

African Journal of Food Science

Volume 9 Number 2, February 2015

ISSN 1996-0794



*Academic
Journals*

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Full Length Research Paper

Survey of the konzo prevalence of village people and their nutrition in Kwilu District, Bandundu Province, DRC

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Received 4 September, 2014; Accepted 7 January, 2015

Konzo is a sudden spastic paraparesis that causes permanent paralysis of the legs and occurs mainly in children and young women. Konzo results from high cyanogen intake and malnutrition caused by a monotonous diet of bitter cassava. The known incidence of konzo in DRC up to 2009 is 3469 cases, but an estimate in 2002 was 100,000 cases. To help resolve this question a konzo survey was made in three health zones in Kwilu District, Bandundu Province, Democratic Republic of Congo (DRC), and the nutrition of those with konzo recorded. Thirty villages (population 22793) in Kwilu District were surveyed for konzo cases, and food consumption scores and mid upper arm circumferences obtained. There were 172 konzo cases with village konzo prevalences of 0.1-17%. The mean konzo prevalence in Masimanimba and Kingandu health zones was much less than in Payikongila health zone, probably because of the higher rate of malnutrition in Payikongila. Since 2009, konzo incidence has increased greatly in Kwilu District and also in 13 villages in nearby Kwango District, where incidence of new konzo cases has been prevented by use of the wetting method. Averaging the data over 495 konzo cases, 48% occurred from 2009 onwards and 52% occurred in the 20+ years before 2009. The very large increase of konzo incidence since 2009 is a public health problem in Bandundu Province, that could be solved by training women to use the wetting method, which removes cyanogens from cassava flour. The wetting method is more direct, effective and cheaper in preventing konzo than broad based interventions.

Key words: Konzo survey, cassava, cyanide, nutrition, sulfur amino acids, food consumption score, mid upper arm circumference.

INTRODUCTION

Cassava contains cyanogens (linamarin and a small amount of lotaustralin) that on consumption liberate cyanide in

the body, which can lead to konzo, an irreversible spastic paraparesis of the legs that occurs suddenly amongst very

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poor village people who live on a monotonous diet of bitter cassava (Cliff et al., 1985; Howlett et al., 1990; Nzwalo and Cliff, 2011). Konzo is an upper motor neuron disease that causes permanent paralysis of the legs and affects mainly children and young women after childbirth. Many of these people suffer from malnutrition with low protein intake, in particular a shortfall of the essential sulfur amino acids methionine and cysteine/cystine that are needed for detoxification of cyanide (CN) to thiocyanate (SCN) in the body (Cliff et al., 1985). Konzo occurs in the Democratic Republic of Congo (DRC), Mozambique, Tanzania, Cameroon, Central African Republic and Angola (Bradbury et al., 2011; Allen, 2010) and because of the very large per capita consumption of cassava in Congo (Nhassico et al., 2008) konzo probably occurs there as well. The total number of konzo cases reported up to 2009 is 6788 (Nzwalo and Cliff, 2011), with 3469 cases in DRC, but this is very much less than the true number, because konzo occurs in isolated areas and during crises such as war and drought. An estimate of 100,000 konzo cases in DRC was made in 2002 (Diasolua Ngudi, 2005). In 2010 Action Against Hunger (ACF) in Kwango District, Bandundu Province, DRC, located 2218 konzo cases (Kasonga and Calo, 2011; Delhourne et al., 2012). Konzo occurs in at least four provinces, Bandundu, Kasai Oriental, Kasai Occidental and South Kivu (Chabwine et al., 2011) (Figure 1), but there has been no country wide survey of konzo in the DRC.

Konzo has been prevented in 13 villages in the DRC with a population of nearly 10000 people by use of the wetting method by village women to remove cyanogens from cassava flour (Banea et al., 2012, 2013, 2014a). The wetting method consists of adding water to cassava flour, spreading the wet flour in a thin layer on a mat for 2 h in the sun or 5 h in the shade to allow the escape of hydrogen cyanide gas produced by breakdown of linamarin by the enzyme linamarase (Bradbury, 2006; Cumbana et al., 2007; Bradbury and Denton, 2010). The damp flour is then cooked in the traditional way to make a thick porridge called fufu. The women readily accepted the wetting method and still continued to use it more than a year after the intervention ceased and the method has spread by word of mouth to other villages (Banea et al., 2014b). Furthermore, a correlation has been found between the monthly percentage incidence of konzo and the monthly cyanide intake as measured by the urinary thiocyanate content of children (Banea et al., 2014a). It is therefore likely that konzo results from high cyanide intake from bitter cassava, coupled with a low intake of sulfur amino acids.

It is important to have a good estimate of the total number of people with konzo in DRC and other countries, so that the prevention methods that we have developed can be systematically applied towards the complete prevention of this disease. In this paper we report the konzo prevalence and nutrition of konzo

cases in 30 villages in three health zones in Kwilu District, Bandundu Province.

