ASSESSING THE PROXIMATE COMPOSITION AND SHELF-LIFE OF DIFFERENT BRANDS OF BREAD ON THE MARKET IN GHANA

Dzomeku Mensh Beloved[1]*, Timuub Benjamin Makimilua[2], Akweiba Peace[2], Addo John.Kwaku[1], Appiah-Kubi Zeppora[1]
[1]CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana, West Africa
[2]College of Agriculture Education, University of Education, Winneba, Mampong Campus, P.O. Box 40, Mampong-Ashanti, Ghana
*E-mail: bmdzomeku@gmail.com

Abstract
This study was conducted to compare the nutritional status of three brands of freshly baked breads available on the Ghanaian market. The proximate composition of the freshly baked bread samples were analysed for moisture, carbohydrate, crude protein, crude fat, crude ash, and crude fibre. The shelf life was determined by storing in refrigerator and under room temperature. In storage microscopic identification of fungal organisms on bread was determined. Results revealed that sugar bread packaged in aluminum foil and old news print stored better for four days under ambient conditions. The fungal species identified to have infected the bread types in storage included Penicilium sp and Rhizopus sp., Aspergillus flavus, and A. niger The high incidence of fungal infestation on butter bread could be attributed to the nutrient level. Also, it could be attributed to the heat generated by the synthetic nature of the polyethylene hence increase in humidity on the package. The proximate analyses of three bread types (sugar, butter and wheat) showed that the raw material (flour) used for the three bread types was composite flour. There was a significant difference between the bread types with regard to carbohydrates, crude protein, crude fibre, and ash contents. There was however no significant difference in the crude protein level between sugar and butter bread. The results further revealed higher crude fat content in wheat than butter bread. There was a significant difference (P>0.05) in the Crude fibre content between wheat and butter bread. There was a significant difference in the ash content between wheat, sugar and butter bread types. Wheat bread had the highest ash content. As regards the moisture content, wheat bread had the highest compared to sugar and butter bread types. There was no significant difference in the moisture content between sugar and wheat.

Keywords: bread, proximate analysis, shelf-life, packaging material, fungi

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1. INTRODUCTION

Bread is not only consumed in all countries of the world but also the most acceptable form of food. It is an important stable food, the consumption of which is increasing. It is however, relatively expensive, being made from imported wheat that is not cultivated in the tropics (Edema *et al*., 2005; Olaoye *et al*., 2006). It has always been a popular and appealing food product due to its superior nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness (Giannou and Tzia, 2007). Wheat flour products have gained wide consumer acceptance for many years in the tropics (Abulude et al., 2005; Tsatsu, 2009). In recent times, emphasis has been on healthy bread with low glycemic index, more protein that will increase the dietary fibre intake, high resistant starch and decrease in calories and carbohydrates of baked goods. Over dependence on wheat bread has adversely affected several economies in the tropics when its production experiences a downward trend. The price of wheat bread shoots up under such conditions as the market price skyrockets. A lot of efforts has been made and still being made to promote the use of composite flours in which flour from locally grown crops and high for use in bread production, thereby decreasing the demand for imported wheat and producing protein seeds replace a portion of wheat flour protein-enriched bread (Giami *et al*., 2004; Olaoye *et al*., 2006). Composite breads are made from blends of wheat and non-wheat

flours. Breads were prepared from composite flours such as fababean, cottonseed and sesame flour (Abdel-Aal *et al.,* 1993); corn, barley and cassava (Khalil *et al.,* 2000); breadnut flour (Oshodi *et al.,*1999); pumpkin and canola seed flour (Mansour *et al.,*1999); soybeans flour (Gahlawat and Sehgal, 1998), legume flour (Sadowska *et al.*, 2003), full-fat or defatted cocoa powder (Aremu *et al.*, 1995), peanuts and sunflower seed (Fagbemi *et al.*, 2005); beniseed (Afolabi *et al.*, 2001); sweet potato flours (Collado *et al.*, 1997); coconut flour (Trinidad *et al.,* 2006). All these ingredients will impart characteristic colours, texture and nutritional value which may be favourable in bakery products, recipes and other food products. The composite flours are advantages to the developing countries because the flour could reduce wheat imports and increased the potential use of locally grown crop (Hugo *et al.,* 2003).

