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Influence of water activity on functional properties of Okra (*Abelmoschus esculentus)* seed flour

Abu J D[1*], Ariahu C C[2], Abu J O[2]
[1] Department of Food Science and Technology, Federal Polytechnic, Idah, Nigeria
[2] Department of Food Science and Technology, University of Agriculture, Makurdi, Nigeria

Abstract
The influence of water activity on functional properties of okra (*Abelmoschus esculentus L*) seed flour was investigated at 30ºC ± 2ºC and eight water activities ranging from 0.11 – 0.84 using standard gravimetric method. The equilibrium moisture content (EMC), oil absorption capacity and foam capacity of the flour increased while water absorption, emulsion activity, emulsion stability, and foam stability decreased with water activity (a_w). Wettability of the flour showed the peak value at 0.30 – 0.50aw. Oil absorption capacity, foam capacity, and EMC varied from 68.0 – 86.0g/100g, 1.0 – 3.0%, and 6.0 – 21.0g/100g respectively within aw of 0.11 – 0.84. Water absorption, emulsion activity, emulsion stability, foam stability and bulk density ranged from 61.0 – 82.0g/100g, 29.0 – 46.0%, 35.0 – 41.0%, 96.0 – 98.0% and 0.49 – 0.54g/ml respectively.

Keywords: Okra seed flour, functional properties, water activity

Introduction

Okra (*Abelmoschus esculentus*) is an annual herbaceous shrub that grows in tropical and sub-tropical countries of the world (Ahmadu and Gupta, 1995) [5]. Worldwide production of okra as fruit vegetable is estimated at six million tonnes per year. India, Costa Rica, Nigeria and Ghana are some of major producers of okra (NARP, 1993) [21].

Dried okra seeds have been reported to contain high levels of protein, fat and carbohydrate (Udayasekhara, 1995; Oyelade *et al*., 2003; Abu, 2005) [2, 28]. The seed is known to be rich in high quality protein especially with regards to its contents of essential amino acids relative to other plant seeds (Oyelade *et al*., 2003) [28]. The dried seeds could be used to produce edible oil and as a substitute for coffee when roasted and ground. Okra cheese prepared from matured okra seed and bakery products in which wheat flour was substituted with okra seed meal at replacement levels of 25 – 100% had acceptable sensory attributes (Martin and Ruberte, 1979) [18]. Despite this, dried okra seeds have not been fully studied in relation to utilization. The potential exists to increase the world food production through the utilization of the okra seed as a source of food.

An important factor in the quality loss of dried foods during storage is the water activity (a_w) which influences the biochemical reactions and stability of dried products (Lamharrar *et al*., 2007) [17]. Processing operations such as mixing of ingredients involve moisture transfers which in turn depend on the state of water in foods. The state of water has direct effect on the quality and stability of foods through its effects on chemical reactions as well as physical, physicochemical or functional characteristics of foods. This underscores the need to investigate the effect of water activity on the functional attributes of okra seeds, since these characteristics largely determine the acceptability and application of okra seed flour as ingredients in food products formulation.

Materials and Methods

Matured and dried okra pods on the parent plants during the May/June season were obtained from Ajaka, Nigeria. 500ml capacity air tight plastic containers (12.5cm diameter x 11.8cm height each), and wire gauzes were purchased from a local market in Makurdi, Nigeria.

Preparation of sample

The seeds were cleaned, sorted and sun-dried to moisture content of 8% (wet basis). The dried seeds were milled using a hand mill (Corona model, China) to pass through a 0.4mm sieve. The flour obtained was dried in an electric oven at 105ºC to constant weight and stored in a polyethylene packaging material at 30 ± 2ºC for analysis.

Preparation of sulphuric acid solution

Eight different concentrations of sulphuric acid solutions (13.60 – 35.33% v/v) were prepared from concentrated sulphuric acid (98%, specific gravity, 1.84) to provide constant water activity environments ranging from 0.11 – 0.84 at 30 ± 2ºC. The water activity data of these acid solutions were obtained from literature (Samuel, 1965; Onwuka, 2003) [25, 30]

Determination of functional properties: To evaluate the effect of water activity on selected functional properties of okra seed flour, duplicate samples, each of 10g were exposed to atmospheres of different relative humidity (11 – 84%) created by sulphuric acid solutions (13.600 – 35.33%) at room temperature (30 ± 2ºC). Sample weights were measured daily until constant values were attained. The equilibrated samples were then used for functional properties evaluation.

