Asian Research Journal of Agriculture

10(2): 1-11, 2018; Article no.ARJA.45491
ISSN: 2456-561X

Physicochemical Properties and Bacteriological Profile of Wheat Flours Produced and (or) Sold in Calabar, Nigeria

Jeffrey A. Nosa-Obamwonyi[1*] and Sylvester P. Antai[1]

[1]Department of Microbiology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between the two authors. Author JAN carried out the analyses, performed the statistical analysis, and wrote the first draft of the article. Author SPA designed the study and prepared the final manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2018/45491
Editor(s):
(1) Dr. Tancredo Souza, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal.
(2) Dr. Bilal Ahmad Wani, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu and Kashmir, India.
Reviewers:
(1) A.O. Dauda, University of Ilorin, Nigeria.
(2) Patrícia Matos Scheuer, Federal Institute of Santa Catarina, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/27796

Original Research Article

Received 17 September 2018
Accepted 02 December 2018
Published 17 December 2018

ABSTRACT

This study was aimed at investigating the physicochemical properties and bacteriological profile of white wheaten flour, wheat semolina and whole wheat meal produced and/or sold in Calabar. Ten (10) samples of each flour type was bought from ten strategic locations and analysed. The following physicochemical parameters of the samples were analysed: pH, moisture, ash, fat, gluten, protein, falling number. Microbiologically, total mesophilic aerobic bacteria (TMAB), total coliform count (TCC), *Escherichia coli, Salmonella* spp*, Bacillus cereus and Clostridium perfringens* counts were determined. Results showed that the ash contents of five white wheaten flour samples and the fat content of one white wheaten sample did not conform to SON (Standards Organisation of Nigeria) standards. TMAB and TCC of all samples conformed to SON standards. *Salmonella* spp was identified in 10% of all samples and *E. coli* was identified in one wheat semolina sample. However, *Bacillus cereus* and *Clostridium perfringens* were not detected in any of the samples. One-way analysis of variance (ANOVA) showed that there were significant differences ($P < 0.01$) between the

**Corresponding author: E-mail: prudentjeff@gmail.com;*

three wheat flour types for ash, fat, gluten and protein, falling number, TMAB and TCC. This study highlights the need for product monitoring by relevant regulatory agencies to ensure conformity to standards.

Keywords: Whole wheat meal; wheat semolina; white wheaten flour; physicochemical analysis; bacteriological analysis; Cross River state.

1. INTRODUCTION

Cereals are grasses (family *Poaceae*, also known as *Gramineae*) grown for their edible parts, which comprises the endosperm, germ, and bran [1]. Cereal and cereal-derived products form a substantial food source for the world's population [2]. Production of wheat in Nigeria is low, hence wheat is imported to meet local demand. Also, there has been a steady increase in wheat imports in Nigeria primarily as a result of increasing urban population and changing consumption pattern [3].

Cereal flours are major raw materials utilised for the production of common food products that are highly acceptable and affordable, with good shelf lives. Among the numerous uses of wheat flours, breadmaking represents the foremost one. The uniqueness of wheat stems from its ability to produce gluten, a protein that impart strength and elasticity to dough; hence it is an indispensable element in the production of baked foods [4].

In terms of the components of wheat grain present in flour—the endosperm, the germ, and the bran or fibre part—there are three general flour types: white wheaten flour, wheat semolina and whole wheat meal. 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 contains slightly more bran and germ.

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 the grain during growth and storage. In addition to wheat processing, handling and packaging could also serve as sources of contamination by pathogenic bacteria.

Physicochemical properties of cereal flours are the major determinants of consumer acceptability and safety [5] and the proliferation of microorganisms depends to a large extent on physicochemical parameters. Although considered a safe or low-risk food because of its low water activity, wheat flour-based mixes have been implicated in several incidents of food-borne illnesses. Hence, this study was carried out to investigate the physicochemical properties and bacteriological profile of wheat flours produced and(or) sold in Calabar, Nigeria vis-à-vis Standards Organisation of Nigeria (SON) standards.

