

1 Mycological & Physico-Chemical Quality of Wheaten White 2 Bread Flour Made for Nigerian Market

3 Akpe¹

4 ¹ Ambrose Alli University

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6

7 **Abstract**

8 Mycological and physico-chemical quality of wheaten white bread flour, made for Nigerian
9 market was examined at room temperature of storage for 120days (about four months).
10 During storage, total fungal count was above the maximum acceptable limit of 100 cfu per
11 gram white bread flour. Fungal counts increased towards the end of the storage period but no
12 significant difference of fungal count was noticed during storage. Also slight ecological
13 succession was noticed amongst the various groups of fungi. The fungal isolates from this
14 study were species of Penicillium, Rhizopus, Mucor, Geotrichum, Oidium, and Saccharomyces.
15 Three of the four brands of flour analysed had a pH of below 6.0 on the 105th day of the
16 study. The ash content of the various brands of flour was above 0.65

17

18 **Index terms**— Mycological, physico-chemical, wheaten, flour, bread

19 **1 INTRODUCTION**

20 heaten white bread flour consists mainly of ground endosperm of the wheat (*Triticum* species) kernel ??Badshah
21 et al., 2005). There are several commercial grade of wheat flour and, the flour is made from different blends of
22 wheat. The composition of the flour is therefore variable and the quality of the flour may differ according to
23 geographical region, milling process, and the quality of the wheat ??Quaglia, 1984).

24 Physico-chemical properties such as fat, carbohydrate, protein, moisture, ash, gluten and pH are of
25 technological and nutritional importance. The proportion of these factors in the flour depends on the variety
26 of wheat grain used and also depends on the standards recommended by the particular country's industrial
27 standards (Adeyemi, 2003; ??adshah et al., 2005).

28 The standards for wheat flour (white flour) as recommended by Standards Organization of Nigeria (SON) and
29 International Standards required that the flour Author : Author :

30 Author :

31 Author : be free from rancidity, objective odour, insects, rodents' hair and any other extraneous material
32 ??SON, 2000).

33 The quality of flour and storage condition after milling is very important in the shelf life of the flour. Studies
34 have revealed that gradual changes of physicochemical properties occur in the flour during storage (Kent-Jones
35 and Amos, 1967; Sur et al., 1993;Hruskova and Machova, 2002).

36 Mould growth has a detrimental effect on the quality of flour (Weidenborner et al., 2000). A number of mould
37 and yeast have been isolated from wheat flour and these fungi are responsible for the enzymatic activity in the
38 flour. In a study in Germany, it was discovered that the overall degree of mould and mycotoxin contamination
39 was lowered with decreasing ash content (Schollenberger et al., 2002). This suggests a localization of the fungi
40 primarily in the outer part of the wheat kernels. The recommendation for total mould count in Nigerian flour
41 is 100 per gram of flour (SON, 2000). There is very little or no information on the Mycological and Physico
42 -chemical quality of flour in the Nigeria market. This survey is intended to augment the scarce information on
43 the Mycogical and Physicochemical quality of Nigeria flour.

44 **2 II.**

45 **3 MATERIALS AND METHODS**

46 **4 a) Sample Collection**

47 Freshly milled wheaten white flours ready for packaging were collected from four mills located at Lagos, Sapele,
48 Ewu and Kano, all in Nigeria. Two samples were collected from each location in clean polythene bags and
49 properly sealed. The samples were kept in the laboratory at room temperature and observed for bacteriological
50 and physico -chemical changes. Samples were aseptically opened and analysed at 15 days intervals for a period
51 of 4 months; this period was based on the assumed shelf -life of 3 -4 months of the flour by the millers.

52 **5 b) Mycological Analysis**

53 The various types and numbers of mould and yeast associated with wheaten white bread flour were enumerated
54 and quantified according to the method described by Harrigan and McCane (1976). Isolation of fungi was carried out using potato dextrose agar (PDA) (LABM) supplemented with chloramphenicol to inhibit
55 bacterial growth. The media were incubated at 35°C for 72 hours. Total fungi were estimated as colony forming
56 units per gram (cfu/g) of flour.

58 **6 c) Characterization And Identification of Isolates**

59 The fungi isolates were identified based on the examination of the conidial heads, phialides, conidiophores and
60 presence or absence of foot cell or rhizoids (Samson and Reemon-Hoekstra, 1888). Wet preparations of actively
61 growing fungi were placed on a glass slide with a methylene blue stain, covered with a cover slip and observed
62 with X40 objective under the microscope.

