



# Influence of Maturity and Drying Methods on the Chemical, Functional and Antioxidant Properties of Breadfruit (*Artocarpus altilis*)

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## Authors' contributions

This study was carried out jointly by all the authors. Authors JAVF and BPJ designed the experiment, carried out the research work in the laboratory and generated all the data. Authors GIP and YOES carried out the statistical analyses and prepared the manuscript. All the authors read and approved the final manuscript.

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## ABSTRACT

This study investigated the effect of maturity and drying methods on some chemical, functional and antioxidant properties of breadfruit (*Artocarpus altilis*) flour. Breadfruit flour was produced from mature and immature breadfruit by hot air oven, sun drying and biomass fuelled dryer at 60°C and the effect of maturity and drying methods on the chemical, functional and antioxidant properties of breadfruit flour were also investigated using standard methods. The results showed that there were significant differences in the properties studied in all the mature and immature samples for all the drying methods. For proximate composition, ascorbic acid, tocopherol, minerals, water absorption capacity, swelling power, solubility and bulk density, matured samples had higher and significant values, while for dispersibility and flavonoid, immature samples had higher and significant values. It was also observed that the chemical properties of breadfruit were significantly retained by air oven

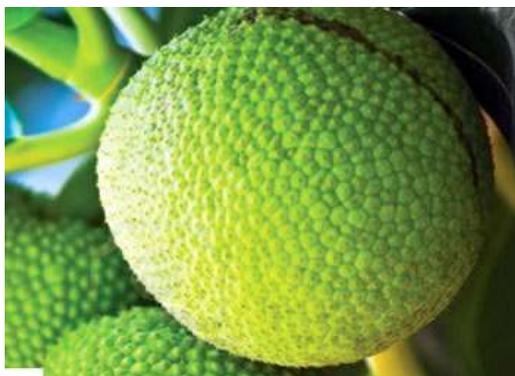
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drying. It was concluded from the results of this study that for better retention of nutritional qualities of bread fruit flour, oven drying method is better and matured fruits should be used.

**Keywords:** Biomass; breadfruit flour; oven drying; sun drying; nutritional qualities.

## 1. INTRODUCTION

Breadfruit (*Artocarpus altilis*) pantropical in its distribution is a multipurpose agroforestry tree crop which is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus, minerals and vitamins [1-3]. It is a beautiful and prolific tree and an essential component of traditional pacific island agriculture. Breadfruit trees grow well on hillsides, protecting watersheds, providing erosion control and windbreaks. The canopy provides beneficial shade for plants and people and the large leaves create mulch [3]. Breadfruit is well adapted to the wet tropics, doing best at temperatures ranging from 21-32°C with an annual rainfall of 1525-2540 mm and adequate drainage. Fully matured breadfruit (Plate 1) are dark-green and their segments are more rounded and smoother than immature fruits, latex stains may be on the skin of the matured fruits. Yellowing of the skin indicates over-maturity (partial ripeness). In some cases, fruits are harvested when fully ripe and ready for consumption. Good quality breadfruits are matured-green, firm, with intact stem and free from defects and decay [4].



**Plate 1. Matured breadfruit**

The tree has been reported to have great productive ability with an average sized tree producing 400-600 fruits per year [5]. It has also been reported that breadfruit yields in terms of food are superior to other starchy staples such as cassava and yam [6]. When fully ripe is soft; the interior is creamy coloured and pasty, also

sweetly fragrant. The mature fruit is a good source of carbohydrate (84%) with starch constituting more than 60% of the total carbohydrate [7,3]. Breadfruit has been processed into many forms for utilization. After peeling, the fruits are sliced into chips dried, milled and reconstituted to form paste and eaten with soups [3]; the fruits are boiled, pounded and eaten with soups just like pounded yam. Processing of breadfruit into starches [8] and flour [9] have also been reported. Although, breadfruit is nutritious, cheap and available in high abundance during its season, it has found limited applications in the food industries [10]. Hence, the study of its functional properties to find better application industrially, becomes pertinent. In Nigeria, it is predominantly found in some parts of the country which include Ibadan, Ogbomosho, Akure and Ikire.

