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Original Research Article

Effect of Fermentation and Variety on Quality Attributes of Okra Seed (*Abelmoschus esculentus* (L) Moench) Flour

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The influence of fermentation on proximate composition, antioxidant capacity and functional properties of okra seeds (NCRI- 05, IFE-1, NGAE- 96-012-3, NGAE -05 and AKURE -2-2) flour were investigated. The proximate composition shows that the moisture ranged from 11.08-13.35%; protein (18.37-19.36%); fat (12.79-13.55%); ash (2.13-4.40%); fibre (5.30-6.56%) and carbohydrate content (44.4-46.96%). The physicochemical properties revealed the bulk density to be between 0.11–0.22 g/cm^3; swelling capacity (1.72–2.91mL); pH (5.28–5.59) and Titratable acidity (0.24–0.48%). The functional properties shows that water absorption capacity as 1.93 to 3.50 g/cm; oil absorption capacity (1.80-2.96 g/cm); emulsion capacity 2.25-3.44%); emulsion stability (1.15-1.81); foam capacity (4.33-6.4 cm) and foam stability (2.11-4.50%). The antioxidant activity shows the range of 18.62 to 38.26 and 25.56 to 152.88 for % inhibition of DPPH and Teac value respectively. Total phenolics and flavonoids 307 – 419.73 µg CE/g 61.33 – 81.72 µg CE/g respectively. The fermented okra seed flour showed an improvement in nutrient composition, antioxidant and some functional properties. Selecting a high protein variety of okra seed can have a good potential of being consumed as a complete diet or incorporated in human food in developing countries where protein foods are not adequate in supply. It can also serve as a functional ingredient in a variety of food formulations. Incorporation of okra seed flour in various food formulations will be beneficial to human health due to the improved antioxidant activity.

Keywords: Okra seeds, Fermentation, Proximate composition, Functional properties, Antioxidant properties.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L) Moench) known as '*gumbo*' in Congo and Angola area of Africa and '*Ila*' in Yoruba of Nigeria, also commonly known as '*bhindi*' in India and Nepal is a well known horticultural crop. The presence of okra in the New World has been attributed to the slaves from Africa (Yamaguchi, 1983). Okra is now grown during the summer in the warmer parts of the temperate region. India is the largest producer of okra in the world with an annual production of 3.24 million tonnes (Negi and Mitra, 1999). Furthermore, Okra has a vast potential as one of the foreign exchange earning crops and accounts for about 60% of the export of fresh vegetables excluding potato, onion and garlic in India (Kalloo, 1998).

In Nigeria, this vegetable is specially valued in different parts of the country for its delicious fruits and it is consumed alone or in combination with other foods. Nutritionally, the richest part of okra plants is the dried seed (Adelakun *et al*., 2009a,b) as it is very rich in protein, oil and antioxidant. Various researchers had documented some quality attributes of okra seed in literatures (Karakoltsidis and Constantinides, 1975; Savello *et al*., 1980; Al-Wandawi, 1983; Bryant *et al*., 1988; Oyelade *et al*., 2003; Calisir *et al*., 2005; Adelakun *et al*., 2012) but to the best of our knowledge none has been able to determine the effect of fermentation on different types of okra seed variety. Fermentation is one of the oldest and most economical

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methods of producing and preserving foods (Steinkraus *et al*., 1983; Cooke *et al*., 1987; Chavan and Kadam, 1989), particularly in the tropical countries where the high temperature and humidity, coupled with unsanitary conditions, favour food spoilage. Under these conditions, lactic acid fermentation inhibits spoilage and pathogenic microorganisms by a combination of factors including production of organic acids, hydrogen peroxide, antibiotics-like substance and lowering of oxidation – reduction potential (Cooke *et al*., 1987; Nout *et al*., 1989; Mensah *et al*., 1991).

