



## Physicochemical Properties and Mycological Quality of Wheat Flours Consumed in Calabar, Nigeria

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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### ABSTRACT

This study was primarily aimed at investigating the physicochemical properties and mycological quality of white wheaten flour, wheat semolina and whole wheat meal produced and/or sold in Calabar. Ten (10) samples of each flour type were bought from ten different locations, and analysed using standard methods. Physicochemical parameters analysed were pH, moisture, crude ash, crude fat, gluten, crude protein and falling number. Mycologically, total fungal count (TFC) was also determined. The results showed that the physicochemical parameters of all but the ash contents of five white wheaten flour samples and the fat content of one white wheaten flour sample did not conform to Standards Organisation of Nigeria (SON) standards. One-way analysis of variance (ANOVA) showed that there were significant differences ( $P < 0.01$ ) between the three wheat flour types for ash, fat, gluten protein, falling number and TFC only Post-hoc analysis using Tukey's honestly significant (HSD) test revealed ash, fat and TFC of the three wheat types were significantly different ( $P < 0.05$ ) from each other; the gluten and protein for both white wheaten flour and wheat semolina were significantly different ( $P < 0.05$ ) from whole wheat meal and; falling number for white wheaten flour was significantly different ( $P < 0.05$ ) from both wheat semolina and whole wheat meal. Although TFCs of the thirty samples conformed to SON

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standards, some fungi species with serious health implications were identified. This study highlights the need for a concerted effort towards ensuring that wheat flours produced conform to standards.

*Keywords: Wheat flour; physicochemical analysis; mycological analysis; Calabar; Nigeria.*

## 1. INTRODUCTION

Cereals and cereal-derived products are basic foods consumed in different households in different processed and unprocessed forms [1] and has been the world's most important source of food for the last 10,000 years [2]. In the developing world, grains like wheat, millet, maize constitute a major part of the diet. The uniqueness of wheat among cereals derives from the cohesiveness and viscoelasticity of dough formed when wheat flour is mixed with water [3].

Based on the type and quantity of wheat components contained—the endosperm, the germ, and the bran parts—flours can be categorised into three general groups. Whole grain or whole wheat meal contains the entire grain, which includes the bran, endosperm, and germ. White wheaten flour contains primarily the endosperm just like wheat semolina, however, white wheaten flour generally contains slightly more bran and germ components than wheat semolina.

During the milling process, wheat grains are subjected to vigorous cleaning. However, not all microorganisms and toxins are removed from the final flour because microorganisms can penetrate the kernel of grains during growth and storage. In addition to wheat processing, handling as well as packaging of the finished product (flour, semolina etc.) could also serve as sources of contamination by pathogenic fungi.

Once the water content of wheat flour exceeds the threshold level of 13-15%, mould proliferation results [4]. This scenario exemplifies how a chemical parameter can impart the microbiological quality of flours [5]. Apart from causing flour spoilage and sometimes producing mycotoxins when environmental conditions are favourable [6], mould growth can reduce flour quality considerably. Other major effects of fungal deterioration of grains include decreased germination, discolouration, development of visible mould growth, musty or sour odours, dry matter loss and nutritional heating and caking [7].

The objective of our study was to investigate the physicochemical properties and mycological quality of wheat flours produced and/or sold in Calabar, Nigeria with a view to ascertaining their conformity to Standards Organisation of Nigeria (SON) standards.

## 2. MATERIALS AND METHODS

### 2.1 Materials

A total of thirty wheat flour samples (10 each of white wheaten flour, wheat semolina and whole wheat meal) were obtained from ten markets, shops and retail outlets in Calabar. The samples were immediately transported to the laboratory and subsequently analysed for physicochemical and mycological parameters.

### 2.2 Physicochemical Analysis

#### 2.2.1 Determination of pH

pH of 10% flour suspension was measured using a pH meter (JENWAY 3310, USA).

#### 2.2.2 Determination of moisture

Determination of moisture content was done according to AACC [8] method No. 44-19.

#### 2.2.3 Determination of crude ash

Crude ash determination was done according to AACC [8] method No. 08-01.

#### 2.2.4 Determination of crude fat

Crude fat extraction was done according to AACC [8] method No. 30-20, using an extractor (FOSS Soxtec 2043, Denmark).

#### 2.2.5 Determination of gluten

Gluten content was measured by using Perten Glutomatic System (Sweden), according to AACC [8] method No.38-12.

