^{a} \pm 0.34$	$14.85^{a} \pm 0.00$	$3.28^{a} \pm 0.59$	$37.11^{e} \pm 0.88$
95:5 TF	$31.50^{d} \pm 1.11$	$2.09^{ab} \pm 0.06$	$3.30^{bc} \pm 0.55$	$10.34^{d} \pm 0.00$	$3.69^{a} \pm 0.00$	$49.09^{b} \pm 0.62$
90.10 TF	$38.07^{a} \pm 0.54$	$2.06^{ab} \pm 0.06$	$2.36^{e} \pm 0.00$	$9.91^{e} \pm 0.00$	$3.07^{a} \pm 0.00$	$44.53^{cd} \pm 0.47$
85:15TF	$37.47^{ab} \pm 0.33$	$1.98^{b} \pm 0.01$	$4.07^{bc} \pm 0.00$	$11.25^{c} \pm 0.00$	$4.07^{a} \pm 1.01$	$41.17^{d} \pm 1.36$

80:20TF	37. 06^{ab} ± 0.26	1.15^{c} ± 0.01	8.54^{a} ± 0.23	14.35^{a} ± 0.00	3.07^{a} ± 1.08	35.85^{e} ± 1.12

Values are mean ± standard deviation of triplicate samples
Mean values bearing different superscripts in the same column differ significantly (p<0.05

3.3 Energy Value of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Energy value was estimated from the contributions of protein, fat and carbohydrate, taking into account the digestibility of each and their heat of combustion (Giami *et al*., 1999). The number of kilocalories (often termed "calories") needed per unit of a person's body weight expresses energy needs (Lawrence et al., 2005). The energy value of bread baked with 100 % wheat flour (control) was significantly higher (p<0.05) with value of 285.28kcal/100g, as shown in Fig. 2. This value was closely followed by those of samples 95:5CF, 80:20TF and 80:20CF, with energy values of 281.26, 277.66 and 274.53kcal/100g, respectively. Decrease in energy value of bread due to substitution of wheat flour with defatted fluted pumpkin seed flour was earlier reported by Kiin-Kabari *et al*. (2020). According to US Nutritional Recommendation (values per 100 g) of white bread consumption for Adults, it is given that it should provide 270 kcal (1110 kJ), 8 g protein, 3 fats, 51 g carbohydrate (Elleuch *et al*., 2011). Relatively higher energy values of 274.53 and 277.66 kcal/100g shown in samples 80:20CF and 80:20TF, respectively were probably due to higher fat and protein content of the substituted croaker and tilapia fish. Akusu *et al*. (2019$_b$) earlier reported increase in energy value of cookies due to increase fat content. A similar result has also been obtained previously by researchers fortifying bread with cumin and caraway flour (Sayed *et al*., 2018). High energy food is desired especially in famine and war-torn locations were the next meal is not easy to come by Ndife *et al*. (2014). As noted by Wardlaw (2004), high-energy foods tend to have a protective effect in the optimal utilization of other nutrients.