MATERIALS AND METHODS

Study area

In order to discover the most affected konzo areas in Kwilu District, discussions were held with Kwilu District health authorities in Kikwit in July 2012. Four health zones were identified as most affected Masimanimba, Kingandu, Payikongila and Djuma, but Djuma health zone was excluded for logistical reasons (Figure 1). Thirty four villages were identified as most affected and konzo cases were found in thirty villages.

Food and diseases of the people

The most important food crops are cassava, maize, peanuts, squash and cowpeas. Cassava is the staple food eaten as fufu, mainly with cassava leaves and vegetables. There is some small livestock, poultry, small scale fishing and fish farming for sale. The dry season is from mid-May to mid-August and there is another short dry season in February-March. There are two lean periods in August to November and in March, when household food security is critical and the number of meals is reduced to one meal per day. The most common diseases are malaria, acute respiratory infection, diarrhea, malnutrition and trypanosomiasis.

Health zone visits and survey methods

Discussions were conducted with the health authorities in each of the three health zones and supervisors were identified in each zone. Four supervisors were given training in Kikwit City by the konzo team of Programme National de Nutrition (PRONANUT). Each trained supervisor was responsible for collecting data on konzo cases in the health areas of his health zone. In each health zone the most affected villages were identified first. A census was taken of each affected village and suspected cases of konzo or those with gait difficulties were identified by the community health workers, who are trained to recognise common diseases including konzo and to refer the case to a health centre. Konzo cases were examined by doctors from PRONANUT following a standardised protocol (World Health Organisation, 1996) as follows: (1) a visible spastic walk or run, (2) an abrupt history of the onset of the disease in less than one week in a person in good health, with no further progression of the disease, (3) a bilateral exaggeration of knee jerks and/or Achilles tendon (ankle clonus). The month and year were recorded when konzo occurred. Five surveyors were trained to administer the food household consumption questionnaire for each family with a case of konzo.

Food consumption score (FCS) in konzo household

The food consumption data of 155 households with konzo (some had multiple cases of konzo) were surveyed in the three health zones and this was used to calculate the World Food program food consumption score (FCS) using the equation:

$$FCS = a_{\text{staple}} X_{\text{staple}} + a_{\text{pulse}} X_{\text{pulse}} + a_{\text{veg}} X_{\text{veg}} + a_{\text{fruit}} X_{\text{fruit}} + a_{\text{animal}} X_{\text{animal}} + a_{\text{sugar}} X_{\text{sugar}} + a_{\text{oil}} X_{\text{oil}}$$

Where x_i = number of days for which each food group was consumed during the past seven days, a_i = weight of each food