There is also a possible increase in the nutritional value or the digestibility of the raw material during fermentation (Adhikari *et al.,* 2013; Chavan and Kadam, 1989). Although different varieties are available in Nigeria, okra seed is still underutilized unless for re-generational purpose. This work, therefore, assessed the influence of fermentation on quality attributes of five varieties okra seed flour.

MATERIALS AND METHODS

Five varieties of okra seeds (Akure – 2 – 2, IFE – 1, NCRI – 05, NGAE – 96 – 012 – 3 and NGAE – 96 – 0061) were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan, Oyo State, Nigeria. The chemicals used were of analytical grade.

Sample Preparation

Each variety (100 g) of okra seeds were cleaned and soaked in 300 mL of distilled water and fermented naturally at ambient temperature (28 ± 2^{o}C) for 36 h (Adelakun *et al*., 2009a). The fermented seeds were then washed and dried in a cabinet dryer at 45 ± 2^{o}C for 24 h to moisture content of 10–13%. The untreated and fermented okra seed of all the five varieties were milled using Kenwood mixer (Model BL350, PK100/AD England). The milled flour was sieved to obtain a flour fraction of less than 250 µm.

Proximate analysis of samples

Moisture, ash, crude fat, crude fiber, crude protein (microKjeldahl N X 6.25) were determined by AOAC (2005) standard method while, carbohydrate was determined by difference.

Determination of some physicochemical properties

Bulk density was determined by the method reported by Wang and Kinsella (1976). The swelling capacity was determined by the method of Sathe and Salunkhe (1981), also, pH and titratable acidity (TTA) values were determined using the standard methods described in AOAC (2005).

Determination of functional properties

Foaming capacity (FC) and foam stability (FS) tests were done by methods of Narayana and Narasinga Rao (1982); water and oil absorption capacities were determined by the method of Sosulski *et al.*, (1976) while emulsion activity and stability were determined by the method of Lin *et al.,* (1974).

Determination of the total phenolic content and total flavonoid content

Determination of total phenolic content of the samples extracts was determined according to the method of Angioloni and Collar (2012). The total flavonoid content was measured according to the method of Hou *et al.,* 2003. Catechin was used as a standard.

Determination of antioxidant activity

The 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) as described by Awika *et al*., 2003 and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays (Shimada *et al*., 1992) were employed to determine the antioxidant activity of samples.

Statistical analysis

The data recorded on the sample were statistically analysed using the Statistica Analysis Software (SAS) package (version 11 of SAS Institute, Inc.).

RESULTS AND DISCUSSION

Proximate composition of different varieties of untreated and fermented okra seed flour

The proximate compositions of fermented and unfermented okra seed flour are given in Table 1 below. The protein content of five varieties ranged from 18.37 ± 0.11 to 19.36 ± 0.03, with variety NGAE-96-012-3 having the highest of 19.36 ± 0.03, followed by variety NGAE-96-0061 (18.88 ± 0.04) while variety Akure-2-2 had the least protein content of 18.37 ± 0.11. These values were generally low when compared with our previous variety ('*iwo agborin*') with protein content of 41.11% (Adelakun *et al*., 2009a). This clearly shows that variety has influence on the protein content. Also, these values are lower than those reported by other researchers: Oyelade *et al*., 2003 (45%); Savello *et al*., 1980 (32.50%); Bryant *et al*., 1988 (24.24%); Karakoltsidis and Constantinides, 1975 (20.58%).

The protein content, however, compare well with that of Calisir *et al*., 2005 (19.10%). After fermentation, the range of 10.00 to 16.33% enhancement to the protein content of okra seed flour was observed when compared with untreated samples. The protein contents of all the five varieties after fermentation were significantly differently ($P < 0.05$) from the untreated varieties. This finding agrees with previous studies (Osundahunsi, 2006; Ikujenlola and Fashakin, 2005; Ade-Omowaye *et al*., 2003) that there might have been protein synthesis during fermentation which contributed to the higher values in fermented samples.