### 2.2.6 Determination of crude protein

Buchi AutoKjeldahl-370 (B 811, Switzerland) instrument was employed in the determination of nitrogen content, according to AACC [8] method No. 46-13 and crude protein was calculated by using a multiplication factor of nitrogen  $\times$  5.83 [9].

### 2.2.7 Determination of falling number

Falling number was determined by using Perten FN 1700 (Sweden) apparatus, according to AACC [8] method No. 02-06.

## 2.3 Mycological Analysis

Twenty-five grams (25g) of each sample was homogenised in 225 ml of sterile peptone water (Oxoid CM 733, Basingstoke, UK) in a sterile 500 ml gas jar cylinder to obtain a ten-fold dilution. The solution was shaken vigorously for a few minutes to allow proper mixing and then left to settle. Agar plates were inoculated in triplicates.

### 2.3.1 Enumeration of total fungi count

Isolation of fungi was carried out using potato dextrose agar (PDA) (CM0139, UK) supplemented with chloramphenicol to inhibit bacterial growth. The media was incubated at 35°C for 72 hrs. Total fungi count was estimated as colony forming units per gram (cfu/g). Wet preparations of actively growing fungi were placed on a glass slide with a methylene blue stain, covered with a coverslip and observed with x40 objective under the microscope. Identification of fungal isolates was carried out according to Samson and Reemon-Hoekstra [10].

## 2.4 Statistical Analysis

Range, mean and standard error of means were used in the presentation of results. The  $\log_{10}$  transformations of microbial counts were carried out to normalise the distributions. Where zero mean counts or standard error of mean were obtained, one (1) was added before transformation across the three varieties of flour. One-way analysis of variance (ANOVA) was used to compare means and Tukey honestly significant difference (HSD) test was used for means separation. Microsoft Excel 2013 (Microsoft Inc.), R Statistical Software (R Software Foundation) were used for the analysis.

## 3. RESULTS AND DISCUSSION

The results of physicochemical analysis and total fungi count (TFC) of samples for white wheaten flour, wheat semolina and whole wheat meal, each in comparison with SON [11] standards, are presented in Tables 1, 2 and 3, respectively.

Microorganisms have different optimal pH values. Yeasts and moulds thrive better in low pH food products where bacteria cannot compete [12]. The pH of all samples in this study ranged from 6.02-6.41 (close to neutral), which is similar to the results of Hendrich and Bryant [12] and Ntuli et al. [13].

Moisture is a very important parameter with regards to the quality of flour and acceptability of flour products. It affects the shelf life and promotes microbial growth during storage [5,14, 15]. The moisture contents of all samples of the three wheat flour types conformed to SON [11] standards. In a similar study with white wheaten flour, Akpe et al. [16] observed moisture contents with the range of 12.0-13.6%. Mahmood [17] stated that the moisture content of wheat is largely dependent on the genetic makeup and is also influenced by the agronomic and climatic conditions of the area. Even though low water activity of dry flour cannot usually support fungal growth or mycotoxin production, slight changes in moisture levels of 1% or 2% have been shown to be sufficient to initiate growth and mycotoxin production [18].

Ash content is an indication of bran level in wheat [19]. It is also a measure of the level of mineral composition in the flour. For white wheaten flour, ash contents of samples bought at Intergro (IGM), Ikot Ishie (IIM), Etim Edem Park (EEP), Watt (WTM1) and Bogobiri (BGB) markets violated SON [11] standards, while all the remaining samples conformed to SON [11] standards.

The innermost germ component of wheat grain is rich in vegetable oils. High fat content may trigger rancidity during storage [20,21], giving rise to off-flavour in the baked or cooked product. Fat contents of all samples conformed to SON [11] standards, except one white wheaten flour sample bought at location one in Watt market (WMT1).

The strength of gluten is mostly a function of the quantity of protein present in wheat flours. Strong wheat flours containing high protein levels make

**Table 1. Physicochemical parameters and TFC of white wheaten flour samples**

Parameters*	Locations										SON standards
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	
pH	6.22	6.09	6.25	6.29	6.32	6.15	6.40	6.40	6.30	6.10	6.0 – 6.8
Moisture (%)	13.2	12.9	12.1	12.5	12.6	12.1	12.5	13.3	12.7	14.0	≤ 14.0
Ash (%)	0.67	0.72	0.68	0.71	0.73	0.76	0.70	0.72	0.69	0.66	≤ 0.70
Fat (%)	1.10	1.24	1.15	1.27	1.31	1.55	1.19	1.28	1.06	1.07	≤ 1.5
Gluten (%)	10.8	11.1	10.7	10.9	10.7	10.2	10.8	10.4	10.5	10.3	≥ 8.0
Protein (%)	12.1	12.3	11.9	12.1	11.9	11.3	12.0	11.6	11.7	12.2	≥ 10.5
Falling number (sec)	314	406	302	283	269	401	259	273	384	264	NIL
TFC (Log <sub>10</sub> cfu/g)	1.15±0.64	1.53±0.64	1.15±0.64	1.15±0.88	1.61±0.00	1.61±0.83	1.15±0.64	1.64±0.88	1.26±0.64	1.32±0.83	2.00