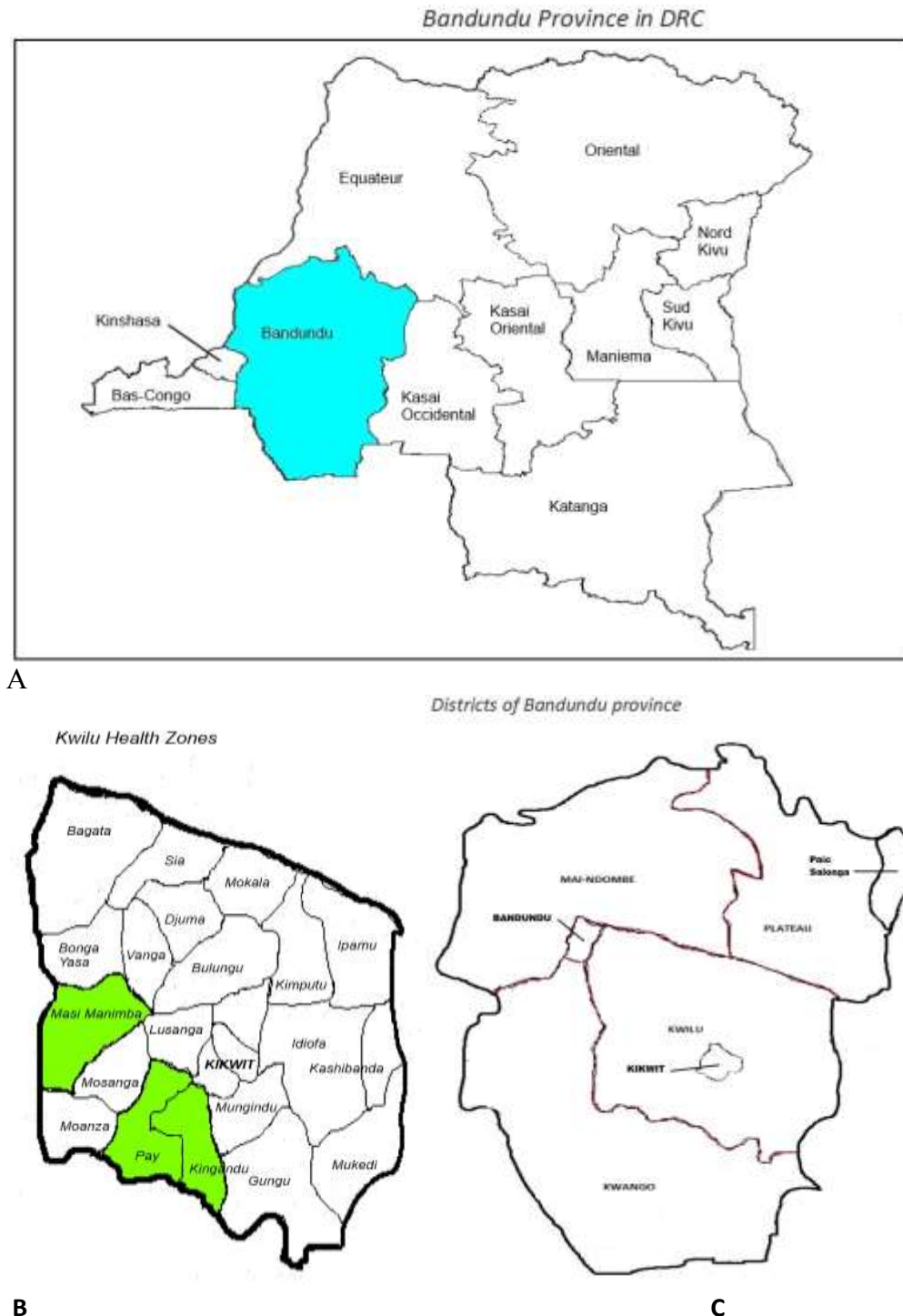


Figure 1. A. The provinces of DRC showing Bandundu, Kasai Occidental, Kasai Oriental and South Kivu where konzo is present; B. Health zones of Kwilu District with the survey ones marked; C. Districts of Bandundu Province.

group as follows: staple (cassava) 2, pulses (beans, peas, groundnuts) 3, vegetables and leaves 1, fruit 1, meat and fish 4, milk 4, sugar 0.5, oils, fats and butter 0.5. The thresholds used were: 0-23.9 for poor food consumption, 24.0-37.9 for borderline food consumption, and ≥ 38 for acceptable food consumption (Interagency Work. Rep. WFP-FAO 2008).

Mid upper arm circumference (MUAC) and nutritional status

The mid upper arm circumference was used in order to identify high-risk malnourished (> 18 years old) konzo patients (Unicef 2012; Projet Sphere). A MUAC of < 210 mm indicated a person with malnutrition. There were 64 konzo patients measured in

Table 1. Percentage konzo prevalence in descending order for thirty villages in Kwilu District.

Village	Population	Number of konzo cases	% Konzo
Kongila Ndola ^a	106	18	17.0
Kongila Kianfu ^a	495	38	7.7
Lubamba ^a	341	9	2.6
Kinzefa ^a	420	8	1.9
Kabanga ^b	564	6	1.1
Kiwawa Kapa ^c	1453	12	0.83
Mulasi ^c	248	2	0.81
Kizefo ^c	1153	9	0.78
Kiwawa Pont ^c	1805	13	0.72
Mvudi ^b	564	4	0.71
Makanga ^c	1047	7	0.67
Mashini ^b	734	4	0.54
Kipongi ^c	1104	6	0.54
Kikonga ^c	751	4	0.53
Kimeso ^c	231	1	0.43
Yoshi ^c	467	2	0.43
Cite Masi ^c	734	3	0.41
Sakani ^b	734	3	0.41
Mbeko ^c	1030	4	0.39
Lunza ^c	805	3	0.37
Bibamba ^c	549	2	0.36
Kifunga Centre ^c	2420	6	0.25
Kikongo ^c	400	1	0.25
Kilombo M ^c	405	1	0.25
Kiwamba ^c	534	1	0.19
Kikiama ^c	564	1	0.18
Kimpembe ^c	564	1	0.18
Kiko ^c	734	1	0.14
Kinzenga Q3 ^c	950	1	0.11
Mudigongo ^c	887	1	0.11
Total	22793	172	

^a Payikongila health zone; ^b Kingandu health zone; ^c Masimanimba health zone

Masimanimba, 13 in Kingandu and 46 in Payikongila.

Ethics statement

The Ministry of Public Health Ethics Committee in DRC approved the survey protocol, which was also approved by Kwilu district health authorities and health zone health authorities. The chiefs of the villages accepted the census and medical examination of their people for konzo and distribution of a questionnaire on food consumption.