Also, for all the five varieties, the fat content ranged from 12.79 ± 0.18 to 13.55 ± 0.34, with variety IFE-1 having the highest of 13.55 ± 0.34, while variety NGAE-96-012-3 had the least fat content of 12.79 ± 0.18. Again, as an established oil seed, these values were generally low when compared with our previous variety ('*iwo agborin*') with fat content of 31.04% (Adelakun *et al*., 2009a) and other researchers such as: Oyelade *et al*., 2003 (20%); Savello *et al*., 1980 (25.57%); Bryant *et al*., 1988 (16.22%); Karakoltsidis and Constantinides, 1975 (20.06%) and Al-Wandawi, 1983 (16.65%). The fat contents, however are higher than that of Calisir *et al*., 2005 (8.21%).

The crude fat content of all the five varieties of the okra seed decreased after fermentation although, no significant difference ($P < 0.05$) were observed. The decrease in crude fat could be due to the utilization of fat for metabolic activity (Achinewhu, 1983). Similar trend was observed by Chaudhary *et al.,* 2014 and Onimawo *et al.,* (2003) in soaked germinated legumes and in fermented pumpkin seed respectively and also in soy and yam flours as reported by Achi (1999).

Table 1: Proximate composition of untreated and fermented flours from samples of five varieties of okra seed (d.b).

Sample	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate by difference (%)
Akure – 2 – 2^U	13.15±0.03^b	18.37±0.11^f	13.16±0.10^b	3.13±0.09^{cd}	5.44±0.03^b	46.76±0.03^a
Akure – 2 – 2^F	11.29±0.05^d	21.37±0.05^b	12.60±0.02^{cde}	4.40±0.06^a	6.22±0.06^a	44.13±0.12^c
IFE – 1^U	13.26±0.20^{ab}	18.82±0.06^e	13.55±0.34^a	2.13±0.04^f	5.30±0.18^b	46.96±0.70^a
IFE – 1^F	11.08±0.01^e	21.71±0.03^a	12.45±0.11^e	4.41±0.02^a	6.20±0.15^a	44.17±0.04^c
NCRI – 05^U	13.23±0.05^{ab}	18.42±0.16^f	13.22±0.07^b	3.02±0.02^{de}	5.32±0.08^b	46.81±0.20^a
NCRI – 05^F	11.33±0.00^d	20.76±0.08^c	12.74±0.09^{cd}	4.36±0.04^a	6.30±0.11^a	44.52±0.08^c
NGAE – 96 – 012 – 3^U	13.35±0.00^a	19.36±0.03^d	12.79±0.18^c	3.17±0.16^c	5.34±0.09^b	46.01±0.09^b
NGAE – 96 – 012 – 3^F	11.29±0.13^d	21.40±0.19^b	12.74±0.1^{cd}	4.21±0.05^b	6.18±0.02^a	44.19±0.14^c
NGAE – 96 – 0061^U	13.11±0.11^b	18.88±0.04^e	13.13±0.13^b	2.91±0.09^e	5.31±0.05^b	46.67±0.07^a
NGAE – 96 – 0061^F	11.55±0.06^c	21.79±0.07^a	12.51±0.10^{de}	4.34±0.11^{ab}	6.36±0.11^a	43.46±0.12^d

Samples with superscript U or F are the Untreated or Fermented samples.
Values are mean ± standard deviation of three determinations.
Values with the same superscript within the same column are not significantly different (P < 0.05).

Fermentation was found to enhance the ash and fibre content of all the five varieties of okra seed flour. Easier removal of the testa of the fermented sample during sieving might have been responsible for ash and fibre content increment (Knorr, 1999). Significant difference (P < 0.05) were observed in the ash and fiber content of fermented and untreated samples. This increased fiber after fermentation will be advantageous as it has been reported to help in the lowering of serum cholesterol, control blood sugar, increase bulk stool which may prevent colon cancer and several digestive disorders (NSRL, 2002).