One major factor limiting its availability is its poor storability, as the fruits undergo rapid physiological deterioration after harvesting. In many food-deficit countries, the need to fully utilize all existing foodstuffs with a view to alleviating poverty and hunger is now receiving considerable attention [3]. One way to minimize post-harvest losses and increase the utilization of breadfruit is through processing into flour, which is a more stable intermediate product [1]. The flour can then be the starting material for its industrial use. Also common synthetic antioxidants such as butylated hydroxytoluene, propylgallate used as supplements in foods are limited by shelves lifespan and adverse side effects [11]. Therefore, the search for natural antioxidants having little or no side effects to replace synthetic antioxidants for use in foods or as medicine is still an active area of research.

Drying methods and the physicochemical changes that occur in tissues during drying affect the quality of the dehydrated products. More specifically, the method used for drying affects properties such as colour, texture, density, porosity and sorption characteristics of materials [3]. High quality, convenient products are obtained efficiently at a fast rate and at competitive costs by several methods of drying. Traditionally, breadfruit slices are mainly dried in the open, under the sun. Sun-drying represents a

low cost processing method of preserving agricultural produce in the tropics. Open sun-drying, however, has some limitations. These include the inability to control the drying process and parameters, weather uncertainties, high labour costs, the requirement of a large drying area, insect infestation, and contamination with dust and other undesirable materials [12]. The drying of wet materials induces a number of physicochemical changes in the product, often reflected by colour. By choosing suitable drying methods and the appropriate conditions, the final product quality can be controlled and functional and antioxidant properties can be studied. Biomass fuelled dryer make use of agricultural waste, the main advantage is that their temperature can be controlled during drying compared with sun-drying.

This study was geared towards production of breadfruit into flour to serve as raw material for industrial use; the main aim of this study therefore was to evaluate the influence of maturity and drying methods on the chemical, functional and antioxidant properties of breadfruit flour.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Matured and immature breadfruit (*Artocarpus altilis*) were obtained from a local farm in Ile Ife, Nigeria. The equipment employed are FUTA slicer, stainless steel knife, bowl, weighing balance, aluminum foil paper, biomass fuelled dryer, hot air oven dryer, attrition milling machine and 0.35 mm mesh size.

### 2.2 Production of Breadfruit Flour

Thoroughly sorted breadfruits were peeled with a stainless knife, and were sliced with FUTA manual slicer to 3 mm thickness and subjected to different drying techniques: Hot air oven (Model IR701SG, Lec Refrigerator Plc, Britian) at 60°C; FUTA biomass fuelled dryer at 60 ± 5°C and Sun drying until constant weights were achieved. The dried samples were milled with attrition mill and sieved with 0.35 mm mesh. The breadfruit flours were packaged in polyethylene bags and stored at 4°C for further use.

### 2.3 Analyses Carried Out

On all the samples, chemical, functional and antioxidant properties of breadfruit flour were carried out.

## 2.4 Proximate Analysis

Analysis of crude protein, crude fat, ash, fibre and carbohydrate were determined by method described by Association of Official Analytical Chemists [13].



Plate 2. Biomass fuelled dryer

## 2.5 Minerals Composition Determination

The mineral analysis was carried by the method described by [13]. The samples were ashed at 550°C. The ash obtained was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 mL standard flask. The filtrate was made up to the mark with de-ionized water. The minerals, sodium (Na) and potassium (K) were determined from the solution using the standard flame emission photometer. NaCl and KCl were used as the standards [13]. Phosphorus was determined calorimetrically using the spectronic 20 [Gallenkamp, UK; 14] with KH<sub>2</sub>PO<sub>4</sub> as the standard. calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and lead (Pb) were determined using an atomic absorption spectrophotometer (AAS, Model SP9, PyeUnicam Ltd, Cambridge, UK). All values were expressed in mg/100 g.

### 2.5.1 Water absorption determination

Water absorption was determined by the modified centrifuge method of [15]. Each sample (M<sub>0</sub>) was transferred into a lagged 50 mL centrifuge tube and weighed as (M<sub>1</sub>). While 30 mL of distilled water at 70°C was added to each sample, the sample and the water was mixed thoroughly for 30 min. The suspension was allowed to rest for 10 min and centrifuged at 1165 rpm for 25 min at 50°C (the centrifuge used was refrigerated and so it makes easy to control the temperature). The tube was cooled in a desiccator and weighed as (M<sub>2</sub>).