RESULTS

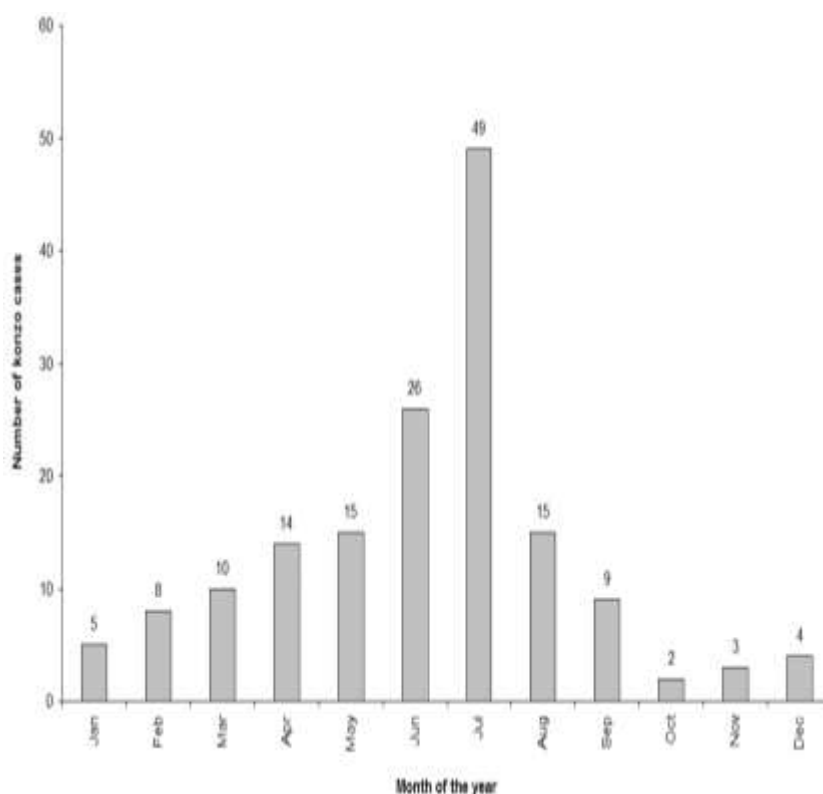
Thirty villages were identified in Masimanimba, Kingandu and Payikongila health zones to be surveyed, 22 in Masimanimba, four in Kingandu and four in Payikongila

health zones. In a population of 22793 people there were 266 people with walking difficulties of whom 172 had konzo, giving a mean konzo prevalence of 0.75% (Table 1). The four villages in Payikongila health zone had the highest konzo prevalences ranging from 17% down to 1.9% with 42% of the total konzo cases. The main group of villages in Kingandu and Masimanimba health zones had konzo prevalences ranging from 1.1% down to 0.11% (with 9 villages recording only one konzo case) and accounting for 58% of the total number of konzo cases. Overall 53% were female and 47% male. Of the women who contracted konzo, 12% were nursing a baby at the time of the konzo attack. The age at onset was 2-4 years, 9%; 5-14 years, 53%; 15-44 years, 34%; 45-55 years, 4%. There was an abrupt onset in less than one day in 83% of cases and the remainder over 2-7 days.

Table 2. Comparison of % konzo prevalence, % malnutrition^a and % nutrition (poor, borderline, acceptable)^b of people in three Kwilu health zones.

Health zone	% Konzo prevalence	% Malnutrition ^a	Nutrition (%) based on food consumption score		
			Poor	borderline	acceptable
Masimanimba	0.44	20	11	28	61
Kingandu	0.66	8	18	6	76
Payikongila	5.4	50	22	38	40

^aBased on mid upper arm circumference (MUAC) measurement of adult konzo patients (Unicef, 2012; Projet Sphere). ^b Based on food consumption score (FCS) of konzo families, see methods (Interagency Work. Rep., WHO-FAO, 2008).

**Figure 2.** Monthly distribution of onset of konzo cases.

Bilateral exaggerated knee jerks were observed in 96% of cases. Speech disorders occurred in 22% of konzo cases, 17% had blurred vision and 2% goitre. It was found that 7% of people with konzo were unable to walk, 25% needed one or two sticks and 68% walked unaided but with a limp. These numbers have been combined with those from six other konzo studies in DRC and Tanzania (Banea et al., 2014a) and the results (mean +/- SD) are 8+/-5 severe, unable to walk; 26+/-13 moderate, needing one or two sticks; 66+/-12 mild, no sticks needed.

Table 2 shows the mean konzo prevalence in each health zone, % malnutrition among konzo cases measured by MUAC and the nutrition measured by a food consumption survey of the konzo families. The monthly

occurrence of konzo cases is shown in Figure 2 and the years in which they occurred in Figure 3. The peeled cassava roots are soaked (retted) in a pond or a stream or at home for 2-3 days, but only for 1-2 days during the dry season. About one third of the konzo households surveyed had difficulty to ensure proper soaking, due to theft during soaking in 87% of cases or lack of water in 13% of cases. One quarter of households had consumed only one meal the day before the survey in July. Survival strategies used during periods of food shortage (August to November and in March) were to fall back on food gathering, use of less preferred foods and also to harvest crops early. Nearly all households (93%) with cases of konzo had houses made of straw and only 12% had