2. MATERIALS AND METHODS

2.1 Materials

A total of thirty (30) wheat flour samples, comprising 10 each of white wheaten flour, wheat semolina and whole wheat meal were obtained from ten strategic markets, shops and retail outlets in Calabar, Nigeria. The samples were immediately transported to the laboratory in chilled containers at 4-6^{0}C and subsequently analysed.

2.2 Physicochemical Analysis

2.2.1 Determination of pH

A pH meter (JENWAY 3310, USA) was used to determine the pH of 10% suspension of flour in water after standardizing with buffer at pH 7.

2.2.2 Determination of moisture

Moisture was determined according to AACC [6] method No. 44-19.

2.2.3 Determination of crude ash

This was determined according to AACC [6] method No. 08-01, using muffle furnace (Brabender M110, Germany).

2.2.4 Determination of crude fat

Crude fat from wheat samples was extracted by adopting AACC [6] method No. 30-20 using an extractor (FOSS Soxtec 2043, Denmark).

2.2.5 Determination of gluten

Gluten content was measured using Perten Glutomatic System (Sweden), according to AACC [6] method No.38-12.

2.2.6 Determination of crude protein

Nitrogen was determined by using Buchi AutoKjeldahl-370 (B 811, Switzerland) instrument, according to AACC [6] method No. 46-13 and crude protein was calculated by using a multiplication factor of nitrogen × 5.83 [7].

2.2.7 Determination of falling number

Falling number was determined by using Perten FN 1700 (Sweden) apparatus, according to AACC [6] method No. 02-06.

2.3 Bacteriological Analysis

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

2.3.1 Enumeration of total mesophilic aerobic bacteria

For the enumeration of total mesophilic aerobic bacteria (TMAB), nutrient agar (Oxoid CM 0003, Basingstoke, UK) was inoculated by pour plating 1 ml of each sample, and colony forming units were determined after incubation for 48 hrs at 35°C.

2.3.2 Enumeration of total coliform count

MacConkey agar (Titan Biotech TM337, Delhi, India) was pour-plated, incubated at 37ºC for 24-48 hrs. Distinct colonies were inoculated into nutrient broth (Merck HG000C42, Germany) at 37ºC overnight, sub-cultured repeatedly on nutrient agar to obtain pure cultures and preserved on nutrient agar slant.

2.3.3 Enumeration of *Escherichia coli*

Distinct colonies of freshly prepared plates from the slant cultures (from determination of coliforms) were inoculated on Eosin-methylene blue (EMB) agar (Sigma-Aldrich 70186, USA), using the pour plate method and incubated at 37ºC for 24 hrs. Colonies that exhibited green metallic sheen on EMB agar were presumptively identified as *E. coli*. These isolates were purified by repeated streaking on nutrient agar and conventional biochemical tests such as Gram's reaction, motility test, IMViC test, indole (I), methyl red (M), Voges Proskauer (V), and citrate (C), catalase formation, carbohydrate fermentation (glucose, lactose and sucrose) were carried out for identification purposes.

2.3.4 Enumeration of *Bacillus cereus*

Bacillus cereus agar (Oxoid CM0617, UK) was pour-plated and incubated at 37ºC for 24 hrs [2]. Biochemical tests (gram staining, Voges Proskauer reaction, gelatin hydrolysis, nitrate reduction, tyrosine degradation and lysozyme test) were carried out for the identification of isolates.

2.3.5 Enumeration of *Salmonella spp*

It was carried out by adding one (1) ml of the diluted sample into 10ml of selenite cystine broth (SCB base plus 0.4% sodium biselenite) (Oxoid CM0395, UK) and incubated at 37°C for 18hrs, then sub-cultured onto *Salmonella-Shigella* agar plates (Oxoid CM0099, UK) for 24 hrs at 37°C [2]. Big, black-centred colonies were identified as *Salmonella* spp. These characteristic colonies were then confirmed using a *Salmonella* latex agglutination test kit (Oxoid FT0203, UK).