$$\text{Water absorption} = \frac{M_2 - M_1}{M_0}$$

### **2.5.2 Swelling capacity determination**

Swelling capacity was determined by modification of the [15] method. Each sample (2 g) was dispersed in 40 mL distilled water. The resultant slurry was heated at a temperature of 70°C for 30 min in a water bath, cooled to room temperature, and centrifuged at 598 rpm for 30 min. The supernatant liquid was decanted and the centrifuge tube was dried for 25 min at 50°C inside a hot air oven. The residue was weighed ( $W_2$ ). The centrifuge tube containing the sample alone was weighed prior to adding distilled water ( $W_1$ )

### **2.5.3 Bulk density determination**

Bulk density was determined using the gravimetric method as described by [16]. Each sample (10 g) was weighed into a 25 ml graduated cylinder. The cylinder was gently tapped 10 times against the palm of the hand. The bulk density was expressed as the sample per volume occupied by the sample.

### **2.6 Dispersibility**

This was determined by the method described by [17]. 10 g of flour was suspended in 100 ml measuring cylinder and distilled water was added to reach a volume of 100 ml. The set up was stirred vigorously and allowed to settle for 3 hours. The volume of settled particles was recorded and subtracted from 100. The difference was reported as percentage dispersibility.

#### **2.6.1 Solubility pattern**

Solubility of starch fraction was determined according to the methods described by [18]. Supernatant liquid (dissolved starch) was poured into a tarred evaporating dish and put in air oven at 100°C for 4 h. Water solubility index was determined from the amount of dried solids recovered by evaporating the supernatant, and was expressed as gram dried solids per gram of sample.

$$\text{Solubility (\%)} = \frac{w_1}{W_s(1-M_c)} \times 100$$

$$\text{Swelling Power} = \frac{W_s \times 100}{W_{dm}(100 - \text{Solubility})}$$

$$\text{Dry Matter Weight (} W_{dm} \text{)} = W_s(1-M_c)$$

where:  $W_1$ ,  $W_2$  = Weight of supernatant and centrifuged swollen granules

$W_s$  = Weight of sample

MC = Moisture content of sample, dry basis (decimal)

$W_{dm}$  = Weight of dry matter

### **2.7 Antioxidant Composition of Breadfruit Flour**

Total flavonoid content of the sample was determined using a colourimeter assay developed by [19]. Vitamin C content was determined using the ascorbic acid as the reference compound using the method of [20], while tocopherol was determined by the method described by [21].

### **2.8 Statistical Analysis**

Data obtained from the experiment was subjected to randomized block design and statistical analysis using SPSS version 20 and mini tab version 16. Where the statistical analysis showed significant differences, the means were separated using the Duncan Multiple Range Test (DMRT).

## **3. RESULTS AND DISCUSSION**

### **3.1 Effect of Maturity and Drying Methods on the Proximate Composition of Breadfruit Flour**

Effect of maturity and drying methods on the proximate composition of breadfruit flour are presented in Table 1. The protein content of Immatured Sun dried (IMSD), Immatured Biomass Fuelled dried (IMBFD) Immatured Oven dried (IMOD), (12.21, 12.19, 13.64) are lesser than those of Mature Sun dried (MSD), Mature Biomass Fuelled dried (MBFD), and Mature Oven dried (MOD) (14.77, 14.38, 15.04)% respectively. The results showed that there is significant difference in the protein content of mature and immatured breadfruit flour which indicated that the proportion of protein is dependent on the maturity of the breadfruit; the results also showed that there is no significant difference in the protein during sun drying and biomass fuelled drying, whereas there is significant increase in protein content when breadfruit flour was produced by air oven drying. This may be due to drying at controlled and regulated temperature where majority of the protein are not denatured during drying. The fat content of IMSD, MSD, IMBFD, MBFD IMOD and MOD were 0.51, 0.83, 0.57, 0.71, 0.55, and

0.86% respectively. The results indicated that fat content of the mature bread fruit is higher than the immature breadfruit flour, while there is no significant difference in the fat content during sun drying, biomass fuelled drying and oven drying off immature breadfruit flour, whereas biomass fuelled drying significantly reduced the fat content of mature breadfruit flour compare to sun drying and oven drying methods (Table 1). The results also showed that carbohydrate content ranged from 79.42% for mature oven dried breadfruit to 84.24% for immaturred sun-dried breadfruit flour, while it showed that carbohydrate content decreased with maturity, it also revealed that there is no significant difference in the carbohydrate content of both drying methods except in mature biomass fuelled breadfruit flour where there is significant increase. The ash content of IMSD, MSD, IMBFD, MBFD, IMOD and MOD were 2.63, 4.00, 2.98, 3.11, 2.90 and 4.10% respectively. The results showed that ash content which defines the amount of minerals present, increased with maturity, it also revealed that there is significant decrease in ash content during biomass fuelled drying of mature breadfruit. The proximate composition of matured and immature breadfruits shows a very significant difference. This trend agrees with earlier report of Akpobome et al. [22], which was supported by Famurewa et al. [3].