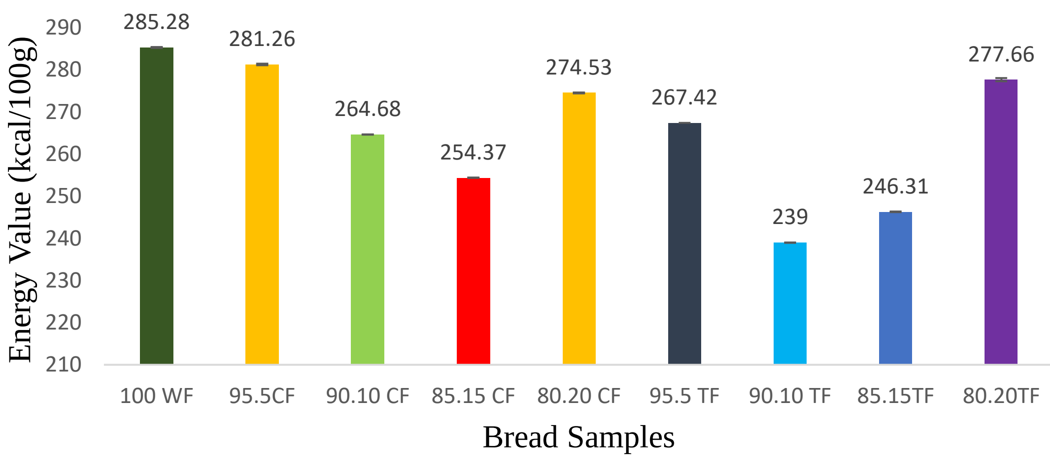

Figure 2 Energy Value of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

3.4 Physical Properties of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

The physical properties in terms of volume, weight and specific volume of bread baked with wheat, croaker and tilapia fish composite flours, as presented in Table 4 showed loaf volume, specific volume and loaf weight ranging from 367.77 - 649.92 cm^3, 2.02-3.88cm^3/g and 167.31 - 187.09g, respectively. The volume of bread baked with 100 % wheat flour and sample 95:5CF were significantly ($p<0.05$) higher, with values of 649.92 and 605.31cm^3, respectively. Shittu *et al*. (2007) reported that loaf weight is affected basically by the quantity of dough baked and amount of moisture and carbon dioxide diffused out of the loaf during baking. The weight of bread baked with 85:15 wheat-tilapia fish composite flour was significantly ($p<0.05$) higher with value of 187.09g. Espinosa-Ramírez *et al*. (2018) reported that high bread volume is related to better retention of carbon dioxide during proofing. Specific volume of bread baked with 100 % wheat flour was significantly ($p<0.05$) higher (3.88cm^3/g), followed by samples 95:5CF and 95:5TF, with values of 3.34 and 3.45 cm^3/g, respectively. The specific volume of the loaves agreed with SV range of 2.82 to 4.14 cm^3 /g reported earlier by Seyer and Gélinas (2009) for 100 % wheat flour loaves, 2.29 to 4.45 ml/g reported by Iwe *et al*. (2014) for cassava starch/wheat flour bread and 2.26 to 4.94 cm^3 /g also reported by Iwe *et al*. (2017) for high quality cassava flour/wheat flour bread. Specific volume of bread indicates the development of bread dough after baking. Higher specific volume indicates more inflated bread mixture after baking (Wahyono *et al*., 2019). Significantly ($p<0.05$) higher weight (187.09g) was observed in loaf baked with 85% wheat and 15% tilapia fish flour. Weight of bread baked with 100 % wheat flour was significantly lower ($p<0.05$) with value of 167.31g.

Table 4 Physical Properties of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Sample	Volume	Specific Volume	Weight
100 WF	$649.92^{a} \pm 8.21$	$3.88^{a} \pm 0.02$	$167.31^{g} \pm 0.46$
95:5CF	$605.31^{ab} \pm 5.21$	$3.34^{b} \pm 0.03$	$181.36^{d} \pm 0.00$
90:10 CF	$489.56^{d} \pm 8.07$	$2.75^{cd} \pm 0.04$	$177.95^{e} \pm 0.00$
85:15 CF	$481.55^{d} \pm 13.84$	$2.63^{de} \pm 0.07$	$183.03^{b} \pm 0.04$
80:20 CF	$367.77^{e} \pm 5.55$	$2.02^{f} \pm 0.03$	$182.35^{c} \pm 0.00$
95:5 TF	$589.92^{b} \pm 10.96$	$3.45^{b} \pm 0.06$	$171.11^{f} \pm 0.00$
90:10 TF	$543.75^{c} \pm 14.55$	$2.98^{e} \pm 0.07$	$182.47^{bc} \pm 0.14$
85:15TF	$466.48^{d} \pm 0.00$	$2.49^{e} \pm 0.00$	$187.09^{a} \pm 0.00$
80:20TF	$445.43^{d} \pm 21.96$	$2.50^{e} \pm 0.12$	$178.34^{e} \pm 0.14$

Values are mean ± standard deviation of triplicate samples
Mean values bearing different superscripts in the same column differ significantly ($p<0.05$)