The carbohydrate content of all the fermented samples were observed to be significantly (P < 0.05) lower than the untreated samples. This could be attributed to the possible bioconversion by the micro-organisms mainly yeast in the soaking water into other substances especially protein, in addition to the portion used as carbon and energy source by the organism (Chavan and Kadam, 1989).

Physicochemical properties of different varieties of untreated and fermented okra seed flour

The physicochemical properties of fermented and untreated okra seed flour is as shown in Table 2. The bulk density of all the variety of okra seed flour ranged from 0.216g/cm^3 to 0.12g/cm^3. Slight decreased in bulk density was observed in all the varieties after fermentation. Considering the result obtained, the unfermented flour would require a larger packaging material while the fermented ones would require smaller ones.

Reduction in the carbohydrates content and subsequent increase in the protein content by the action of micro-organisms present during fermentation might be responsible for the decrease in bulk density (Chavan and Kadam, 1989; Okezie and Bello, 1988). The results of the swelling capacity of okra seed flour showed that fermented samples had higher swelling capacity than the untreated sample (Table 2). Swelling capacity is another procedure for measuring the amount of water absorbed by food products (Iwe, 2003). The titratable acidity value (TTA) of the untreated sample vary from 0.24% (IFE-1) to 0.40% (NGAE-96-0061) while values for fermented samples ranged between 0.40% (IFE-1) – 0.48% (NGAE-96-012-3). There was an increase in the TTA for all the varieties investigated after fermentation.

A corresponding decrease in the pH value between the untreated varieties and all the fermented samples exist. This might be caused by the action of the lactic acid bacteria in the steep water during fermentation (Akingbala *et al.,* 1994). Significant differences (P < 0.05) were however observed to exist for most of the physicochemical properties of fermented and untreated okra seed flour.

Functional Properties of different varieties of untreated and fermented okra seed flour

The results of the functional properties for the five varieties of the okra seed flour determined are as shown in Table 3. The water absorption capacity (WAC) varies from 1.93g/cm (IFE-1) to 3.11g/cm (AKURE-2-2) in raw dried okra seed flour for all varieties to 2.42g/cm (IFE-1) to 3.50g/cm (AKURE-2-2) for all samples in the fermented okra seed flour. The oil absorption capacity (OAC) in all the raw dried okra seed flour also, varies from 1.80g/cm to 2.94g/cm with IFE-1 variety having the lowest and NCRI – 05 having the highest. After fermentation however, the OAC for all samples vary from 2.21g/cm (NGAE-96-0061) to 3.42g/cm (AKURE-2-2). Results show that pretreatment by fermentation increased the WAC and the OAC. The improved WAC and OAC may be due to increased solubility of protein and could be explained by proteolytic activity of micro-organisms (Iwe, 2003).

The reduction in emulsion capacity and stability after fermentation may be attributed to the decrease in fat content of the samples (Table 1). Invariably this may lower its functionality in terms of use in preparing comminuted meats like sausages, cake buffers, mayonnaise and salad dressing (Akubor *et al*., 2000) most especially for variety IFE-1 which has the lowest emulsion capacity of 2.25%. Foam capacity and stability were reduced by fermentation as the unfermented proteins appeared to have a positive influence on foamability. It was expected that higher protein value would increase foamability, but fermentation that occurred in the soaked samples appeared to interfere with the capacity of the protein to foam thereby leading to the production of a light and spongy sample (Achi, 1999).

Table 2: Physicochemical properties of untreated and fermented flours from samples of five varieties of okra seed (d.b).