2.3.6 Enumeration of *Clostridium perfringens*

Zero-point-one (0.1) millimetre of the sample dilutions was spread-plated on tryptose-sulfite-cycloserine (TSC) agar (Oxoid CM 587, UK) containing egg yolk emulsion (Oxoid SR 047, UK) and the plates were overlaid with 10ml of TSC agar without egg yolk emulsion after the inoculum had been absorbed (i.e. after about 5 mins) [2]. When the agar had solidified, the plates were placed in an upright position and incubated for 20-24 hrs at 35°C under anaerobic conditions [8].

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 normalize the distributions. Where zero mean counts or standard error of mean were encountered, one (1) was added before transformation across the three varieties of flour. A one-way analysis of variance (ANOVA) was used to compare means and Tukey's honestly significant difference (HSD) test was used for mean separation. Microsoft Excel 2013 (Microsoft Inc.) and R Statistical Software (R Software Foundation) were used to carry out statistical analysis.

3. RESULTS AND DISCUSSION

The results of physicochemical and bacteriological analysis of samples for white wheaten flour, wheat semolina and whole wheat meal, each in comparison with SON standards, are presented in Tables 1, 2 and 3, respectively.

The physicochemical properties of wheat flours are the major determinants of consumer acceptability, safety [5] and microbiological parameters. Most bacteria grow best at about pH 7 and poorly at pH below 4 [9]. Yeasts and moulds thrive better in low pH food products, where bacteria cannot compete [10]. The pH of all samples in this study ranged from 6.02-6.41 (close to neutral), which agrees with the findings of Hendrich and Bryant [10] as well as that of Ntuli et al. [5], who had a pH range of 5.8-6.5.

Moisture affects shelf life and microbial growth during storage [2,11,12]. Mahmood [12] reported that wheat moisture is majorly dependent on the genetic makeup and is also influenced by the agronomic and climatic conditions of the production area. The moisture contents of all samples conformed to SON [13] standards.

Ash content is an indication of bran in wheat [14]. It is also a measure of the mineral composition of the flour. Wheat variety and growing atmospheric conditions are important determinants of ash content [15]. Wheat varieties with low ash contents contain more endosperm and ultimately yield more flour [16]. 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 standards, while the remaining samples conformed to SON [13] standards.

The germ layer of wheat grain is rich in oils. High fat content may trigger rancidity during storage [17], giving rise to off-flavour in baked or cooked flour product. Fat contents of all samples conformed to SON standards, except one white wheaten flour sample bought at Watt market (WMT1). This violation may be due to the wheat type milled, the growing climatic conditions for the milled wheat [18], or quality control issues.

Wheat variety and environmental conditions are important determinants of gluten content [19]. The amount of gluten is largely determined by the amount of protein content in wheat flours. Flours from strong wheat with higher protein contents produce greater quantities and stronger gluten compared to weak flours [16]. The gluten contents of all samples conformed to SON [13] standards.

Protein has a huge influence on nutritional, functional and technological attributes of flour [18]. Protein quantity is an important factor in the evaluation of wheat quality [19]. Wheat protein has a major impact on the rheological properties of wheat flour dough [20]. According to SON [13], 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 in this study were of good quality in terms of protein content.

The falling number (FN) test is used to measure the level of alpha-amylase activity in wheat or flour, as a means of detecting sprout damage and determining the proper supplementation rates of barley malt, or other amylase enrichment [21]. Sprouting in wheat results in a higher than normal level of alpha-amylase in the flour. Falling number has an inverse relationship with alpha-amylase activity, meaning the higher the alpha-amylase activity the lower the FN value, and vice-versa. Alpha-amylase can cause extensive damage to the structural integrity of starch, making sprouted wheat unsuitable for use in food production [22].

Wheat forms a major component in the dietary intake of inhabitants of Calabar. Flours obtained from it are used for sundry food products. Although considered a safe or low-risk commodity because of its low water activity, wheat flour-based products have been implicated in several food safety incidents.