3.5 Mineral Content (mg/100g) of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

From the result in Table 5, Calcium and Iron content ranged from 3.35 - 63.21mg/100g and 0.66 - 0.97mg/100g, respectively. Significantly ($p<0.05$) higher calcium value of 63.21 mg/100g was sample 80:20TF (bread baked with 20 % substitution of tilapia fish flour). There was no significant difference ($p<0.05$) in the iron content of all the bread samples. Calcium content increased with increase substitution of fish flour. Calcium content also corroborated with 14 – 35 mg/100g reported by Bostos *et al*. (2014) for wheat-fish flour bread. Calcium is a mineral involved in a large number of vital functions, such as bone health and blood pressure regulation (WHO, 2004; Ross *et al*., 2011). An adequate dietary calcium intake has been associated not only with the prevention of hypertensive disorders of pregnancy and blood pressure reduction but also with low-density lipoprotein (LDL) cholesterol levels and prevention of osteoporosis and colorectal adenomas (Heaney, 2006; Omotayo *et al*., 2018; Onakpoye *et al*., 2011). It also protects against bile-induced mucosal damage and experimental bowel carcinogenesis (Pence, 1993). Iron content was higher than 0.54 – 0.64 mg/100g reported by Bostos *et a*. (2014). Zinc and phosphorus content ranged from 2.04 - 10.16 mg/100g and 3.01 - 4.49 mg/100g, respectively. Significant differences ($p<0.05$) were seen in the zinc content of the bread samples, with the control (100% wheat bread) given significantly ($p<0.05$) higher value of 10.16 mg/100g. The body depends on a regular zinc supply provided by the daily diets and improvement of zinc in the diet as obtained in the formulated complementary foods may help decrease the prevalence of stunting, as linear growth was reported (FAO/WHO, 2001) to be affected by zinc supply. Phosphorus content of the bread samples were significantly different ($p<0.05$). Phosphorus content of sample 80:20TF and 85:15TF were significantly ($p<0.05$) higher, with values of 4.49 and 4.30 mg/100g, respectively. These values were however, not significantly different ($p>0.05$) from 4.14 mg/100g phosphorus seen in sample 80:20CF. Phosphorus content increased significantly ($p<0.05$) as the percentage substitution of fish flour increased, more with tilapia fish powder. Phosphorus is needed for bone and teeth health (Mona *et al*., 2013).

Table 5 Mineral Content (mg/100g) of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Sample	Ca	Fe	Zn	P
100 WF	$3.35^{i} \pm 0.00$	$0.67^{a} \pm 0.00$	$10.16^{a} \pm 0.01$	$3.23^{cd} \pm 0.14$
95:5CF	$32.37^{h} \pm 0.14$	$0.90^{a} \pm 0.14$	$2.20^{fg} \pm 0.00$	$3.25^{cd} \pm 0.14$
90:10 CF	$36.45^{f} \pm 0.01$	$0.85^{a} \pm 0.07$	$2.37^{ef} \pm 0.02$	$3.33^{cd} \pm 0.00$
85:15 CF	$42.96^{e} \pm 0.00$	$0.87^{a} \pm 0.00$	$2.81^{C} \pm 0.00$	$3.81^{b} \pm 0.04$
80:20 CF	$50.47^{c} \pm 0.02$	$0.97^{a} \pm 0.00$	$3.19^{b} \pm 0.00$	$4.14^{ab} \pm 0.00$
95:5 TF	$34.41^{a} \pm 0.02$	$0.76^{a} \pm 0.00$	$2.04^{g} \pm 0.00$	$3.35^{c} \pm 0.00$
90:10 TF	$44.98^{d} \pm 0.00$	$0.74^{a} \pm 0.14$	$2.40^{f} \pm 0.14$	$3.01^{d} \pm 0.00$
85:15TF	$54.56^{b} \pm 1.41$	$0.66^{a} \pm 0.00$	$2.50^{de} \pm 0.00$	$4.30^{a} \pm 0.14$
80:20TF	$63.21^{a} \pm 0.14$	$0.82^{a} \pm 0.37$	$2.65^{cd} \pm 0.02$	$4.49^{a} \pm 0.05$

Values are mean ± standard deviation of triplicate samples
Mean values bearing different superscripts in the same column differ significantly ($p<0.05$)

3.6 Invitro Protein Digestibility of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Protein digestibility is important in evaluating the nutritive quality of a food protein. It is a primary determinant of the availability of amino acids in food (Hassan, 2011). The percentage invitro protein digestibility (IVPD) of bread produced from 90:10, 85:15 and 80:20 wheat/ tilapia fish flour blends were significantly ($p<0.05$) higher, with values of 19.17, 18.84 and 19.41%, respectively. Followed by sample 85:15CF with percentage IVPD of 13.36 (Fig. 3). This result indicates that increasing tilapia powder content in composite bread increased the IVPD significantly. Fifteen percent substitution of croaker fish powder also increased the percentage IVPD to 18.36 %. This result corroborated with the report of Desai *et al*. (2018) for increased percentage IVPD of bread enriched with salmon fish flour. Among the indices that are used to evaluate the nutritional value of foods, IVPD is considered to be a global predictor of the protein quality (Lorusso *et al*., 2017). Similarly, wheat bread enriched with faba bean and wheat bread enriched with legume lupin significantly increased the percentage IVPD (Villarino *et al*., 2015; Coda *et al*., 2017).