Bulk Density

This was determined by the method of Narayana and Narasinga Rao (1984) [20]. Calibrated centrifuge tubes were weighed and flour samples were filled to 5ml mark by constant tapping until there was no further change in volume. The centrifuge tubes and contents were weighed and from the difference in weight, the bulk density of sample was calculated as weight of sample per unit volume of sample (g/ml). Measurements were made in triplicate.

Wettability
Wettability was evaluated using the method described by Okezie and Kosikowski (1981) [24]. One gram of each sample was placed in 10ml graduated cylinder with a diameter of 1cm. A finger was placed over the open end and the cylinder was inverted and clamped at a height of 10cm from the surface of a 600ml beaker containing 500ml distilled water. The finger was removed to allow the test sample to be dumped, and the time required for the sample to become completely wet was recorded. Triplicate measurements were made.

Water and Oil Absorption Capacities
The method described by Beuchet (1977*)* was used for water and oil absorption capacities determinations. A gram of flour sample was added to 10ml distilled water or vegetable oil (Gino brand, density 0.91) in a weighed centrifuge tube and allowed to stand at ambient temperature (30 ± 2ºC) for 1hr. Thereafter it was centrifuged at 2000 x g for 30 min. The water or oil was decanted and the centrifuge tube inverted to drain dry. Water or oil absorption capacity was expressed as grammes water or oil absorbed by 100 grams flour.

Foam Capacity (FC) and Foam Stability (FS)
Foam capacity and stability were evaluated by the method of Narayana and Narasinga Rao (1982) [19]. Two-gram flour sample was homogenized with 40ml distilled water in a micro blender (JVC, Japan). The dispersion was transferred into a 100ml measuring cylinder. The blender jar was rinsed with 10ml distilled water which was then gently added to the graduated cylinder. After mixing and shaking to foam, the volume of foam after 30 seconds was recorded. The FC was expressed as a percentage increase in volume using the formula:

$$FC(\%) = \frac{volume\ of\ form\ after\ whipping - volume\ of\ form\ before\ whipping}{volume\ of\ form\ before\ whipping}\ x\ 100$$

The foam volume was recorded one hour after whipping to determine the FS as a percentage of initial foam volume

$$FS(\%) = \frac{volume\ of\ form\ after\ 1hr}{initial\ volume\ of\ form}\ x\ 100$$

Emulsion Activity (EA) and Emulsion Stability (ES)
Emulsion activity and stability were evaluated using the method described by Narayana and Narasinga Rao (1982) [19]. One gram of flour was dispersed in 20ml distilled water in a conical flask using a magnetic stirrer set at high speed for 20min. Ten-millimeter vegetable oil (Gino brand, density 0.91) was added over a period of 5min with continuous stirring. The mixture was transferred to a calibrated centrifuge tube and heated at 80ºC for 15min in a water bath (Gallenkamp, UK). The tube was removed, cooled to 30ºC and centrifuged (Hettech, Italy) at 2000 x g for 15 min. The ratio of the height of the emulsified layer to the total height of the liquid layer in the tube was calculated as the emulsion activity expressed in percentage. The ES expressed in percentage was calculated as the height of the emulsified layer to the height of liquid layer after heating the tubes at 80ºC for 30 min, cooling for 15 min and centrifuged (Hettech, Italy) at 2000 rpm for 15 min.

Statistical Analysis
All experiments in this study were performed in triplicate. The correlation coefficients of various functional characteristics as affected by water activity were determined using Minitab statistical software (Minitab Inc. USA). Least significant difference (LSD) procedures were also used to separate means, and differences reported as significant ($p \leq 0.05$) (Steel and Torrie, 1980) [33].

Results and Discussion
The functional properties of okra seed flour studied were influenced by water activity. The water absorption capacity, emulsion activity, emulsion stability, and foam capacity decreased with increase in water activity. The oil absorption capacity and foam stability decreased with increase in water activity. The bulk density was unaffected by water activity while wettability was low at low and high-water activities respectively.