The idea of substituting part of wheat with other starchy crops is not new. Several institutions have carried out research designed to find ways of partially substituting wheat flour with other sources of flour or replacing wheat altogether (Bokanga, 1995). With the constant increasing consumption of bread and other baked products in many countries, the composite flour programme promises to save significant amount of foreign exchange, provide a traditional nutritious food to more people at lower cost and to utilize indigenous crops to a greater extent. The product is basically made of hard wheat flour, yeast, fat sugar, salt and water. Different bread types exist in Ghana; it could be butter, sugar or salt based. It could also be composite flour bread. Nonetheless, they all have wheat flour base. Most often these composite bread types are not labelled as such for consumers to know what they are purchasing. The consumption of bread in Ghana as a staple food has steadily been on the increase, especially with explosions in population and changing life style patterns. Hence bakers take advantage and sell composite bread as sole wheat bread. It is worth noting that bread is one food that knows no social stratification, as such is consumed by all and sundry irrespective of their per capita income. Bread from the wheat flour has certain desirable aesthetics due to its gluten content (Nickerson and Ronsivalli, 1980).

Fresh bread is prized for its taste, aroma, quality and texture. Retaining its freshness is important to keep it appetizing. Bread that has stiffened or dried past its prime is said to be stale. During storage, bread undergoes staling; staling of bakery food generates major concerns. Economically, losses to the baking industry from stale unsalable bread are estimated in the order of 8 % of total production.The objective of this study was to evaluate the proximate composition and shelf-life of bread brands on the Ghanaian market.

2. MATERIALS AND METHOD

Three types of bread (wheat, butter and sugar) were purchased from three sources in Koforidua and Nkawkaw in the Eastern region of Ghana. Samples were sent to the laboratory in Kumasi for the storage, microbiological and proximate analysis. Shelf-life study was conducted using packaging materials as news print, aluminium foil and polyethylene. Bread samples were packaged in news print, aluminium foil and polyethylene bags. The study was a 3 x 3 x 2 factorial in randomised complete block design with three replicates. The samples were stored under room temperature conditions (25^{o}C) and refrigerator condition (4^{o} C) for 24 days.

The treatments were replicated three times. Samples of the fresh bread were earlier collected for proximate analysis before storage. In storage samples were collected at intervals for microscopic identification of fungal growth. The samples were examined under the stereomicroscope for habit characters of the fungi present. Appearance, type and pattern of growth, arrangement of conidia on conidiophores, conidia shape and colour and the extent of growth were some of the traits that were examined. Identification of the fungal species and the level was done using the compound microscope.

Once, a fungus is identified to the species level, the extent of growth was scored on a scale of 1 -5 interpreted as
1 – 0-5% coverage on sample
2 – 6%-20% coverage on sample
3 – 21%- 50% coverage on sample
4 – 51%-70% coverage on sample
5 – Whole sample covered
Proximate composition of the freshly baked bread samples were analysed using AOAC (1990) for moisture, carbohydrate, crude protein, crude fat, crude ash, and crude fibre. Samples of the bread types were taken again 10 days in storage to determine proximate composition. Data was analyse using ANOVA. The means were separated by Least Significant Difference (LSD) at P>0.05.

3. RESULTS AND DISCUSSION

Three brands of bread samples were randomly collected from three companies for proximate composition and shelf life study. The study revealed that sugar bread packaged in aluminum foil and paper and stored for four days under ambient conditions did not develop bread mould (Table1).