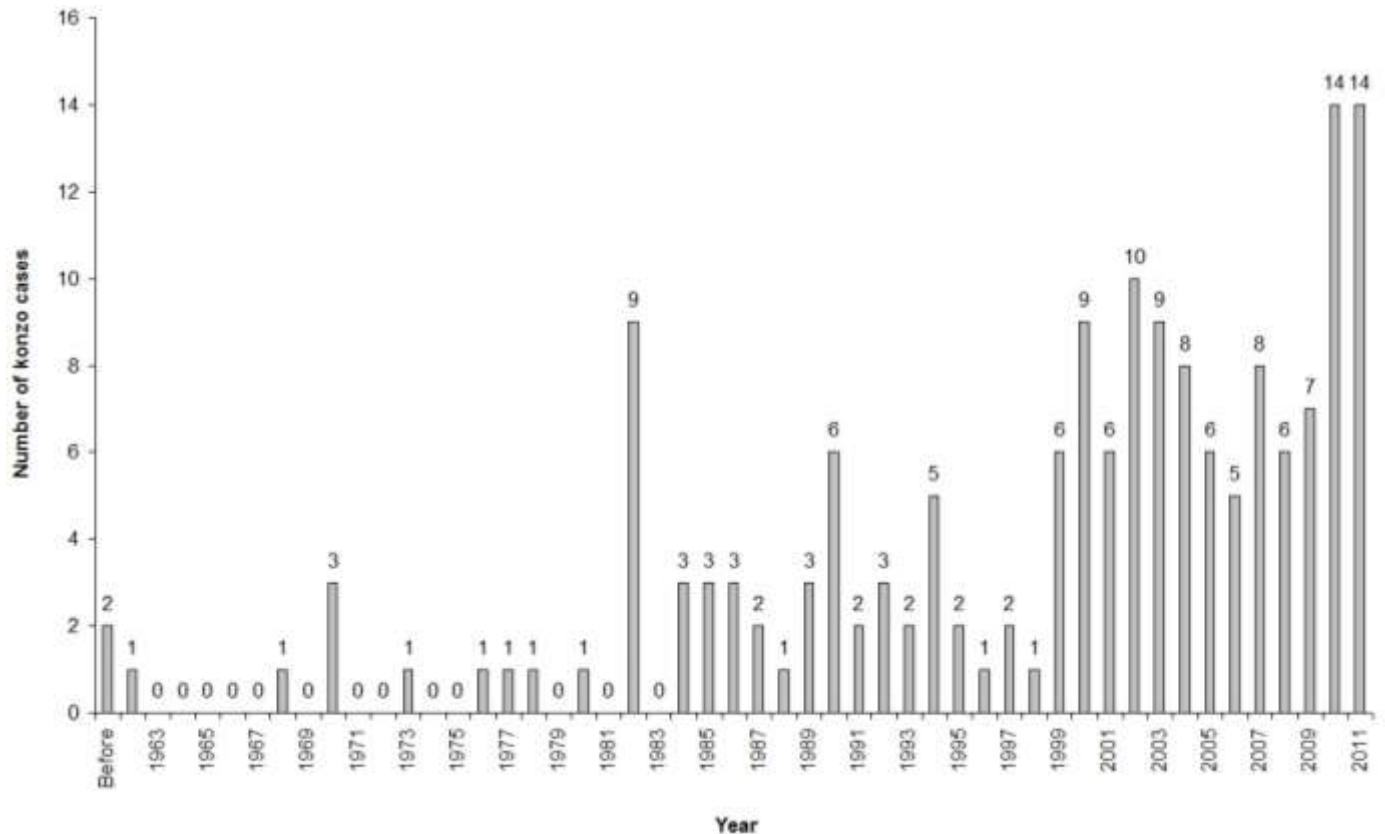


Figure 3. Annual distribution of onset of konzo cases.

access to a source of safe drinking water.

DISCUSSION

The data in Table 1 show that konzo prevalence is very high in four villages of Payikongila health zone with 42% of total konzo cases and much less in the 26 villages of Kingandu and Masimanimba health zones with 58% of total konzo cases. The high konzo prevalence in Payikongila health zone is probably due to the much poorer nutrition and higher degree of malnutrition of konzo cases in Payikongila compared with konzo patients from the other health zones (Table 2). Unfortunately, we do not have the urinary thiocyanate content of the school children, which would have allowed comparison between the cyanide intake of children from each health zone.