Water Absorption Capacity
The influence of water activity on selected functional properties of okra seed flour is shown in Table 1. The water absorption capacity decreased with increase in water activity and varied from 88.0 – 61.0g/100g solids over water activity range 0.11–0.84.
The values were also significantly different ($p \geq 0.05$) from each other over the same range of water activity (Table 2). In addition, there exist a negative correlation between the equilibrium moisture content and water absorption capacity of okra seed flour at specific water activity. High equilibrium moisture content at high water activity reduced the amount of water absorbed by okra seed flour.
The water absorption capacity of okra seed flour increased with decrease in the water activity. At low water activity range (< 0.30), the particles of the flour are free flowing and possess high surface area for enhanced moisture absorption or binding. Between 0.30 and 0.60a_w, the water content of the flour is deemed to be sufficient for the establishment of contacts between particles and the flour is at the funicular stage of caking (Gutman, 1992) [13]. The attendant reduction in surface area could result in observed decrease in the amount of water absorbed by the flour. At high water activity range (0.60 – 0.84), the flour agglomerate as moisture is high enough to form liquid bridges between particles. The subsequent water absorption ability is further decreased due to reduction in surface area.
The range of values (61 – 88%) for water absorption capacity obtained in this study were systematically lower than the value of 241% reported for dried okra seed flour elsewhere (Adelakun *et al.*, 2010) [4]. This systematic difference could partially be explained by differences in the sample treatments and methods used for the determination. Water absorption capacity is a critical function of proteins. Abu (2005) [2] reported that okra seed flour contained 11.7% moisture, 21.8% protein, 23.0% fat, 1.7% fiber, 4.0% ash, and 37.8% carbohydrate. The degree of protein interaction with water and their conformational characteristic (Buth and Batool, 2010) also account for this observed variation. Water absorption is also related to the degree of dryness and porosity of foods (Oyelade *et al*., 2001, Akpapunam and Achinewhu, 1985) [7, 27].
Furthermore, the water absorption profile tended to correlate well with existence of bound, semi-bound, and free water in

the okra flour. Flour samples maintained at 0.11 – 0.30a_w had equilibrium moisture contents that correspond to the existence of bound water. On the other hand, samples kept at 0.60 – 0.84 a_w had high equilibrium moisture levels that parallel the occurrence of free water in the flour.

Oil Absorption Capacity

Oil absorption capacity increased with increase in water activity and ranged from 68.0g/100g solids at 0.11a_w to 86.0g/100g solids at 0. 84aw.There were also significant differences ($p \geq 0.05$) between the values of oil absorption capacity of okra seed flour at various water activities. In addition, the oil absorption decreased with increase in equilibrium moisture content over the range of water activity studied.

The range of values for oil absorption capacity obtained in this work is comparable to that of cowpea flour (68 – 98%) and pigean pea (89.7%) (Prinyawiwataal *et al*., 1996; Oshodi and Ekperigin, 1989) [26], but lower than 229.4% reported for okra seed flour (Adelakun *et al*.,2010) [4]. Okra flour samples equilibrated at relative humidity >60% could have attained equilibrium moisture content sufficient to induce swelling of proteins and carbohydrate components leading to conformational changes that exposed the hydrophobic groups of these molecules to enhanced oil absorption.

Oil absorption involves a physical entrapment of oil related to the non-polar side chains of proteins. Nature of primary structure and conformational features, hydrophobic amino acid proportions and protein content all contribute to the oil-retaining properties of food materials. Any treatment that influences these parameters would tend to influence the oil absorption characteristics of the food system (Njintang *et al*., 2001) [23].

The effect of water activity on water and oil absorption capacities of okra seed flour was negatively correlated (Table 2). At low water activity region, the high values observed for oil absorption could be due to insufficient moisture to mask the hydrophobic components (non-polar groups in proteins, carbohydrates and lipids) of the flour, leaving these groups available for enhanced oil absorption. Conversely, the decreased oil absorption capacity of the flour at high water activity region could be attributed to enhanced blockage of non-polar components by readily available free water molecules. Okra seed flour stored at high relative humidity may be useful in ground meat formulation, meat replacers and extenders, pancakes and baked goods, where oil holding capacity is of prime importance (Ahmedna *et al*., 1999) [6].