Table 1. Wheat bread storage in three packaging materials under ambient conditions

Days in storage	Type of packaging material		
	Aluminium foil	News Print	Polyethylene
4	Zero	Zero	Mould
7	Mould	Zero	Mould
11	Mould	Zero	Mould
19	Mould	Zero	Mould
24	Mould	zero	*Penicelium* (2)

Figures in parenthesis show the concentration level on the scale 0-5. Where 0 is nil and 5 is 100% inoculum

Table 2. Sugar bread storage in three packaging materials under ambient conditions

Days in storage		Type of packaging material			
	Aluminium foil		News Print	Polyethylene	
		Percentages (%) cover			% Coverage
4	Zero	0	Zero	Zero	
7	*Penicilium* *Rhizopus*	6-20 6-20	Zero	*Penicilium* Mould	6-20
11	*Penicilium* *Aspergillus flavus* A. niger	21-50 21-50 6-20	Zero	*Penicilium* *Aspergillus flavus*	6-20 6-20
19	*Penicilium* *Aspergillus flavus* *A. niger*	21-50 21-50 21-50	Zero	*Penicilium* *Aspergillus flavus* *A. niger*	21-50 21-50 21-50
24	*Penicilium* *Aspergillus flavus* *A. niger*	51-70 21-50 21-50	Zero	*Penicilium* *Aspergillus flavus* *A. niger Penicilium*	*21-50* *21-50* *0-5* *6-20*

However, wheat and butter bread stored in polyethylene developed bread mould after four days (Table 1). This phenomenon could be attributed to the nutrient level of the butter and the wheat. Also, it could be attributed to the heat generated by the synthetic nature of the polyethylene hence increase in humidity on the package.
The study further revealed that wheat bread packaged in aluminium foil and stored under ambient conditions for seven days developed bread mould; whereas sugar and butter under the same storage conditions experienced the

growth of *Penicellium sp* and *Rhizopus sp*. (Table1 and Table 2). Seven days in storage the *Penicellium* and *Rhizopus* concentrations were at five percent of the sample size. Butter bread under the same condition for seven day had a complex of fungal (*Aspergillus flavus*, *A. niger* and *Rhizopus sp)* that covered 50% of the sample (Table 3).

The mycelium was very grayish. *Aspergillus* species for example *A. niger* and *Penicilium* species are known to produce gluconic acid when they colonize carbohydrate substrate. For example *Aspergillus niger* converts glucose to gluconic acid in a single enzymatic reaction. Commercial production of gluconic acid using *A. niger* employs a submerged culture process. *A. niger* is initially grown to form a sufficient amount of mycelia after which conversion of glucose to gluconic acid, mediated by fungi enzyme glucose oxidate is purely enzymatic reaction.

The complex of fungal growth could be attributed to the nutritional quality of the butter bread. During the kneading process of the flour several shortenings and margarine are added which could be the source of attraction for the fungi complex

The study showed a complex of growth of fungi (*Penicilium, Aspergellus flavus, A. niger* and *Rhizopus)* for butter bread stored in Aluminium foil and polyethylene under ambient condition from the seventh day in storage. The inoculum levels increased with the days in storage (Table 3). The use of old news print as a storage material for butter bread under ambient conditions did not show any growth of fungus until 19 days in storage. Newsprint therefore shows itself as the best storage material for butter bread (Table 3).

From the results, old newsprint stood up as the best packaging material for all the bread types under ambient conditions. The cocktail of fungal growth on butter bread could be attributed to the high nutrient level in the bread. The high nutritional level attracts the fungi. Also the butter bread is more moistened hence the ability of the fungi to grow than the wheat and sugar bread types.

The high level of *Penicilium* sp showed that the bread types could be used as substrate for the production of the drug penicillin for use in the pharmaceutical industry. However, the high inoculum levels of *A. flavus A. niger* and *Rhizopus* showed that storage of bread types for a long time in polybags is not recommended as these fungi can produce aflatoxins that are poisonous to mankind.