Total mesophilic aerobic bacteria (TMAB) is widely used to evaluate the general hygienic quality and microbiological load of foodstuffs [23]. It also provides valuable insights into shelf life or changes in organoleptic properties [11]. In this study, none of the samples had TMAB exceeding

Table 1. Physicochemical and bacteriological parameters of white wheaten flour samples

Parameters*	Locations										SON
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	standards
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	314	406	302	283	269	401	259	273	384	264	-
TMAB	1.72 ±0.88	1.85 ±0.83	1.70 ±0.83	1.86 ±0.88	2.72 ±0.99	2.42 ±0.88	1.60 ±0.83	2.46 ±0.99	1.36 ±0.64	2.31 ±1.19	3.00
TCC	1.04± 0.83	1.04 ±0.00	1.15 ±0.64	1.49 ±0.83	2.37 ±0.99	1.94 ±0.88	1.38 ±0.64	2.03 ±0.99	1.04 ±0.83	1.79 ±1.04	-
E. coli		-	-	-	-	-	-	-	-	-	NIL
Salmonella spp	-	-	-	1.23 ±0.82	-	1.43 ±0.52	-	-	-	-	NIL
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	NIL
C. perfringens	-	-	-	-	-	-	-	-	-	-	NIL

**Units: pH = no unit, falling number = sec, other physicochemical parameters = %, bacteriological parameters = Log_{10} cfu/g*

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, TMAB = Total mesophilic aerobic bacteria, TCC = Total coliform count, NIL = Not to be detected

Table 2. Physicochemical and bacteriological parameters of wheat semolina samples

Parameters*	Locations										SON
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	standards
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	401	426	391	503	407	386	433	419	494	422	-
TMAB	1.11 ±0.64	1.00 ±0.83	1.00 ±0.00	1.00 ±0.83	1.80 ±0.64	1.70 ±0.83	1.23 ±0.64	1.99 ±0.64	1.00 ±0.83	1.00 ±0.00	3.00
TCC	0.00 ±0.00	0.60 ±0.64	0.90 ±0.88	0.60 ±0.64	1.26 ±0.64	1.26 ±0.88	0.60 ±0.64	1.64 ±0.99	0.00 ±0.00	0.00 ±0.00	-
E. coli	-	-	-	-	-	-	-	1.63 ±0.82	-	-	NIL
Salmonella spp	-	-	-	-	-	-	-	1.11 ±0.52	-	-	NIL
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	NIL
C. perfringens	-	-	-	-	-	-	-	-	-	-	NIL

**Units: pH = no unit, falling number = sec, other physicochemical parameters = %, bacteriological parameters = Log_{10} cfu/g*

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, TMAB = Total mesophilic aerobic bacteria, TCC = Total coliform count, NIL = Not to be detected

Table 3. Physicochemical and bacteriological parameters of whole wheat meal samples

Parameters*	Locations										SON
	IEM	IGM	IAO	IIM	EEP	WTM1	WTM2	BGB	MBM	MRM	standards
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	406	389	356	407	389	391	418	372	402	411	-
TMAB	2.20 ±0.99	2.36 ±0.99	2.17 ±0.88	2.99 ±1.19	2.56 ±1.19	2.66 ±1.21	2.51 ±0.99	2.20 ±1.10	2.27 ±0.99	2.43 ±1.10	3.00
TCC	1.79 ±0.83	2.15 ±1.04	1.64 ±0.99	2.34 ±0.83	2.42 ±0.99	2.29 ±0.99	1.96 ±1.04	1.94 ±0.88	2.09 ±0.99	1.89 ±0.99	-
E. coli	-	-	-	-	-	-	-	-	-	-	NIL
Salmonella spp	-	-	-	-	-	-	-	-	-	-	NIL
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	NIL
C. perfringens	-	-	-	-	-	-	-	-	-	-	NIL

**Units: pH = no unit, falling number = sec, other physicochemical parameters = %, bacteriological parameters = Log_{10} cfu/g*

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, TMAB = Total mesophilic aerobic bacteria, TCC = Total coliform count, NIL = Not to be detected

the recommended SON [13] limit of 1.0 x 10^3 cfu/g. In a similar work carried out by Berghofer et al. [24] in Australia, only six (6) out of six hundred and fifty (650) samples had TMAB exceeding the limit. This reveals that the number of samples exceeding total aerobic counts of 10^4cfu/g are usually very low as long as hygienic and processing conditions, storage conditions and handling of products are not poor. This, however, does not imply that TMAB below limit is indicative of flour safety, as the presence of certain coliforms even at low levels could result in food poisoning and cause other food-related diseases.