In Figure 2, the monthly incidence of konzo throughout the year shows a strong maximum incidence in July, the peak cassava harvesting season in the dry season, which agrees with previous work (Banea et al., 2012, 2013). The survey of konzo families showed that short soaking for 1-2 days instead of 3-4 days (Banea et al., 1992) was common especially in the dry season, which together with a peak in consumption of cassava, caused a peak in both

cyanogen intake and konzo. The yearly incidence of konzo (Figure 3) shows that konzo cases have occurred every year since 1984, with much larger annual numbers since 1999, particularly in 2010 and 2011. This confirms the very concerning results found in 13 villages in Popokabaka and Boko health zones in nearby Kwango district, where konzo incidence has greatly increased since 2009 in this area of Bandundu Province (Banea et al., 2012, 2013, 2014a). By combining the data from this survey and the four interventions, it was found that 48% of the konzo incidences were in the three years from 2009 onwards and 52% in the preceding 20+ years.

By the successful use of the wetting method by village women to remove cyanogens from cassava flour we have prevented konzo in 13 villages with 9191 people (Banea et al., 2012, 2013, 2014a). Furthermore, we have shown that there is a significant correlation between month by month percentage konzo incidence and monthly cyanide intake, as measured by the percentage of children with high urinary thiocyanate content (Banea et al., 2014a). It is therefore likely that konzo arises from high cyanide intake and malnutrition, specifically a shortfall of sulfur amino acids, also being a factor (Nzwalo and Cliff, 2011). We have prevented konzo by decreasing cyanide intake, but the results in Table 2 suggest that malnutrition may

be an important factor. The importance of malnutrition was evident in three unrelated konzo epidemics in which people of the same ethnic group as those who had high konzo prevalence, but living only about 5 km away had virtually zero konzo prevalence. Those who were protected from konzo in the Mozambique konzo epidemic lived near the sea and consumed fish (Ministry of Health, 1984), as did those in Tanzania who lived near Lake Victoria (Howlett et al., 1992) and those protected in DRC lived in the forest and ate animals from the forest (Banea Mayambu, 1993). Thus, improvement of the diet particularly by inclusion of animal protein is probably a means of preventing konzo. This is part of the cross-sectoral approach used by Action Against Hunger (ACF) (Kasongo and Calo, 2011). The intervention of ACF has reduced konzo incidence by 84% between 2010 and 2011 (Delhourne et al., 2012). The reduction of cyanide intake by educating the women about the cause of konzo and how to use the wetting method to remove cyanogens, is direct, effective and less expensive than the cross-sectoral approach which involves improvement of the diet and other factors. Ideally, reduction of cyanide intake and reduction of malnutrition should be used together (Cardoso et al., 2005).

Conclusion

In three health zones of Kwilu District there were 172 cases of konzo in 30 villages. The villages of Payikongila health zone had much higher konzo prevalences and the konzo cases much poorer nutrition, than in the villages of Masimanimba and Kingandu health zones. Since 2009 there has been a large increase of yearly konzo incidence in four interventions to prevent konzo using the wetting method in nearby Kwango District and also in this survey in Kwilu District. Using the data from the four interventions and this survey it was found that 48% of konzo cases occurred from 2009 onwards and 52% of cases in 20+ years preceding 2009. The use of the wetting method to reduce cyanogen intake has prevented konzo in 13 villages costing an average \$27 per person and is more effective in preventing konzo and cheaper than broad based interventions.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGMENTS

We thank the Kwilu district health authorities and the health zone authorities for Masimanimba, Kingandu and Payikongila health zones. We also thank the village chiefs and all the people involved in this konzo and nutrition survey.