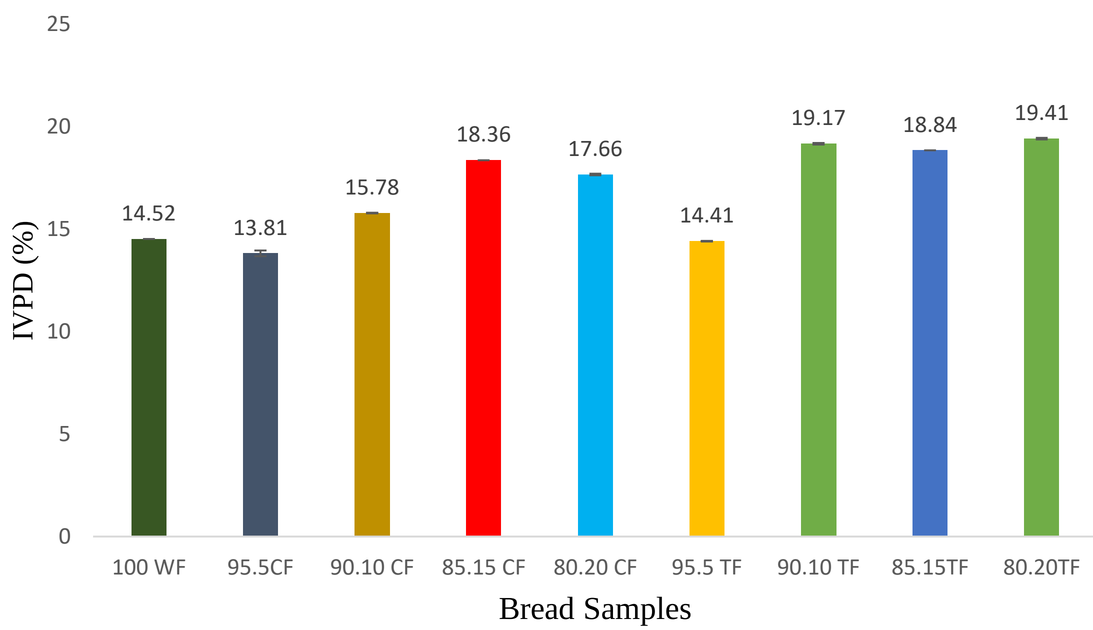

Figure 3 Invitro Protein Digestibility of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

3.7 Sensory Values of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Result for the sensory properties of bread produced from wheat, croaker and tilapia fish flour blends is presented in Table 6. Colour, taste and aroma scores are shown ranging from 5.15 - 7.58, 5.95 - 7.32 and 6.00 - 7.21, respectively. Colour scores for samples 90:10CF was high with value of 7.58. This score was however not significantly different ($p>0.05$) from those of sample 95:5CF, 100WF (control) and 85:15CF. The perception of sensory attributes, such as aroma, taste and flavour are affected by colour and appearance as reported by Hutching (1999). There was no significant difference ($p>0.05$) in the taste scores for all the bread samples, there was also no significant difference in the aroma scores for the bread samples. Sensory scores for textures and overall acceptability ranged from 6.20 - 7.21 and 6.00 - 7.53, respectively. There was no significant difference (p>0.05) in the texture scores for the bread samples. Zebib *et al*. (2020) observed burnt colour in the surface of bread when the proportion of fish flour increased from 15% to 20%, creates due to easily damaged tissue found in fish during baking process. Adeleke and Odedeji (2010) also good sensory acceptability for wheat bread fortified with tilapia fish flour T 5 and 20 % level of substitution. Cercel *et al*. (2016) also reported good sensory rating for bread produced from wheat substituted with fish protein concentrate. Enhanced Sensory characteristics of breads substituted with chicken meat and chicken meat powder had earlier been reported (Cakmak *et al*., 2013).