\*pH = no unit

Key: IEM = Ikot Ekpo market, IGM = Intergro market, IAO = Ikot Ansa outlet, IIM = Ikot Ishie market, EEP = Etim Edem park, WTM = Watt market, BGB = Bogobiri, MBM = Mbukpa market, MRM = Marian market, SON = Standards Organisation of Nigeria, TFC = Total fungi count, NIL = Not to be detected

**Table 2. Physicochemical parameters and TFC of wheat semolina samples**

Parameters*	Locations										SON standards
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	
pH	6.04	6.15	6.25	6.13	6.30	6.08	6.20	6.34	6.18	6.02	6.0 – 6.8
Moisture (%)	14.0	12.8	13.1	12.7	13.0	13.1	13.2	13.7	12.9	13.3	≤ 14.0
Ash (%)	0.54	0.59	0.61	0.65	0.52	0.58	0.58	0.55	0.60	0.54	≤ 0.70
Fat (%)	0.70	0.75	0.92	1.08	0.68	0.74	0.81	0.73	0.73	0.92	≤ 1.5
Gluten (%)	10.2	9.9	10.5	10.3	10.1	10.6	9.8	10.8	10.4	11.0	≥ 8.0
Protein (%)	11.3	10.9	11.7	11.4	11.2	11.8	11.1	11.8	11.6	12.8	≥ 10.5
Falling number(sec)	401	426	391	503	407	386	433	419	494	422	NIL
TFC (Log <sub>10</sub> cfu/g)	0.60±0.64	0.60±0.64	-	0.90±0.64	1.15±0.64	1.04±0.83	-	1.85±0.83	0.90±0.64	1.32±0.83	2.00

\*pH = no unit

Key: IEM = Ikot Ekpo market, IGM = Intergro market, IAO = Ikot Ansa outlet, IIM = Ikot Ishie market, EEP = Etim Edem park, WTM = Watt market, BGB = Bogobiri, MBM = Mbukpa market, MRM = Marian market, SON = Standards Organisation of Nigeria, TFC = Total fungi count, NIL = Not to be detected

**Table 3. Physicochemical parameters and TFC of whole wheat meal samples**

Parameters*	Locations										SON standards
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	
pH	6.18	6.22	6.29	6.21	6.24	6.36	6.05	6.26	6.25	6.41	6.0 – 6.8
Moisture (%)	12.9	13.0	12.7	12.8	13.1	13.3	12.8	13.2	13.0	12.9	≤ 14.0
Ash (%)	1.48	1.63	1.72	1.66	1.53	1.59	1.70	1.68	1.55	1.47	≤ 2.00
Fat (%)	1.41	1.57	1.62	1.59	1.49	1.60	1.61	1.55	1.52	1.48	≤ 3.0
Gluten (%)	9.4	8.6	9.9	9.6	8.9	8.7	9.8	10.1	9.2	9.1	≥ 7.5
Protein (%)	13.1	12.9	13.4	13.6	13.3	13.7	13.2	13.5	13.0	12.8	≥ 11.5
Falling number (sec)	406	389	356	407	389	391	418	372	402	411	NIL
TFC (Log <sub>10</sub> cfu/g)	1.79±0.83	1.73±0.88	1.81±0.88	1.85±0.83	1.71±0.83	1.73±0.64	1.73±0.88	1.87±0.64	1.83±0.64	1.85±0.83	2.00

\*pH = no unit

Key: IEM = Ikot Ekpo market, IGM = Intergro market, IAO = Ikot Ansa outlet, IIM = Ikot Ishie market, EEP = Etim Edem park, WTM = Watt market, BGB = Bogobiri, MBM = Mbukpa market, MRM = Marian market, SON = Standards Organisation of Nigeria, TFC = Total fungi count, NIL = Not to be detected

stronger gluten in comparison with weak wheat flours [22]. The gluten contents of all samples conformed to SON [11] standards.