Total coliform counts (TCC) and *Escherichia coli* counts provide information about the general hygienic conditions of flours. The presence of *E. coli* in finished, ready-to-eat foods can be of public health concern, as it may indicate deficiencies in process control, inadequate processing or post-process recontamination [25]. *Escherichia coli* O1157: H7 can lead to fatal illnesses when present at levels as low as 10 cfu/gram [26]. The contamination of the sample (wheat semolina sample obtained from Bogobiri (BGB) market) with *E. coli* can be attributed to post-process contamination arising from exposure of the sample, unhygienic storage conditions as well as constant contact of the product with the vendor during dispensing. Conversely, all other wheat semolina samples which were bought in sealed packs showed zero presence of *E coli.*

According to SON [13] standards for wheat flours, *Salmonella* spp should not be detected in 25 grams of sample. WFP [22] reports that *Salmonella* spp in foodstuff accounts for more than 50% of all food poisoning cases. In this study, *Salmonella* spp was detected in 10% (3) of samples investigated. In studies by other authors, *Salmonella* spp were not detected in Australian (n = 650) [27] and Turkish flours (n = 27) [28].

Bacillus cereus can be isolated from a variety of foods, especially from carbohydrate-rich foods (rice, cooked pasta), puddings, salads, and vegetable sprouts [29]. Berghofer et al. [24] reported low levels (0.3 MPN g^{-1}) of *B. cereus* in Australian flour. In a study by Aydin et al. [2], only 6 (4.2%) samples of a total of 142 wheat flour samples from the Thrace region contained *B. cereus* and all counts were below the acceptable limit of the Turkish Food Codex. In this study, *B. cereus* was not detected in any of the thirty samples analysed, which is in conformity with SON [13] standards for the three wheat flour types.

Clostridium perfringens strains are easily isolated from raw food samples [30] and are known to be a common cause of food poisoning. However, in this study, none of the samples analysed contained *C. perfringens.* This is similar to the result of a Turkish study carried out by Alp et al. [28] on wheat flour samples, where *C. perfringens* was not detected in a total of 27 samples analysed.

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 three wheat flour types because the final moisture content of flours is largely dependent on the amount of water added during tempering with the aim to producing flours which conform to specific standards. In this case, all three wheat flour types have the same standard of 14.0%.

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

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	4.66×10^{-13}**
Gluten	29.01	1.88×10^{-7}**
Protein	51.171	6.52×10^{-10}**
Falling number	18.44	8.93×10^{-6}**
TMAB	24.36	8.99×10^{-7}**
TCC	22.46	1.8×10^{-6}**

***Significant at 1% alpha level (two-tailed)*
KEY: TMAB = Total mesophilic aerobic bacteria, TCC = Total coliform count

There was a significant difference between the ash contents of the three wheat types and Tukey's HSD test revealed significant differences between all three possible comparisons of the three wheat flour types. This reflects the different levels of bran present in the 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

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[a]	6.17±0.03[a]	6.25±0.03[a]
Moisture	12.79±0.18[a]	13.18±0.13[a]	12.97±0.06[a]
Ash	0.70±0.01[a]	0.58±0.01[b]	1.60±0.03[c]
Fat	1.22±0.05[a]	0.81±0.04[b]	1.54±0.02[c]
Gluten	10.64±0.09[a]	10.36±0.12[a]	9.33±0.16[b]
Protein	11.91±0.10[a]	11.56±0.17[a]	13.25±0.10[b]
Falling	316±18.63[a]	428±12.66[b]	394±5.99[b]
TMAB	2.00±0.90[a]	1.28±0.59[b]	2.44±1.06[c]
TCC	1.53±0.77[a]	0.69±0.53[b]	2.05±0.96[c]

**Mean ± standard error of the mean*

[+]Means in the same row with different superscripts are significantly different (P < 0.05)

KEY: TMAB = Total mesophilic aerobic bacteria, TCC = Total coliform count, WWF = White wheaten flour, WS = Wheat semolina, WWM = Whole wheat meal