Table 1: Effect of water activity on selected functional properties of Okra seed flour

Water activity	Equilibrium moisture content (g/100g)	Water absorption capacity (g/100g)	Oil absorption capacity (g/100g)	Emulsion activity (%)	Emulsion stability (%)	Foaming capacity (%)	Foaming stability (%)	Bulk density (g/ml)	Wettability (seconds)
0.11	6.0[e]	88.0[a]	68.0[f]	46.0[a]	41.0[a]	1.0[c]	98.0[a]	0.54[a]	26.0[d]
0.20	8.0[d]	82.0[b]	76.0[e]	46.0[a]	40.0[a]	1.0[c]	98.0[a]	0.54[a]	30.0[c]
0.30	11.0[c]	78.0[c]	78.0[d]	44.0[b]	37.0[b]	1.0[c]	98.0[a]	0.54[a]	50.0[a]
0.40	12.0[c]	78.0[c]	78.0[d]	44.0[b]	37.0[b]	1.0[c]	98.0[a]	0.54[a]	50.0[a]
0.50	12.0[c]	72.0[d]	80.0[c]	42.0[c]	37.0[b]	1.0[c]	98.0[a]	0.54[a]	50.0[a]
0.60	17.0[b]	17.0[e]	82.0[b]	40.0[d]	36.0[c]	2.0[b]	97.0[b]	0.53[a]	48.0[e]
0.70	18.0[c]	61.0[f]	85.0[a]	31.4[e]	36.0[bc]	2.0[b]	97.0[b]	0.49[a]	23.0[e]
0.84	21.0[a]	61.5[f]	86.0[a]	29.0[f]	35.0[c]	3.0[a]	96.0[c]	0.51[a]	20.0[f]
$LSD_{0.05}$	1.0	1.4	1.1	1.4	1.2	0.6	0.4	0.06	1.6

Values in columns with the same superscripts are not significantly different at $p \geq 0.05$

Table 2: Correlations coefficient of effect of water activity on functional properties of Okra seed flour

	a_w	EMC	WAC	OAC	EA	ES	FC	FS	BD
EMC	-0.983[a] 0.000[b]								
WAC	-0.972 0.000	-0.9520.000							
OAC	0.940 0.001	0.937 0.001	-0.959 0.000						
EA	-0.925 0.001	-0.919 0.001	0.938 0.001	-0.836 0.010					
ES	-0.910 0.002	-0.920 0.001	0.883 ,0.004	-0.928 0.001	0.755 0.030				
FC	0.868 0.005	0.9010.002	-0.812 0.014	0.747 0.033	-0.915 0.001	-0.686 0.060			
FS	-0.868 0.005	-0.901 0.002	0.812 0.014	-0.747 0.033	0.915 0.001	0.686 0.060	-1.000 *		
BD	-0.758 0.029	-0.765 0.027	0.838 0.009	-0.704 0.051	0.908 0.002	0.564 0.145	-0.752 0.031	0.752 0.031	
W	-0.208 0.622	-0.210 0.618	0.255 0.542	-0.076 0.858	0.529 0.178	-0.145 0.732	-0.523 0.183	0.523 0.183	0.622

a Correlation coefficient, b P-Value, aw = water activity, EMC = equilibrium moisture content, WAC = water absorption capacity, OAC = oil absorption capacity, EA = emulsion activity, ES = emulsion stability, FC = foam capacity, FS = foam stability, BD = bulk density, W = wettability

Emulsion Activity and Stability

The emulsion activity decreased with increase in water activity and ranged from 29% at 0.84a_w to 46% at 0.11a_w. Within the water activity range, differences between the values of emulsion activity were significant ($p \geq 0.05$). Similarly, the emulsion stability decreased with increase in water activity and varied from 35-41% over 0.11-0.84a_w.

The emulsion activity was influenced by water activity. Below a_w = 0.70, the emulsion activity values of okra seed flour were similar to 44% reported elsewhere (Adelakun *et al*., 2010) [4]. At high water activity region, increased equilibrium moisture content and the resultant free water content of the flour could physically block the active non-polar sites of globular proteins which consequently decreased surface activity and adsorption at the oil-water interphase (Nir *et al*., 1994) [22]. This may reduce the functionality of okra flour stored at high a_w in terms of use in preparing commuted meats like sausages, mayonnaise and salad dressing (Akubor *et al*., 2000) [9]. The emulsion stability (Table 1) was generally higher than 30.5% reported by Adelakun *et al*. (2010) [4]. The stability of the emulsion could be partly dependent on the sensitivity of the emulsion systems to heating and or centrifugation. In this regard, the chemical nature of emulsifiers, and other components of the system seemed to play a dominant role in stability of the emulsion rather than water activity. The high emulsion stability of the flour indicates its suitability in stabilizing fat/water phase in many baked foods.