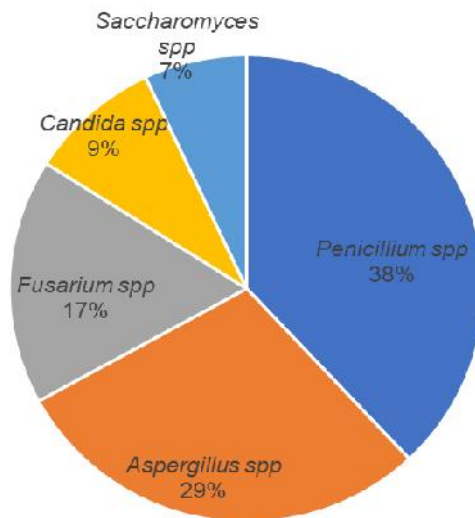
Protein content is of great significance, especially with regards to texture and palatability of the final product of wheat flour [23]. The results of this study agree with studies by Ekinic and Unal [19] and Aydin et al. [5], who recorded protein contents of wheat flour ranging from 7-13.5%. According to SON [11], high protein contents above 9%, 10.5% and 11.5% dry weight for semolina, wheat flour and whole wheat meal, respectively indicates good quality. Therefore, all samples analysed can be considered to be of good quality in terms of protein content.

The falling number test is a measure of the degree of alpha-amylase activity in wheat flour, as a way of measuring sprout damage and as a means to determine the right amylase supplementation rates [24]. When wheat sprouts, it leads to an elevated level of alpha-amylase in flour. If harvesting is done before sprouting takes place, wheat will usually have a small quantity of alpha-amylase [25]. As falling number increases the level of alpha-amylase decreases, and vice-versa, indicating an inverse relationship. Bread flours that have normal diastatic activity (milled from good, unspouted wheat that has received alpha-amylase supplementation) generally have falling number values ranging from 220 to 250 sec. Flours that lack diastatic activity will

normally have falling number values exceeding 400 sec and flours that have received over-supplementation or flours produced from wheat with sprout damage can have falling number as low as 60 sec.

The level of contamination of flour by yeast and moulds is of paramount importance when considering the quality and safety of food [13]. All samples of the different types of flours conformed to recommended SON [11] limits for TFC. Food decomposition occurs due to the presence of moulds and many species of moulds are known to produce mycotoxins [25]. Diseases caused by mycotoxin-producing fungi include gangrenous ergotism and reproductive cancers in women and children [26]. There are different sources for moulds present in flour, for example, fungi prevailing in the grain, the mill machinery itself, and/or a unhygienic practices [18,27].

In this study, *Penicillium* was predominant (38%) over *Aspergillus* (29%) (Fig. 1). Ntuli et al. [13] reported in their study that the predominant and frequently isolated fungi were *Aspergillus* (33%) and *Penicillium* (25%). Weidenborner et al. [28] also reported 15% *Penicillium* of the numerous mould counts ( $1.730 \times 10^3$  cfu/g) of white wheaten flour. The identified fungi in this study are of great importance in food safety, as they produce mycotoxins, which have diverse implications for health and the economy [16].



**Fig. 1. Isolation frequency of different genera of fungi sp. in white wheaten flour, semolina and whole wheat flour**

High isolation frequency of *Penicillium* and *Aspergillus* in this study may be due to unhygienic handling. Storage moulds such as *Aspergillus* and *Penicillium*, and the soil moulds penetrate well into the kernel of grain and produce toxins which can be difficult to remove during food processing. Thus, that a product does not contain microscopic mycelial fungi is not an indication that it is mycotoxin free. Contrarily, that a product contains fungi that produce mycotoxins is not sufficient evidence to presume that mycotoxins are present [13].

*Fusarium* spp is widespread in soil and on plants. Mycotoxins produced by some species in cereals can negatively impact human and animal health. Its presence in processed food indicates the ineffectiveness of food processing because some strains are harmful to humans and can cause foodborne illnesses [13].

Results of one-way analysis of variance (ANOVA) and Tukey's honestly significant (HSD) test are presented in Tables 4 and 5, respectively. There was no significant difference between the moisture contents of the different flour types because the final moisture content of flours is largely dependent on the amount of water added during tempering with the aim of producing flours which conform to specific standards. In this case, all three wheat flour types have the same SON [11] limit of 14.0%.