Sample	Bulk density g/cm^3	Swelling capacity (ml)	pH	TTA
Akure – 2 – 2^U	0.216±0.01^{abc}	2.77±0.05^b	5.50±0.02^{bc}	0.31±0.01^c
Akure – 2 – 2^F	0.20±0.10^a	2.91±0.01^a	5.43±0.02^{cd}	0.47±0.04^a
IFE – 1^U	0.21±0.01^{bc}	1.82±0.02^e	5.44±0.02^{cd}	0.24±0.03^d
IFE – 1^F	0.15±0.06^{abc}	1.95±0.03^d	5.28±0.01^e	0.40±0.01^b
NCRI – 05^U	0.13±0.01^{abc}	1.72±0.04^f	5.57±0.03^{ab}	0.32±0.02^c
NCRI – 05^F	0.12±0.01^{bc}	2.08±0.02^c	5.43±0.05^{cd}	0.43±0.01^b
NGAE – 96 – 012 – 3^U	0.18±0.01^{ab}	1.72±0.01^f	5.59±0.03^a	0.29±0.02^c
NGAE – 96 – 012 – 3^F	0.16±0.01^{abc}	1.92±0.02^d	5.44±0.04^{cd}	0.48±0.02^a
NGAE – 96 – 0061^U	0.12±0.01^{bc}	1.73±0.01^f	5.37±0.1^d	0.40±0.01^b
NGAE – 96 – 0061^F	0.11±0.01^c	1.91±0.01^d	5.41±0.02^d	0.44±0.02^b

Samples with superscript U or F are the Untreated or Fermented samples.
Values are mean ± standard deviation of three determinations.
Values with the same superscript within the same column are not significantly different (P < 0.05).

Table 3: Functional properties of untreated and fermented flours from samples of five varieties of okra seed (d.b).

Sample	Water absorption (g/cm)	Oil absorption (g/cm)	Emulsion capacity (%)	Emulsion stability (%)	Foam capacity (cm^3)	Foam stability (%)
Akure – 2 – 2^U	3.11±0.11^c	2.87±0.03^c	3.44±0.01^a	1.81±0.01^a	6.11±0.11^c	4.21±0.01^b
Akure – 2 – 2^F	3.50±0.00^a	3.42±0.02^a	2.47±0.02^i	1.47±0.02^e	4.56±0.04^i	2.29±0.15^g
IFE – 1^U	1.93±0.03^i	1.80±0.01^l	3.24±0.02^c	1.33±0.01^g	6.44±0.03^a	3.88±0.02^c
IFE – 1^F	2.42±0.02^g	2.32±0.02^f	2.25±0.01^j	1.31±0.01^g	4.77±0.03^g	2.11±0.02^h
NCRI – 05^U	3.23±0.03^b	2.94±0.02^b	2.92±0.03^e	1.62±0.02^c	5.92±0.03^d	4.42±0.02^a
NCRI – 05^F	3.13±0.03^c	2.96±0.02^b	2.86±0.01^f	1.21±0.01^h	4.33±0.02^j	2.3±0.02^g
NGAE – 96 – 012 – 3^U	2.52±0.02^f	2.41±0.02^e	3.32±0.02^b	1.57±0.02^d	6.23±0.03^b	4.5±0.01^a
NGAE – 96 – 012 – 3^F	2.94±0.04^d	2.82±0.02^d	2.65±0.02^h	1.15±0.01^i	5.04±0.04^f	3.03±0.03^e
NGAE – 96 – 0061^U	2.23±0.03^h	2.14±0.05^h	3.19±0.02^d	1.77±0.01^b	5.73±0.01^e	3.78±0.03^d
NGAE – 96 – 0061^F	2.81±0.01^e	2.21±0.01^g	2.75±0.01^g	1.40±0.02^f	4.65±0.02^h	2.91±0.01^f

Samples with superscript U or F are the Untreated or Fermented samples.
Values are mean ± standard deviation of three determinations.
Values with the same superscript within the same column are not significantly different (P < 0.05).

Table 4: Antioxidant activity and Total phenolic content of untreated and fermented flours from samples of five varieties of okra seed (d.b).