63 **7 d) Determination Of Physico -Chemical Properties of Flour
pH**

65 A pH meter (JENWAY 3310) was used to determine the pH of 10% suspension of flour in water after standardizing
66 with buffer at pH 7. A standard buffer 7 powder was prepared into 200ml solutions with distil and ionise in a
67 volumetric flask. The buffer solution was poured into a beaker and the pH electrodes immersed in and regulated
68 to stabilize at pH 7. There after, the electrodes were removed and introduced into the filtrate from the 10% flour
69 suspension and allowed to stabilize and the final pH reading to be taken.

70 **8 Moisture**

71 Moisture content was determined using the dry oven method (Polemeranz and Meloan, 1996).

72 **9 Gluten**

73 Extraction of gluten was done according to the ICC (international cereal chemistry) -Standards No 106/1. Protein
74 Analysis of protein content was done using the Kjeldahl method. The sample was heated in sulphuric acid and
75 digested until the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed into
76 ammonium sulphate. The concentrated sodium hydroxide is added and the digest heated (distillate) to drive off
77 the liberated ammonia into a known volume of standard acid solution. The unreacted acid is determined and
78 the results are transformed by calculation with factor 5.7 into a percentage of protein in the flour sample.

79 **10 Carbohydrate**

80 This was estimated according to the ICCstandard No. 123, method for the determination of starch content by
81 hydrochloric acid dissolution.

82 **11 Fat**

83 Extraction of fat was performed by the Soxtec method in automatic fat extraction unit using diethyl ether.
84 Determination of flour ash was carried out according to the ICC -standards No. 104, for the determination of
85 flour ash at 900°C. The difference in weight was used to estimate the crude ash; based on the moisture content
86 of the flour, the ash on dry matter of the flour was calculated.

87 **12 e) Statistical Analysis**

88 Changes in bacteriological and physicochemical qualities due to duration of storage for the different brands were
89 analysed for statistical significance using the chi -square goodness of fit. Differences in the above qualities among
90 the different flour brands were tested for statistical significance using the Single Factor Analysis of variance
91 (ANOVA). Where significant differences were detected, the Duncan's Multiple Range (DMR) test was used to
92 separate means on the basis of significance. All statistical tests were carried out using the "SPSS10.0 package".

93 13 f) Results

Average fungi counts for the brands of flour ranges from 3.357×10^3 cfu/g (Brand 4) to 10.144×10^3 cfu/g (Brand 1) (Table 10). Significant difference ($P = 3.153$) was recorded in fungi count flour brands. During storage, significant difference ($\chi^2 = 55.988$) was recorded for fungi count only in flour Brand 1, with day 0 having 27.65×10^3 cfu/g total fungal counts. Brands 2, 3 and 4 show no significant difference in fungal counts during storage (table 1). One yeast and five moulds were isolated (Table 2). The difference in the moisture content of the individual brands of flour is highly significant ($P = 21.966$) but there is no significant difference in moisture content of flour during storage. There was no significant difference ($P = 0.479$) in pH of individual flour. The pH ranges from 6.03 (brand 1) to 6.12 (brand 3) (table 10). Protein and gluten content of the individual flour shows highly significant difference ($P = 18.517$). Protein and gluten for brand 2 is 11.47% and 10.23% and for brand 4 is 10.24 and 8.64 respectively. Gluten content correlates with the protein content. Carbohydrate content was between 65 -66% in all the brands of flour with no significant difference ($P = 0.248$). Ash content increases for the individual brands of flour during storage, but statistically, there is no significant difference (table 8).

115 14 ISCUSSION

116 Total fungi counts of the flour during storage (Table 1) show no significant difference for only Brand 1 ($x\bar{ } =$
117 55.988), but no significant difference was observed for the other flour Brands. Fungal counts from among the
118 various Brands of flour show a significant difference ($P = 3.153$), flour Brand 1 has above 10^4 cfu/g and the
119 other Brands below 5×10^3 cfu/g (Table 10).

Fungi thrive better at lower pH and this was reflected in the increase in the fungal counts at day 105 as the pH in all the flour Brands (Tables 1 and 4). Flour Brand 1 shows an increase in fungal count from 13.5×10^3 cfu/g 9day 90) to 14.0×10^3 cfu/g day 105) with a corresponding decrease in pH from 6.20 to 5.64. Corresponding in fungal counts were also observed in the other flour Brands (Tables 1 and 4). The increase in fungal counts for all the flour Brands at day 60 can be associated with the increase in moisture content of the flours. This finding is in agreement with the reports of Mashhood et al. (2000) that mould growth in flour is favoured by high moisture content. Penicillium species was isolated throughout the storage period of the flour. This finding corresponds with previous studies that Penicillium species are among the dominant fungi in wheat and wheaten flour. Kent-Jones and Amos ??1967) reported about 90% Penicillium of the total mould isolated from white flour and Weidenborner et al. (2000) also reported 15% Penicillium of the numerous mould counts (1.730×10^3 cfu/g) of white wheat flour.