The metabolites produce by these fungi are named AFB1, AFB2, AFG1, and AFG2, all which occur naturally. Of the four, AFB1 is found in highest concentration followed by AFG1, AFB2, and AFG2. *Aspergillius flavus* is known to only produce AFB1 and AFB2 and *Aspergillius parasiticus* produces these same metabolites along with G1 and G2. Aflatoxins are secondary metabolites that are highly mutagenic and toxic for human and also animal (Sorenson *et al*., 1984; Howard *et al*., 1990). It is a common phenomenon to see people peel off the moulds on loaves of bread and start to consume. The results showed that these practices could be damaging to the health the consumers of such stuff. Some of these may be the causes of food-borne diseases.

Rhizopus species are known to be among the fungi causing the group of infections referred to as zygomycosis. Zygomycosis is now the preferred term over mucormycosis for this angio-invasive disease. Zygomycosis infection includes mucocutaneous, rhinocerebral, genitourinary, gastrointesternal, pulmonary and disseminated infections. The most frequent predisposing factors for zygomycosis include diabetic (Howard, 1990).There is evidence to indicate that children expose to aflatoxin breast milk and dietary items such as unrefined groundnut oil, may develop cirrhosis. Malnourished children are also prone to childhood cirrhosis on consumption of contaminated food. Several investigators have suggested aflatoxins as an aetiological agent of Reye's syndrome in children in Thailand, New Zealand etc. Though there is no conclusive evidence as yet. Epidemiological studies have shown the involvement of aflatoxins in kwashiorkor mainly in malnourished children. The diagnostic features of kwashiorkor are

edema, damage liver etc. These outbreaks of aflatoxicosis in man have been attributed to ingestion of contaminated food such as maize, groundnut etc. Hence it very important to reduce the dietary intake of aflatoxins by following the procedures for monitoring levels of aflatoxins in foodstuff (Boateng, 2011).

The proximate analyses of three bread types (sugar, butter and wheat) are presented in Table 4. It shows that there was a significant difference between the bread types with regards to carbohydrates content. Wheat bread had the highest level of carbohydrate compared to sugar and butter bread (Table 4). There was a significant difference in the crude protein level between wheat and butter bread. There was however no significant difference in the crude protein level between sugar and butter bread (Table 4). The results further revealed higher crude fat content in wheat than butter bread. There was a significant difference ($P>0.05$) in the Crude fibre content between wheat and butter bread (Table 4). There was a significant difference in the ash content between wheat, sugar and butter bread types. Wheat bread had the highest ash content. As regards the moisture content, wheat bread had the highest compared to sugar and butter bread types. There was no significant difference in the moisture content between sugar and wheat (Table 4).

The study has revealed that the carbohydrate content of the three bread types compared favourably with the results of Oluwamukomi et al**.,** (2011) where wheat flour was substituted by cassava flour. The results revealed a suspicion that the bakeries were using composite flour instead of refined wheat flour for the bread types. The results of the study corroborated very well with the study of Oluwamukomi et al**.,** (2011) in all the parameters (crude protein, crude fat, crude fibre, ash and the moisture content) assessed showing that the bread types used in were made from composite flour (Table 4).

The results of the proximate composition of butter bread stored under ambient and refrigerated conditions are presented in Table 5. From the results there was an increase in the carbohydrate content with storage with a significant difference between refrigeration and the ambient. There was an interaction between treatments for all the parameters measured. The moisture content of butter bread increased with storage in refrigerator and under ambient conditions (Table 5). However, the crude fat content reduced in storage. The significant reduction in the crude fat content could be attributed to the growth of the fungal complex. The nutrient-rich characteristic of the butter bread attracted the fungi. The sources of the fungal could also be attributed to the unhygienic nature of handling bread.

The results of the proximate composition of sugar bread stored under ambient and refrigerated conditions are presented in Table 6. From the results there was an increase in the carbohydrate content with storage with a significant difference between refrigeration and the ambient. There was an interaction between treatments for all the parameters measured. There was no significant difference in the crude protein content among the storage conditions for sugar bread (Table 6). There was a reduction in the crude fat content of sugar bread in storage compared to the control. The moisture content also increased with storage (Table 6).