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 contain the endosperm. However, during milling of white wheaten flour, parts of the germ may also 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 significant differences between the gluten contents of white wheaten flour and whole wheat meal, wheat semolina and whole wheat meal, whereas no significant difference was obtained between wheat semolina and white wheaten flours. These significant Tukey's HSD results can be attributed to the fact that gluten proteins are contained in the endosperm of wheat from which wheat semolina and white wheaten flours are obtained. The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of total flour protein and confer properties of elasticity and extensibility that are essential for the functionality of wheat flours [31], while the remaining percentage comes from germ and bran proteins.

Results of analysis of variance and Tukey's HSD test for crude protein are the same as for gluten. The significant Tukey's HSD results between whole wheat meal and wheat semolina and between whole wheat meal and white wheaten flour can be attributed to the fact that both white wheaten flour and wheat semolina contain mainly gluten proteins, while whole wheat meal contains germ and bran proteins in addition to gluten proteins.

There was a significant difference between the falling number values 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. These significant Tukey's HSD is likely due to supplementation of white wheaten flour with enzyme enrichments or additives, resulting in higher amylase activity, however, wheat semolina and whole wheat meal are usually not supplemented with amylase.

There was a significant difference between TMAB of the three wheat flour types and a similar result was also obtained for TCC. Tukey's HSD test showed that TMAB and TCC of the three wheat flour types were significantly different from each other. These can be attributed to high ash contents resulting from the bran, which harbours a wide range of microorganisms. Flours that are low in bran or ash will normally have low levels of microorganisms [32] due to milling (dehulling) which concentrate over 90% of total microorganisms present on the wheat bran. Wheat semolina had the least ash content and microbial count, whereas the whole wheat meal had the highest ash and microbial count. This means that an increase in percentage ash content is directly proportional to an increase in microbial contents of samples.

The inner semolina fraction has low levels of microorganisms and is the cleanest mill product. Removal of bran in wheat reduces the level of ash (biomass) in the final product, concomitantly reducing the levels of microorganisms, thereby improving product safety. Nevertheless, important minerals and nutrients are lost as the

ash content of wheat grains is reduced but this is usually solved by food fortification [5].

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.

REFERENCES

1. Food and Agricultural Organization (FAO) 2006. FAOSTAT Database. Accessed on 9 September 2010. 2006; Available:www.fao.org
2. Aydin A, Paulsen P, Smulders FJ. The physicochemical and microbiological properties of wheat in Thrace. Turkish Journal of Agriculture and Forestry. 2009;33:445-454.
3. Food and Agricultural Organization (FAO). Africa Report No. 1, 5;2005.
4. Akpe AR, Usuoge PO, Enabulela OI, Esumeh FI, Obiazi HA, Amhanre IN, et al. Bacteriological and physicochemical quality of wheaten white bread flour made for Nigerian market. Pakistan Journal of Nutrition. 2010;9:1078-1083.
5. Ntuli V, Mekibib SB, Molebatsi N, Makotoko M, Chatanga P, Asita OA. Microbial and physicochemical characterization of maize and wheat flour from a milling company, Lesotho. Internet Journal of Food Safety. 2013;15(2013):11-19.
6. American Association of Cereal Chemists (AACC). Approved methods of the American association of cereal chemists. 10th ed. St. Paul, USA; 2000.
7. Watt BK, Merril AL. Composition of Foods: Raw, Processed and Prepared (Ag Handbook No. 8). Washington DC: United States Department of Agriculture; 1975.
8. Rhodehamel EJ, Harmon SM. *Clostridium perfringens*. In: Bacteriological analytical manual online (Chapter 16). Accessed on 9 September 2012. 2001; Available:http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070878.htm.
9. ICMSF. Microorganisms in foods: microbial ecology of food commodities. London: Blackie Academic and Professional; 1998.
10. Hendrich S, Bryant CM. Food mycotoxins: An update. Journal of Food Science. 2006;71:51-65.
11. Batool SA, Rauf N, Tahir SS, Kalsoom R. Microbial and physicochemical contamination in the wheat flour of the twin cities of Pakistan. International Journal of Food Safety. 2012;14:75-82.
12. Mahmood A. Acid-PAGE gliadin composition and cluster analysis for quality traits for different wheat varieties. Doctoral dissertation, Department of Food Technology, University of Agriculture Faisalabad, Pakistan; 2004.
13. SON (Standards Organization of Nigeria). Standards for wheat flour. NIS: 121; 2000.
14. Ekinci R, Unal S. Some properties of flour types produced in different regions of Turkie II. Rheological properties. Gıda/The Journal of Food. 2003;3:201-207.
15. Buriro M, Oad FC, Keerio MI, Gandahi AW, Laghari GM. Impact of storage sources on physico-chemical properties of various wheat varieties. Sarhad Journal of Agriculture. 2012;28(2):185-190.
16. Keran H, Salkic M, Odobasic A, Jasic, M, Ahmetovic N, Sestan I. The importance of determination of some physical-chemical properties of wheat and flour. Agriculturae Conspectus Scientificus. 2009;74(3):197-200.
17. Shobha D, Prasanna F, Kumar MK, Sreemasetty TA. Effect of antioxidant on the shelf life of quality protein maize flour. Indian Journal of Fundamental and Applied Life Sciences. 2011;1:129-140.
18. Iqbal Z, Pasha I, Abrar M, Masih S, Hanif, MS. Physico-chemical, functional and rheological Properties of wheat varieties. Journal of Agricultural Research. 2015;53(2).