131 Newer fungal groups begin to emerge as the storage progresses as a result of ecological succession with the yeast
 132 Saccharomyces being isolated at day 30 and 60 (Table 2). The isolation of yeast can be supported with increase
 133 in starch content at day 30 and 60. This finding correlates with previous report that yeast multiplication in flour
 134 occurs as a result of the high starch content (Kent-Jones and Amos, 1967). Other fungi such as the Odium, Mucur,
 135 Geotrichum were later isolated, this also is supported by previous studies that ecological succession occurs during
 136 storage of flour with Penicillium appearing after Aspergillus and followed later with Mucur, Odium, Geotrichum
 137 e.t.c. (Kent-Jones and Amos, 1967; Weidenborner et al., 2000 and Schollenbeger et al., 2002) a number of
 138 fungal species had been isolated from white wheat flour (Weidenborner et al., 2000). Moulds usually contaminate
 139 the wheat from the field, despite the screening the wheat may pass through, the spores of the moulds cannot
 140 be completely eliminated since they are more resistant to heat and other chemicals. The effect can easily be
 141 observed after few days of storage of baked goods and even in the flour itself when left for long in the store.
 142 Mould growth usually produce undesirable odour in the flour products.

143 The increase in ash content of the flour may have encouraged proliferation of fungi. High ash content shows
144 that there is much bran (the outer covering of the wheat grain) in the flour. This finding corresponds with the
145 report of Schollenberger et al. (2002), that overall mould contamination was lower with decreasing ash content
146 suggesting the localization of the fungi in the outer part of the wheat kernel. Total mould counts in all the flour
147 Brands and during storage were above the standards of < 100 cfu/g (10 2 cfu/g) (Tables 1 and 10) recommended
148 for Nigerian wheaten white flour ??SON, 2000). Initially the ash contents for Brands 1 and 2 (Table 8) was
149 within the acceptable limit value of < 0.65% ??SON, 2000). Ash content however increased above the acceptable
150 level for all the flours as from day 90 of storage. Flour Brands 3 and 4 have ash contents above 0.65 throughout
151 the storage period (Table 8). There is however a significant difference in the ash content of the individual Brands
152 of flour ($P = 7.292$) with flour Brands 1 and 2 having values of 0.56% and 0.63% respectively and Brands 3 and
153 4 of 0.69% and 0.80% respectively (Table 10).

15 CONCLUSION

154 The average fat content in the different brands flour shows no significant difference ($P = 0.915$) (Table ??).
155 The value obtained for fat is acceptable as regarded $<1.5\%$ fat content for Nigerian white wheat flour ??SON,
156 2000). Intermittent was noticed protein content of the various brands of flour during storage. Flour brand 4
157 shows decrease in protein content from 10.02% (day 75) to 9.85% (day 105) and flour brand 2 shows a decrease
158 in protein content from 11.64% (day 30) to 11.34% (day 90). The decrease noticed in the protein content of
159 the flour corresponds with earlier reports that protein content flour decreases during storage (Sur et al., 1993;
160 Hruškova and Machova, 2002).

161 The changes in protein content of the flour was however not significant, but average protein content for the
162 individual brands of flour shows highly significant difference ($P = 18.517$) with brand 1 having 11.46% and brand
163 4; 10.24% (Table 10). Gluten content was seen to correlate with the total protein content as it also decreased
164 slightly with storage (Table 6 and 7). This finding corresponds with previous reports of Sur et al, (1993) and
165 Hruškova and Machova (2002).

166 IV.

167 15 CONCLUSION

168 Wheaten white flour also referred to as the "all purpose" flour, because of unequal ability to produce gluten
169 is used for several bakery products such as bread, pizzas, cakes and pastries, which are major supplements for
170 breakfast. Wheat flour has high nutritional value, and hence is highly susceptible to spoilage. Fungi are primarily
171 responsible for deterioration of grain especially when conditions of storage are favourable. This can be observed
172 from the isolation of several moulds and yeast and the occurrence of ecological succession. There is therefore
need to develop on methods to improve on and ^{1 2}