The results of the proximate composition of wheat bread stored under ambient and refrigerated conditions are presented in Table 7. From the results there was an increase in the carbohydrate content with storage with a significant difference between refrigeration and the ambient. There was an interaction between treatments for all the parameters measured. There was an increase in the moisture content for the refrigerated wheat bread.

The results also showed an increase in the carbohydrate content of wheat bread stored in the refrigerator. Wheat bread stored under ambient condition had a slight increase in the carbohydrate content. There was however a reduction in the crude fibre content for refrigerated wheat bread compared to the control and the ambient condition (Table 7).

Table 4. Mean Values of the proximate analysis of different bread types produced in the Eastern region of Ghana

READ TYPES	PARAMETERS					
	Carbohydrate	Crude protein	Crude fat	Crude fibre	Crude ash	Moisture content
Wheat	73.52a ± 0.09	10.49a ± 0.21	11.8± 0.02	0.41a ± 0.11	2.22b ± 0.08	12.46a ± 0.24
Sugar	67.11b± 0.10	10.15ab ± 0.21	9.7± 0.02	0.39a ± 0.11	1.42a ± 0.08	11.84a ± 0.24
Butter	63.87c ± 0.09	9.77b ± 0.21	8.6± 0.02	0.29b ± 0.11	1.2a ± 0.08	4.88b ± 0.24
LSD (P>0.05)	0.6	0.7	0.1	0.1	0.3	0.8
CV %)	0.9	6.5	1.3	18.5	18.5	8.4

Figures with same letters in a column are not significantly different (P>0.05). Figures with different letters in the same column are significantly different at P>0.05

Table 5. Mean values of proximate Analysis of Butter Bread Stored under Two conditions (Refrigeration and Ambient).

Treatment	Parameters					
	Carbohydrate	Crude protein	Crude fibre	Crude fat	Crude ash	Moisture content
Fresh	65.12	10.44	0.46	11.99	1.97	4.67
Refrigerated	69.52	10.85	0.32	10.32	1.14	13.25
Ambient	66.48	8.96	0.30	8.21	1.41	14.64
LSD (P>0.05)	1.04	1.14	0.12	0.22	0.52	1.41
CV (%)	0.9	6.5	18.5	1.3	18.5	8.4

Table 6. Proximate Analysis of Sugar Bread Stored Under Two Conditions (Refrigerated and Ambient)

Treatment	Carbohydrate	Crude protein	Crude fibre	Crude fat	Crude ash	Moisture content
Fresh	59.76	8.64	0.30	8.49	2.78	4.04
Refrigerated	73.67	9.48	0.47	5.90	1.28	9.21
Ambient	63.87	9.61	0.21	6.77	1.39	12.74
LSD (P>0.05)	0.6	0.66	0.67	0.13	0.30	0.82
CV (%)	0.9	6.5	18.5	1.3	18.5	8.4

Table 7. Proximate Analysis of Wheat Bread Stored Under Two Conditions

Treatment	Moisture content	Carbohydrate	Crude protein	Crude fibre	Crude fat	Crude ash
Fresh	5.93	66.73	10.22	0.41	14.82	1.90
Refrigerated	13.07	77.37	11.13	0.45	12.81	1.17
Ambient	9.99	70.98	11.88	0.35	10.69	1.46
LSD (P>0.05)	0.8	0.6	0.7	0.7	0.1	0.3
CV (%)	8.4	0.9	6.5	18.5	1.3	18.5

4. CONCLUSION

The study has shown that the three brands of bread on the market were made from composite flour. The bread types did not have the constituents on the labels as recommended by the Foods and Drugs Board and the Standard Board. It was evident that news print was a better wrapper for storage of bread compared to polyethylene bags. The aluminium foil could have been ideal for storage of bread but the cost would be too high for the ordinary person. Bread storage in refrigeration was favourable than ambient condition. However, the high cost of running a refrigerator does not present itself as a good candidate for storing bread. Storage of bread in a refrigerator makes the bread stale. The high nutritional level of the bread types attracts the fungi. Also the butter bread was more moistened hence the ability of the fungi to grow than those without butter as an ingredient.

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