### 3.2 Effect of Maturity and Drying Methods on Mineral Composition of Breadfruit Flour

Effect of maturity and drying methods on mineral composition of breadfruit flour are presented in Table 2. The calcium content of IMSD, MSD, IMBFD, MBFD, IMOD and MOD were 2.20, 2.40, 1.60, 1.80, 2.20 and 2.50 mg/100 g respectively. The results showed that calcium content increased significantly with maturity. This

significant increase with respect to maturity is also observed in sodium, potassium, phosphorus, magnesium, zinc, lead and copper. The results also revealed that the amount of calcium content decreased significantly due to biomass fuelled drying of immature and mature breadfruit flour. The result is consistent with the report of Ragone and Cavaletto [1] who reported that the extreme variation in the nutritional contents, especially mineral contents may be due to differences in the maturity of the fruit tested or analyzed, production systems, environmental factors, inconsistent analytical method and cultivars tested. Results also indicated that there is significant decrease in the potassium, magnesium content of breadfruit flour by biomass fuelled drying compare to sun drying and oven drying methods. This could be due to drying at relatively high and uncontrolled high temperature.

### 3.3 Effect of Maturity and Drying Methods on the Functional Composition of Breadfruit Flour

Effect of maturity and drying methods on the functional composition of breadfruit flour are presented in Table 3. Water absorption capacity of IMSD, MSD, IMBFD, MBFD, IMOD, and MOD were 3.60, 4.80, 3.60, 5.00, 4.00 and 5.00% (g/g) while bulk density were 0.53, 0.54, 0.53, 0.52, 0.60 and 0.59 g/cm<sup>3</sup> respectively. Solubility were 5.38, 6.68, 4.47, 6.47, 6.83 and 7.12% (g/g), swelling power were 1.20, 2.30, 1.20, 2.40, 2.10 and 2.40 g while dispersibility were 67.00, 63.00, 66.00, 64.00, 72.00 and 69.00% (g/g) respectively. Dried flour from matured breadfruit had a better water absorption capacity, solubility, swelling power when compared with the immature flour. While there is a significant reduction in water absorption capacity of mature breadfruit flour by sun drying.

**Table 1. Effect of maturity and drying methods on the proximate Composition of breadfruit flour (%)**

Samples	Ash	Fat	Crude fiber	Crude protein	Carbohydrate
IMSD	2.63 <sup>a</sup> ±0.00	0.51 <sup>a</sup> ±0.03	0.55 <sup>c</sup> ±0.01	12.21 <sup>a</sup> ±0.01	84.24 <sup>c</sup> ±0.04
MSD	4.00 <sup>c</sup> ±0.09	0.83 <sup>c</sup> ±0.12	0.22 <sup>a</sup> ±0.17	14.77 <sup>c</sup> ±0.01c	80.18 <sup>a</sup> ±0.08
IMBFD	2.98 <sup>a</sup> ±0.01	0.57 <sup>a</sup> ±0.25	0.44 <sup>b</sup> ±0.15	12.19 <sup>a</sup> ±0.02	83.82 <sup>c</sup> ±0.03
MBFD	3.11 <sup>b</sup> ±0.03	0.71 <sup>b</sup> ±0.01	0.21 <sup>a</sup> ±0.02	14.38 <sup>c</sup> ±0.11	82.59 <sup>b</sup> ±0.02
IMOD	2.90 <sup>a</sup> ±0.08	0.55 <sup>a</sup> ±0.02	0.40 <sup>b</sup> ±0.05	13.64 <sup>b</sup> ±0.01	83.11 <sup>c</sup> ±0.02
MOD	4.10 <sup>c</sup> ±0.01	0.86 <sup>c</sup> ±0.01	0.22 <sup>a</sup> ±0.12	15.04 <sup>d</sup> ±0.02	79.78 <sup>a</sup> ±0.08