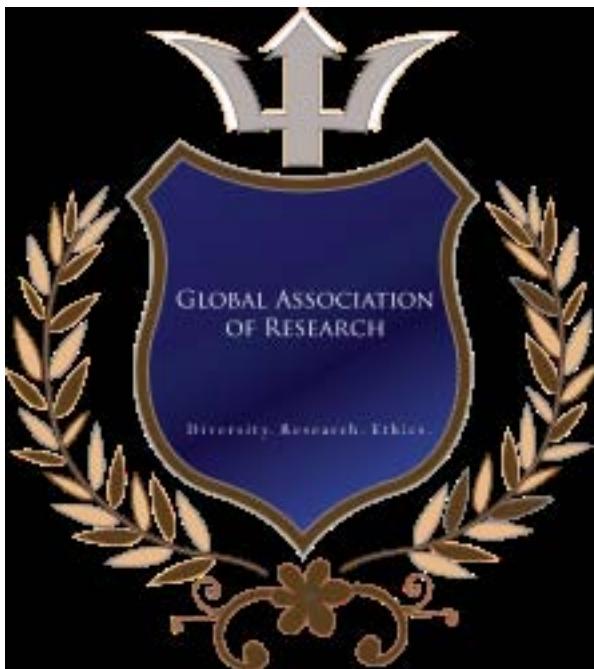


Figure 1: Ash

1

NOTE : $P > 0.05$ = not significantly different

$P < 0.001$ = highly significantly different

Figure 2: Table 1 :

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Figure 3: Table 2 :

3

FLOUR BRANDS

MOISTURE CONTENT (%)
 DAY DAY DAY
 0 30 45 60 75
 DAY
 15

1 12.92 ± 0.02 12.85 ± 0.01 12.48 ± 0.37 12.26 ± 0.06 12.92 ± 0.05 13.03 ± 0.00 13.16 ± 0.06 12.97 ± 0.16
 2 13.00 ± 0.06 12.67 ± 0.04 12.53 ± 0.19 12.15 ± 0.01 12.79 ± 0.02 12.98 ± 0.09 13.00 ± 0.01 13.00 ± 0.00
 3 11.93 ± 0.08 11.89 ± 0.31 11.25 ± 0.05 11.60 ± 0.44 12.02 ± 0.05 12.27 ± 0.40 11.91 ± 0.10 11.92 ± 0.08 P > 0.05
 4 13.65 ± 0.08 13.23 ± 0.01 13.19 ± 0.01 13.22 ± 0.02 13.71 ± 0.03 13.82 ± 0.13 13.80 ± 0.07 13.85 ± 0.00

NOTE : $P > 0.05$ = not significantly different

Figure 4: Table 3 :

4

Figure 5: Table 4 :

5

Figure 6: Table 5 :

6

NOTE : $P > 0.05$ = not significantly different

Figure 7: Table 6 :

7

NOTE : $P > 0.05$ = not significantly different

Figure 8: Table 7 :

8

NOTE : $P > 0.05$ = not significantly different

Table 9 : Changes in Fat Content (%) of Wheaten White Bread Flour During Storage

$P > 0.05$ = not significantly different

Figure 9: Table 8 :

10

NOTE :

PARAMETERS

BRAND 1

BRAND 2 BRAND 3 BRAND 4 SIGNIFICA

$\bar{N} \pm SD$

$\bar{N} \pm SD$

$\bar{N} \pm SD$

$\bar{N} \pm SD$

MOISTURE

$12.82 b \pm 0.11$ $12.77 b \pm 0.31$ $11.97 a \pm 0.58$ $13.56 c \pm 0.29$ $P < 0.001$

pH

6.03 ± 0.09 6.07 ± 0.04 6.12 ± 0.04 6.05 ± 0.03

$P >$

0.05

CARBOHYDRATE

65.31 ± 0.97 65.46 ± 0.65 65.87 ± 1.13 66.26 ± 1.12

$P >$

0.05

PROTEIN

$11.46 b \pm 0.04$ $11.47 b \pm 0.04$ $11.09 b \pm 0.15$ $10.24 a \pm 0.11$ $P < 0.001$

GLUTEN

$9.96 b \pm 0.08$ $10.23 b \pm 0.37$ $10.28 b \pm 0.48$ $8.64 a \pm 0.19$

$P <$

0.001

ASH

$0.56 a \pm 0.07$ $0.63 b \pm 0.06$ $0.69 c \pm 0.07$ $0.80 c \pm 0.02$

$P <$

0.001

FAT

0.94 ± 0.02 0.94 ± 0.03 0.92 ± 0.04 0.98 ± 0.11

$P >$

0.05

FUNGAL COUNT (X 10³ CFU/g) $10.14 b \pm 3.13$ $4.50 a \pm 1$ 24 $3.50 a \pm 0.87$ $3.36 a \pm 0.71$ $P < 0.05$

NOTE : Those with similar alphabet are not significantly different from each other.

$P > 0.05$ = not significantly different

$P < 0.05$ = significantly different

$P < 0.001$ = highly significantly different

Figure 10: Table 10 :

-
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