**Table 4. Analysis of variance of the effect of wheat flour type on physicochemical parameters and TFC**

Parameters	Source of variation Wheat type	
	F	P-value
pH	1.934	0.164
Moisture	2.123	0.139
Ash	875.8	$< 2 \times 10^{-16}^{**}$
Fat	97.11	$4.66 \times 10^{-13}^{**}$
Gluten	29.01	$1.88 \times 10^{-7}^{**}$
Protein	51.171	$6.52 \times 10^{-10}^{**}$
Falling number	18.44	$8.93 \times 10^{-6}^{**}$
TFC	18.26	$9.62 \times 10^{-6}^{**}$

**\*\*Significant at 1% alpha level (two-tailed)**  
Key: TFC = Total fungi count

There was a significant difference between the ash contents of the three wheat types and Tukey's HSD test revealed that the ash contents of the three wheat flour types were significantly different from each other. This reflects the

different levels of bran present in the three wheat flour types. There was a significant difference between the crude fat contents of the three wheat flours types and Tukey's HSD test revealed that the crude fat contents of the three wheat flour types were significantly different from each other. This is because the germ component of the wheat, which is very rich in polyunsaturated fats, is retained in whole wheat meal, whereas the white flour and semolina mainly contains the endosperm. However, during milling of white wheaten flour, part of the germ may escape into the flour.

There was a significant difference between the gluten contents of the three wheat flour types and Tukey's HSD test revealed that there was a significant difference between the gluten contents of white wheaten flour and whole wheat meal; wheat semolina and whole wheat meal, whereas no significant difference was observed between wheat semolina and white wheaten flours. This is because gluten is contained in the endosperm of wheat from which semolina and white flours are obtained. Results of analysis of variance and Tukey's HSD test for crude protein were the same as for gluten. This is because both white wheaten flour and wheat semolina contain mainly gluten proteins while whole wheat meal also contains germ and bran proteins apart from gluten proteins.

There was a significant difference between the falling number of the three wheat flour types and Tukey's HSD test revealed significant differences between white wheaten flour and each of wheat semolina and whole wheat meal, but no significant difference between wheat semolina and whole wheat meal. The difference is likely due to the supplementation of white wheaten flour with enzymes or additives, resulting in higher amylase activity, but wheat semolina and whole wheat meal are usually not supplemented with amylase.

Analysis of variance showed a significant difference between the TFC of the three wheat flour types and Tukey's HSD test showed that TFC of the three wheat flour types were significantly different from each other. This can be attributed to the higher ash content of whole wheat meal, resulting from the bran which harbours a wide range of microorganisms. Flours that are low in bran or ash usually have low levels of microorganisms [29].

**Table 5. Tukey's HSD test for means separation**

Parameters	Wheat type**		
	White wheaten flour	Wheat semolina	Whole wheat meal
pH	6.25±0.04 <sup>a</sup>	6.17±0.03 <sup>a</sup>	6.25±0.03 <sup>a</sup>
Moisture	12.79±0.18 <sup>a</sup>	13.18±0.13 <sup>a</sup>	12.97±0.06 <sup>a</sup>
Ash	0.70±0.01 <sup>a</sup>	0.58±0.01 <sup>b</sup>	1.60±0.03 <sup>c</sup>
Fat	1.22±0.05 <sup>a</sup>	0.81±0.04 <sup>b</sup>	1.54±0.02 <sup>c</sup>
Gluten	10.64±0.09 <sup>a</sup>	10.36±0.12 <sup>a</sup>	9.33±0.16 <sup>b</sup>
Protein	11.91±0.10 <sup>a</sup>	11.56±0.17 <sup>a</sup>	13.25±0.10 <sup>b</sup>
Falling number	316±18.63 <sup>a</sup>	428±12.66 <sup>b</sup>	394±5.99 <sup>b</sup>
TFC	1.36±0.66 <sup>a</sup>	0.84±0.57 <sup>b</sup>	1.79±0.79 <sup>c</sup>

\*Mean ± standard error of the mean

\*\*Means in the same row with different superscripts are significantly different ( $P < 0.05$ )

Key: TFC - Total fungi count

#### 4. CONCLUSION

The ash contents of five white wheaten flour samples and the fat content of one white wheaten sample did not conform to SON standards. Also, some flour samples were contaminated with microbial pathogens. Higher levels, more than the legal limits for *E. coli*, *B. cereus*, *Salmonella* spp and *Clostridium* spp and total coliforms in flour compromise the safety, storage and organoleptic characteristics of the final product. The presence of *E. coli* in wheat semolina sample from Bogobiri renders the flour unsafe for human consumption. *Salmonella* spp was detected in 10% of the samples analysed, rendering them hazardous to health and unfit for human consumption, according to SON standards. *Bacillus cereus* and *Clostridium perfringens* levels of all samples investigated conformed to SON standards.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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