*Immatured Sun dried (IMSD); Matured Sun dried (MSD); Immatured Biomass Fuelled dried (IMBFD) Matured Biomass Fuelled dried (MBFD); Immatured Oven dried (IMOD); Matured Oven dried (MOD). Values are means of 3 replications. Values along the same column followed by the same superscripts are not significantly difference (p<0.05)*

**Table 2. Effect of maturity and drying methods on mineral composition of breadfruit flour (mg/100 g)**

<b>Samples</b>	<b>Na</b>	<b>K</b>	<b>P</b>	<b>Ca</b>	<b>Mg</b>	<b>Zn</b>	<b>Pb</b>	<b>Cu</b>
IMSD	298.00 <sup>b</sup> ±0.26	1022.00 <sup>b</sup> ±0.34	0.52 <sup>c</sup> ±0.25	2.20 <sup>c</sup> ±0.23	0.80 <sup>b</sup> ±0.15	0.23 <sup>a</sup> ±0.21	0.80 <sup>a</sup> ±0.20	0.12 <sup>b</sup> ±0.01b
MSD	355.00 <sup>d</sup> ±0.15	1278.00 <sup>d</sup> ±0.24	0.44 <sup>b</sup> ±0.23	2.40 <sup>d</sup> ±0.34	0.90 <sup>c</sup> ±0.15	0.87 <sup>c</sup> ±0.15	1.80 <sup>b</sup> ±0.23	0.50 <sup>d</sup> ±0.31b
IMBFD	261.00 <sup>a</sup> ±0.53	885.00 <sup>a</sup> ±0.35	0.61 <sup>d</sup> ±0.38	1.60 <sup>a</sup> ±0.21a	0.70 <sup>a</sup> ±0.38	0.23 <sup>a</sup> ±0.36	1.80 <sup>b</sup> ±0.56	0.14 <sup>b</sup> ±0.52b
MBFD	345.00 <sup>c</sup> ±0.38	1060.00 <sup>c</sup> ±0.28c	0.49 <sup>b</sup> ±0.04	1.80 <sup>b</sup> ±0.16	0.80 <sup>b</sup> ±0.22	1.10 <sup>c</sup> ±0.24	2.10 <sup>c</sup> ±0.44	0.19 <sup>c</sup> ±0.15b
IMOD	341.00 <sup>c</sup> ±0.16	1010.00 <sup>b</sup> ±0.36	0.53 <sup>c</sup> ±0.15	2.20 <sup>c</sup> ±0.42	0.70 <sup>a</sup> ±0.31	0.21 <sup>a</sup> ±0.25	2.08 <sup>c</sup> ±0.15	0.06 <sup>a</sup> ±0.12a
MOD	358.00 <sup>d</sup> ±0.28	1151.00 <sup>c</sup> ±0.55	0.35 <sup>a</sup> ±0.32	2.50 <sup>a</sup> ±0.25	1.00 <sup>d</sup> ±0.25	0.26 <sup>b</sup> ±0.18	2.40 <sup>d</sup> ±0.50	0.18 <sup>c</sup> ±0.05b

*Immatured Sun dried (IMSD); Matured Sun dried (MSD); Immatured Biomass Fuelled dried (IMBFD); Matured Biomass Fuelled dried (MBFD); Immatured Oven dried (IMOD); Matured Oven dried (MOD).*

*Values are means of 3 replications. Values along the same column followed by the same superscripts are not significantly difference ( $p < 0.05$ )*