Foaming Capacity and Stability

The foaming capacity of okra seed flour was poor and unaffected by water activity < 0.60. However, the increase above 0.60 a_w was marginal. Generally, the foam stability of okra seed flour was very high and unaffected by water activity below 0.6. The foams formed were very stable (> 90%).

Okra seed flour possessed very low foaming ability with high stability over the range of water activity studied. It has been documented elsewhere that flours with low foaming capacity could bring about the formation of smaller air bubbles surrounded by thicker and more flexible protein films, which discouraged the coalescence of air bubbles and consequently increased the foaming stability (Jitngarmkusol *et al*., 2008) [14]. Kinsella *et al*. (1985) [16] attributed low foaming capacity to inadequate electrostatic repulsions, lesser solubility and hence, excessive protein – protein interactions. The foaming capacity could be attributed to the solubility states of the protein (Kinsella, 1979) [15], concentration effect (Abbey and Ibeh, 1987; Sathe and Salunkhe, 1981) [31] or probable low albumin and globulin contents of the proteins in the flour (Derphande *et al*., 1983). The effect of water activity on the foaming properties even though significant (p > 0.05), could not be considered an overriding factor in the determinations.

The ability of okra seed flour to form stable foams can be harnessed in formulation of whipped toppings, frozen desserts and sponge cakes (Yu *et al*., 2007) [35]. In these products, varying emulsifying and stabilizing capacity are required because of the different compositions and stresses to which these products are subjected (Adebowale *et al*., 2005) [3].

Bulk Density

The bulk density of okra seed flour was generally low and stable, and ranged from 0.49-0.54g/ml over 0.11-0.84a_w. The effect of water activity on the bulk density of okra seed flour was insignificant (p >0.05). Generally bulk density is affected by moisture content and particle nature/size of flour. The value of bulk density of okra seed flour is within the range of 0.42 to 0.61 g/cm^3 reported elsewhere for Mucuna species (Adebowale *et al*., 2005) [3], lower than the values for cowpea varieties (0.71g/cm^3) and pigean pea (0.68g/m^3) reported by Butt and Batool (2010) [11].

In a powder with particles of the same size/diameter, the air space (interstitial air) between the particles will be very large, resulting in low bulk density. With a wide particle size distribution with enough small particles to fill out the spaces between the medium and large particles, the bulk density of the powder will be high. Agglomeration also contributes to reduced bulk density of flour. It appeared that the counteracting influences of moisture and particle size could explain the similarity in the bulk density of okra seed flour at various water activities. Okra seed flour is therefore suitable for infant food formulation where low bulk is required. Low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999) [8].

Wettability

The wettability of okra seed flour varied from 20-50 seconds and was low at both low (< 0.30) and high (>0.60) water activities respectively. Between these extremes, the wettability values of the flour increased and did not vary significantly (p≤ 0.05) from each other.

The high wettability rate of okra seed flour (Table 1) at very low water activity region (0.11 – 0.20) could be ascribed to available high surface area of free-flowing particles. At high water activity range (0.60 – 0.84), the fast rate of wettability could however, be due to the existing films of free water that easily penetrated the capillaries of the flour. Between these extremes, the low wettability could be caused by reduction in surface area of particles occasioned by formation of contacts between particles of the flour. Wettability depends on the nature of the surfaces of the agglomerates or single particles, and interfacial tension between the particle surface and the water. Presence of surface-active agents such as lecithins and phospholipids on surfaces of food particles facilitates wetting. Wettability is a desirable property in the production of instant food powders.

Conclusion

This study has revealed that functional properties of okra seed flour are affected by water activity of storage environments. Water and oil absorption capacities, foam stability, and bulk density of the flour irrespective of water activity are suitable in ground meat, meat replacers and extenders, baked and complementary food formulations.

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