REFERENCES

- Allen TJ (2010). Konzo in Angola. CCDN News No 16, 3-4.
- Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Foster MP, Kuwa N, Tshala Katumbay D (2014a). Prevention of konzo in the DRC using the wetting method and correlation between konzo incidence and percentage of children with high urinary thiocyanate level. *Afr. J. Food Sci.* 8:297-304.
- Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Kuwa N, Tshala Katumbay D (2013). Control of konzo by detoxification of cassava flour in three villages in the Democratic Republic of Congo. *Food Chem. Toxicol.* 60:506-513.
- Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Kuwa N, Tshala Katumbay D (2014b). Effectiveness of wetting method for control of konzo and reduction of cyanide poisoning by removal of cyanogens from cassava flour. *Food Nutr. Bull.* 35:28-32.
- Banea JP, Nahimana D, Mandombi C, Bradbury JH, Denton IC, Kuwa N (2012). Control of konzo in DRC using the wetting method on cassava flour. *Food Chem. Toxicol.* 50:1517-23.
- Banea JP, Poulter NH, Rosling H (1992). Shortcuts in cassava processing and risk of dietary cyanide exposure in Zaire. *Food Nutr. Bull.* 14:137-143.
- Banea Mayambu JP (1993). Cassava processing, dietary cyanide exposure and konzo in Zaire. Masters Thesis. Uppsala University, Uppsala, Sweden, p. 55.
- Bradbury JH (2006). Simple wetting method to reduce cyanogen content of cassava flour. *J. Food Comp. Anal.* 19:388-93.
- Bradbury JH, Cliff J, Denton IC (2011). Uptake of wetting method in Africa to reduce cyanide poisoning and konzo from cassava. *Food Chem. Toxicol.* 49:539-542.
- Bradbury JH, Denton IC (2010). Rapid wetting method to reduce cyanogen content of cassava flour. *Food Chem.* 121:591-94.
- Cardoso AP, Mirione E, Ernesto M, Massaza F, Cliff J, Haque MR, Bradbury JH (2005). Processing of cassava roots to remove cyanogens. *J. Food Comp. Anal.* 18:451-460.
- Chabwine JN, Masheka C, Balol'ebwami Z, Maheshe B, Balegamire S, Rutega B, wa Lola M, Mutendela K, Bonnet MJ, Shangalume O, Balegasmire JM, Nemery B (2011). Appearance of konzo in South Kivu, a wartorn area in the Democratic Republic of Congo. *Food Chem. Toxicol.* 40:644-649.
- Cliff J, Martensson J, Lundquist P, Rosling H, Sorbo B (1985). Association of high cyanide and low sulphur intake in cassava induced spastic paraparesis. *Lancet* 11:1211-13.
- Cumbana A, Mirione E, Cliff J, Bradbury JH (2007). Reduction of cyanide content of cassava flour in Mozambique by the wetting method. *Food Chem.* 101:894-97.
- Delhourne MM, Mayans J, Calo M, Guyot-Bender C (2012). Impact of cross-sectoral approach to addressing konzo in DRC. *Field Exch.* 44:50-54.
- Diasolua Ngudi D (2005). Konzo and cassava toxicity: a study of associated nutritional factors in the Popokabaka District, DRC. Ph D Thesis, Universiteit Gent, Belgium.
- Howlett WP, Brubaker G, Mlingi N, Rosling H (1992). A geographical cluster of konzo in Tanzania. *J. Trop. Geogr. Neurol.* 2:102-108.
- Howlett WP, Brubaker GR, Mlingi N, Rosling H (1990). Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain* 113:223-35.
- Interagency Workshop Report WFP-FAO (2008). Measures of Food Consumption—Harmonizing Methodologies. April 2008, Rome. Italy.
- Kasonga E, Calo M (2011). A cross-sectoral approach to addressing konzo in DRC. *Field Exch.* No 41, 3-5.
- Ministry of Health Mozambique (1984). Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. 1. Epidemiology and clinical laboratory findings in patients. *Bull. WHO* 62:477-484.
- Nhassico D, Muquingue H, Cliff J, Cumbana A, Bradbury JH (2008). Rising African cassava production, diseases due to high cyanide intake and control measures. *J. Sci. Food Agric.* 88:2043-49.
- Nzwalo H, Cliff J (2011). Konzo: from poverty, cassava and cyanogen intake to toxico-nutritional neurological disease. *PLoS Negl. Trop. Dis.* 5(6): e1051. doi:10.1371/journal.pntd.0001051.
- Projet Sphere : La charte humanitaire et les standards minimum de

l'intervention humanitaire, page 258.

Unicef (2012). Unicef/Ministère de la Santé publique: Protocole national de Prise en charge intégrée de la malnutrition aiguë (PCIMA), Kinshasa, Avril 2012.

World Health Organisation (1996). Konzo: a distinct type of upper motor neuron disease. *Wkly. Epidemiol. Rec.* 71:225-232.

Full Length Research Paper

The mycological content of ready to eat garri in Amassoma, Bayelsa State

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Received 4 December, 2014; Accepted 15 January, 2015

Garri is consumed by several millions of people in the West African sub-region and in Nigeria in particular, regardless of ethnicity and socio-economic class. However, production and handling methods have not been standardized resulting in garri product with varying mycological contamination. The objective of this study was to assess the mycological safety and mycological contamination of garri marketed in Amassoma, Bayelsa State. A total of forty-four samples comprising of both freshly prepared garri and displayed garri in the open market were used for this study. The samples were collected with sterile polythene bags adopting standard procedures and transported to the laboratory for analysis within 12 h. The result of this study clearly showed fungal contamination resulting from its display in the open market, *Aspergillus* sp. and *Penicillium* sp. had the highest frequency of occurrence (33.3%) in white garri while in yellow garri, *Aspergillus* sp. (33.3%) was the fungi most frequently isolated. Other fungi species isolated in the garri samples were *Fusarium* sp, *Mucor* sp, *Alternaria* sp, *Cladosporium* sp and *Rhizopus* sp. The relationship between fungi spp. and type of garri (white or yellow) was not statistically significant as the calculated value was greater than the p value of 0.05. Since this product harbor arrays of fungi, strategy to antagonize their growth and survival in this commodity in order to neutralize the potential of these organisms serving as agents of food borne diseases should be adopted.

Key words: Disease, fungi, garri, microbiology, production, occurrence.