Table 6 Sensory Values of Bread Produced from Wheat, Croaker and Tilapia fish Flour Blends

Sample	Colour	Taste	Aroma	Texture	Overall Acceptability
100 WF	$7.58^{a} \pm 1.21$	$7.32^{a} \pm 1.33$	$7.21^{a} \pm 1.35$	$7.21^{a} \pm 1.65$	$7.53^{a} \pm 1.01$
95:5CF	$6.90^{abc} \pm 1.44$	$7.16^{a} \pm 1.46$	$6.52^{a} \pm 2.01$	$7.05^{a} \pm 1.07$	$6.91^{ab} \pm 1.23$
90:10 CF	$6.32^{abcd} \pm 1.70$	$6.68^{a} \pm 1.60$	$6.68^{a} \pm 1.56$	$6.42^{a} \pm 1.64$	$6.53^{ab} \pm 1.36$
85:15 CF	$6.47^{abcd} \pm 0.84$	$6.53^{a} \pm 1.77$	$6.26^{a} \pm 2.18$	$6.05^{a} \pm 1.92$	$6.33^{ab} \pm 1.26$
80:20 CF	$5.42^{cd} \pm 1.53$	$6.47^{a} \pm 2.09$	$6.53^{a} \pm 1.83$	$6.26^{a} \pm 1.93$	$6.14^{ab} \pm 1.45$
95:5 TF	$5.90^{bcd} \pm 1.89$	$6.30^{a} \pm 1.49$	$6.00^{a} \pm 1.62$	$6.35^{f} \pm 1.69$	$6.78^{ab} \pm 1.16$
90:10 TF	$7.20^{b} \pm 1.15$	$6.95^{a} \pm 1.43$	$6.45^{a} \pm 1.63$	$6.50^{a} \pm 1.79$	$6.00^{b} \pm 1.26$
85:15TF	$5.80^{bcd} \pm 1.43$	$5.95^{a} \pm 1.95$	$6.05^{a} \pm 2.30$	$6.20^{a} \pm 1.76$	$6.36^{ab} \pm 1.47$
80:20TF	$5.15^{d} \pm 1.75$	$6.80^{a} \pm 1.98$	$6.70^{a} \pm 1.65$	$6.50^{a} \pm 1.64$	$6.29^{ab} \pm 1.53$

Values are mean ± standard deviation of triplicate samples
Mean values bearing different superscripts in the same column differ significantly (p<0.05)

4 Conclusion and Recommendations

The study was focused on evaluating the physiochemical, nutritional and sensory properties of bread produced from the blends of wheat, croaker and tilapia fish flours.
The results showed that Ash, fat, protein and crude fiber content of the wheat, croaker and tilapia composite flour increased with increase substitution of croaker and tilapia fish flour. Significantly higher protein (24.35 %) was obtained when substituted with 20 % tilapia fish flour, while substitution with croaker flour at 20 % level gave significantly higher fat (6.66 %). Ash content of the composite flour increased to 1.98 % when substituted with 15 % tilapia fish flour. The Ash, fat, protein and crude fiber content of enriched bread produced from these composite flours also increased accordingly. Though carbohydrate content of the composite flour and bread decreased as the percentage substitution of fish flour increased, but energy value increased due to increased fat and protein content. With higher energy values of 277.66 kcal/100g and 274.54 kcal/100g, respectively, when substituted with 20 % tilapia and croaker fish flour.

Bulk density of the composite flour increased, while swelling power and oil absorption capacity decreased with increased substitution of fish flour. There was no significant change in the solubility and water absorption capacity of the wheat, croaker and tilapia composite flour.
Loaf volume and specific volume decreased with substitution of fish flour in the composite blend. However, loaf weight increased significantly. Calcium and Phosphorus content of the bread increased as fish flour substitution increased. Percentage invitro protein digestibility of the composite bread was significantly high when substitution with 10, 15 and 20 % tilapia fish flour. Bread produced from the composite flour all received equal acceptability with 100 % wheat flour bread. Use of fish flour as substitute to enhance the nutrient content of composite flours, for baking purposes and use of tilapia fish flour at 15 % and 20 % level of substitution with wheat flour to enhance the mineral, protein, fat and energy value of bread are here recommended.

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