19. Khan MR, Anjum FM, Zahoor T, Nawaz H. Biochemical and technological characterization of Pakistani spring wheats. Pakistan Journal Agricultural Sciences. 2009;46(4):271-279.
20. Gulzar A, Saeed MK, Ali MA, Ahmad I, Ashraf M, Haq IU. Evaluation of physiochemical properties of different wheat (*Triticum aestivum* L.) varieties. Pakistan Journal of Food Sciences. 2010;20(1-4):47-51.
21. Doty NC. The falling number method. Bakers Digest. 1981;4:23-25.
22. Dencic S, DePauw R, Kobiljski B, Momcilovic V. Hagberg falling number and rheological properties of wheat cultivars in wet and dry preharvest periods. Plant Production Science. 2013;16(4):342-351.
23. Morton RD. Aerobic plate count. In: Downes P & Ito K, editors. Microbiological examination of foods. American Public Health Association, Washington DC; 2001.
24. Berghofer LK, Hocking AD, Miskelly D, Jansson E. Microbiology of wheat and flour milling in Australia. International Journal of Food Microbiology. 2003;85:137-49.
25. Deibel KE, Swanson KM. Cereal and cereal products. In: Downes PF, Ito K, editors. Microbiological examination of foods. American Public Health Association, Washington D.C.; 2001.
26. World Food Program (WFP). Food quality control. Accessed 15 November 2012; 2012. Available: www.wfp.org.
27. Abang D, Zaidel N, Chin Y, Yusof A, Abd Rahman R. Analysis and correlation studies on gluten quantity and quality during production. Journal of Applied Sciences. 2009;9:1686-1694.
28. Alp A, Vural A, Erkan ME, Alp SY. The microbiological and physico-chemical quality properties of wheat samples, in Turkey, In: Proceedings II. National Veterinary Food Hygiene Congress, Istanbul, pp. 769-770; 2006.
29. Bennett R, Belay N. *Bacillus cereus*. In: Downes P, Ito K, editors. Microbiological examination of foods. American Public Health Association, Washington DC; 2001.
30. Ugur M, Nazlı B, Bostan K. Food Hygiene. Istanbul: Technical Publications; 2001.
31. Shewry PR., Tatham AS, Barro F, Barcelo P, Lazzeri P. Biotechnology of breadmaking: Unravelling and manipulating the multi-protein gluten complex. Biotechnology. 1995;13:1185–1190.
32. Sperber W. Role of microbiological guidelines in the production and commercial use of milled cereal grains: A practical approach for the 21st century. Journal of Food Protection. 2007;70:1041-1053.

© 2018 Nosa-Obamwonyi and Antai; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27796