Sample	Antioxidant activity (% inhibition of DPPH)	Antioxidant activity (Teac value)	Total phenolic content (µg CE/g)	Flavonoid content (µg CE/g)
Akure – 2 – 2^U	19.72±0.79^e	26.42±6.31^c	345.34±20.51^c	77.73±6.82^a
Akure – 2 – 2^F	34.31±1.19^c	148.86±1.26^a	404.13±5.47^a	62.96±3.41^c
IFE – 1^U	22.44±2.56^d	26.93±5.81^c	360.21±13.89^{bc}	81.72±3.91^a
IFE – 1^F	37.02±1.50^{ab}	151.59±0.49^a	415.05±6.37^a	64.93±2.51^c
NCRI – 05^U	18.62±0.39^e	25.56±6.07^c	307.29±5.47^d	76.66±6.56^{ab}
NCRI – 05^F	32.77±1.55^c	140.38±7.70^b	375.73±19.02^b	61.33±3.12^c
NGAE – 96 – 012 – 3^U	20.74±0.69^{de}	28.56±6.07^c	353.68±28.85^{bc}	79.04±7.20^a
NGAE – 96 – 012 – 3^F	38.26±0.25^a	152.88±0.80^a	412.29±4.05^a	68.79±0.63^{bc}
NGAE – 96 – 0061^U	20.44±1.21^{de}	26.56±3.07^c	355.93±23.28^{bc}	80.26±5.01^a
NGAE – 96 – 0061^F	34.88±0.61^{bc}	150.74±3.14^a	419.73±1.31^a	64.54±4.99^c

Samples with superscript U or F are the Untreated or Fermented samples.
Values are mean ± standard deviation of three determinations.
Values with the same superscript within the same column are not significantly different (P < 0.05).

Antioxidant activity and Total phenolic content of untreated and fermented flours from samples of five varieties of okra seed

The percent inhibition of DPPH, Trolox equivalent antioxidant capacity (TEAC) and the total phenolic content for all the samples is as shown in Table 4. The ABTS-radical scavenging capacity of all samples ranged from 25.56 – 152.88 µg TE/g with sample NCRI-05 having the lowest and sample NGAE-96-012-3 having the highest. The percent increase of the range of 435.29 – 467.55% was observed after fermenting the samples. The percentage inhibition of DPPH for all the samples also ranged between 18.62– 38.26 % with sample NCRI-05 having the lowest and sample NGAE-96-012-3 having the highest. Total phenolics and flavonoid content were higher in fermented samples than all untreated samples. Generally, sample NCRI-05 had the lowest value for all the analysis performed (Table 4). From this study, there is a possibility that structural breakdown of okra seed cell wall occurred leading to the release of several bioactive compounds which caused the increase in antioxidant capacity. Studies have reported an increase in antioxidative value of herbs after lactic acid

fermentation (Ibrahim *et al*., 2014). Bioactive compounds of these okra seeds were probably modified during fermentation. Modification of grains by amylases and proteases derived from microbes during fermentation thereby causing release of phenolics via enzymes treatment have been reported (Katina *et al.*, 2007). The work of Ibrahim *et al*., (2014) which evaluated and compared the antioxidant capacity between freshly prepared and lactic fermented Malaysian tea showed that, all lactic acid fermented herbal teas exhibited higher phenolic contents, flavonoid contents and antioxidant properties compared to the freshly prepared herbal teas. Natural fermenting microorganisms during fermentation may have triggered a pH reduction and activated some enzymes which are involved in hydrolyzing complex polyphenols, producing a much simpler and active polyphenols (Duenas *et al.*, 2005).

CONCLUSIONS

Five varieties of okra seeds: NCRI- 05, IFE-1, NGAE- 96-012-3, NGAE -05 AND AKURE -2-2 have been examined for their proximate composition, antioxidant and some of their functional properties. The fermented seed flour showed an improvement in nutrient composition, total phenolic contents, flavonoid contents, antioxidant properties and some functional properties. In conclusion, if a high protein variety of okra seed is selected, it can have a good potential of being consumed as a complete diet or incorporated in human food in developing countries where protein foods are not adequate in supply. It can also serve as functional ingredient in a variety of food formulations.

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