**Table 3. Effect of maturity and drying methods on the functional composition of breadfruit flour**

Samples	WAC % (g/g)	Bulk density (g/cm <sup>3</sup> )	Solubility % (g/g)	Swelling power g	Dispersibility % (g/g)
IMSD	3.60 <sup>a</sup> ±0.14	0.53 <sup>b</sup> ±0.34	5.38 <sup>a</sup> ±0.02	1.20 <sup>a</sup> ±0.14	67.00 <sup>c</sup> ±0.25
MSD	4.80 <sup>b</sup> ±0.38	0.54 <sup>b</sup> ±0.16	6.68 <sup>b</sup> ±0.12	2.30 <sup>c</sup> ±0.32	63.00 <sup>a</sup> ±0.12
IMBFD	3.60 <sup>a</sup> ±0.23	0.53 <sup>b</sup> ±0.24	4.47 <sup>a</sup> ±0.36	1.20 <sup>a</sup> ±0.21	66.00 <sup>c</sup> ±0.27
MBFD	5.00 <sup>c</sup> ±0.10	0.52 <sup>a</sup> ±0.28	6.47 <sup>b</sup> ±0.23	2.40 <sup>c</sup> ±0.15	64.00 <sup>b</sup> ±0.34
IMOD	4.00 <sup>b</sup> ±0.26	0.60 <sup>c</sup> ±0.05	6.83 <sup>b</sup> ±0.14	2.10 <sup>b</sup> ±0.31	72.00 <sup>e</sup> ±0.18
MOD	5.00 <sup>c</sup> ±0.17	0.59 <sup>c</sup> ±0.18	7.12 <sup>c</sup> ±0.25	2.40 <sup>c</sup> ±0.16	69.00 <sup>d</sup> ±0.55

*Immatured Sun dried (IMSD); Matured Sun dried (MSD); Immatured Biomass Fuelled dried (IMBFD); Matured Biomass Fuelled dried (MBFD); Immatured Oven dried (IMOD); Matured Oven dried (MOD).*

*Values are means of 3 replications. Values along the same column followed by the same superscripts are not significantly difference (p<0.05)*

**Table 4. Effect of maturity and drying methods on antioxidant composition of breadfruit flour**

Samples	Flavonoid (mg/ml)	Tocopherol (mg/ml)	Ascorbic acid (mg/ml)
IMSD	1.86 <sup>d</sup> ±0.15	1.54 <sup>b</sup> ±0.07	0.61 <sup>c</sup> ±0.11
MSD	1.54 <sup>c</sup> ±0.23	1.93 <sup>d</sup> ±0.11	1.06 <sup>d</sup> ±0.57
IMBFD	1.45 <sup>c</sup> ±0.31	1.08 <sup>a</sup> ±0.03	0.33 <sup>a</sup> ±0.05
MBFD	1.30 <sup>b</sup> ±0.16	1.62 <sup>c</sup> ±0.14	0.56 <sup>b</sup> ±0.05
IMOD	1.24 <sup>b</sup> ±0.05	1.42 <sup>b</sup> ±0.78	0.56 <sup>b</sup> ±0.05
MOD	1.06 <sup>a</sup> ±0.21	1.66 <sup>c</sup> ±0.22	0.61 <sup>c</sup> ±0.05

*Immatured Sun dried (IMSD); Matured Sun dried (MSD); Immatured Biomass Fuelled dried (IMBFD); Matured Biomass Fuelled dried (MBFD); Immatured Oven dried (IMOD); Matured Oven dried (MOD)*  
*Values are means of 3 replications. Values along the same column followed by the same superscripts are not significantly difference (p<0.05)*

### 3.4 Effect of Maturity and Drying Methods on Antioxidant Composition of Breadfruit Flour

Effect of maturity and drying methods on antioxidant composition of breadfruit flour are presented in Table 4. Total flavonoid of IMSD, MSD, IMBFD, MBFD, IMOD, MOD were 1.86, 1.54, 1.45, 1.30, 1.24, and 1.06 mg/ml; Tocopherol were 1.54, 1.93, 1.08, 1.62, 1.42 and 1.66 mg/ml while Ascorbic acid were 0.61, 1.06, 0.33, 0.56, 0.56 and 0.61 mg/ml respectively. Results indicated that Total flavonoid decreased with respect to maturity while there is significant decrease by oven drying method. This could be due to drying at regulated and controlled temperature which has significant effect on the Total flavonoid of breadfruit flour. Results also showed that Tocopherol and Ascorbic acid increased with respect to maturity while there is significant reduction in Tocopherol and Ascorbic acid by biomass fuelled drying.

### 4. CONCLUSION

The present study has demonstrated the effects of maturity and drying methods on chemical, functional and antioxidant properties of breadfruit flour. The study has shown that maturity significantly increased the protein, calcium, potassium, tocopherol and ascorbic acid in breadfruit flour which is of health and nutritional significance. The study also revealed the advantageous effect of the oven drying method over sun drying and biomass fuelled drying method which has a significant improvement on the proximate, functional and antioxidant properties of breadfruit flour. It could be deduced from the research however that maturity and oven drying significantly improves the nutritional qualities of bread fruit flour.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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