INTRODUCTION

Garri is made from peeled, washed, grated, fermented and roasted fresh cassava tuber (*Manihot esculenta* Crantz) (Ernesto et al., 2000). It is the most popular fermented cassava products in Africa (Oluwole et al., 2004; Ernesto et al., 2000) and it is consumed by several millions of person in West Africa where it forms major part of their diet (Edem et al., 2001; Kostinek et al., 2005; Ogiehor et al., 2007). In Nigeria, its acceptability cuts

across the various ethnic and socio-economic classes, making it the commonest food among the rich and the poor (Jekayinfa and Olajide, 2007; Ogiehor et al., 2007).

Garri is stored and marketed in a ready-to-eat form and prepared into a stiff paste or dough-like called 'Eba' by adding the granules into hot water and stirring to make a paste of varied consistency. Eba can be consumed with local soups or stews of various types by chewing or

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swallowing in morsels (Asegbeloyin and Onyimonyi, 2007; Ogiehor et al., 2007). Garri can also be deliciously consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moimoi, kuli-kuli, or taken as a fast food when soaked in cold water (Ogugbue and Obi, 2011). Sometimes, it is taken with beverages mixed with cold water or warm water with salt or sugar depending on the choice of the individual. Garri is rich in starch, has high fibre content and contains some essential vitamins (Adepoju et al., 2010). Its high fibre content makes it very filling and in the prevention or at least in reducing the likelihood of constipation and bowel diseases (Adepoju et al., 2010). Microbial growth, deterioration and spoilage of garri are major cause of food borne illnesses and threat to public health (Ogiehor et al., 2007). However, some unhygienic practices involved in production, processing of cassava to garri and post processing handling such as spreading on the floor and mats after frying, displaying in open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from rural to urban areas and the use of bare hands during handling and sales may lead to microbial contamination due to deposition of bio-aerosols on exposed products and transfer of infectious agent during handling (Ogiehor et al., 2007; Ogugbue and Obi, 2011; Ogugbue et al., 2011).

The main biological agents that contaminate and spoil garri are moulds, bacteria, insects and mites (Igbeka, 1987; Ogiehor et al., 2005). Garri is rich in carbohydrate and therefore, suitable for fungal growth. Moulds such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Mucor* have been associated with garri during storage and distribution (Ekundayo, 1984; Ogugbue et al., 2011). Several reports have revealed high occurrence of microorganisms in market samples of garri (Ijabadeniyi, 2007; Amadi and Adebola, 2008; Ogiehor et al., 2007). The growth of moulds in garri results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage of the food product (Efiuvwevwe and Isaiah, 1998). Some moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium* sp. can also produce aflatoxins (SubbaRao, 2000; Frazier and Westhoff, 2000; Ogiehor et al., 2007), which can have serious effects on human health depending on the dosage consumed. The objective of the study was to assess the mycological contamination of garri freshly prepared and marketed in Amassoma community which is a major producer of garri in this part of the country and this information will help in the formulation of policy that will ensure the mycological safety of the product.

MATERIALS AND METHODS

Sample collection

The study was carried out in Amassoma located in Niger Delta

region of Bayelsa State, Nigeria. Amassoma is a community with a population of about twenty thousand people who are mainly farmers, fishermen, traders and civil servant and constitute one of the largest areas production and consumption of garri in Bayelsa state.

Garri samples used for this study were obtained from sellers in the open markets and from local garri processors. A total of 44 garri samples were collected for the analysis. Freshly prepared garri samples and garri displayed in the market were collected without bias from every first compound and shop in the community.

Microbiological analyses of garri samples

The garri samples were processed by weighing 1 g proportion of each sample aseptically (after thorough mixing) into 9 ml of (w/v) sterile peptone water in a beaker, and allowed to stand for 30 min, stirring occasionally using a sterile wooden applicator stick as described by Ogiehor and Ikenebomeh (2005). A drop of the garri suspension was dispensed with a sterile pipette onto the centre of the sterile Petri dish containing Saboroud dextrose agar and spread with a sterile hockey stick (Lugauskas, 2005). The plates were incubated at 25°C for 7 days and observed each day for fungal growth (Appendix plates 1 to 7). Identification of fungi was done by both macroscopic and microscopical methods (Reennen-Hoekstra, 1998).

Macroscopic examination of fungi were based on the following characteristics; colony colour (obverse/reverse), colony size, exudate and soluble pigment. Microscopical examination of fungi was done by teasing small portions of the fungal pure culture and mounting in lactophenol cotton blue dye on a clean slide, covered with a clean cover slip and observed under the microscope (Fawole and Osho, 1995) with 10 x objective lens and confirmed with 40x objective lens. Royal Horticultural Society (RHS) mini colour chart was used in this study as a guide for morphology identification.

Statistical analysis

Statistical analysis was performed with the SPSS version 17 statistical software package. Comparisons between groups were analyzed by Pearsons Chi square to determine the relationship between fungi contamination and garri type (yellow or white) at the significance level, $p < 0.05$.

RESULTS

A total of seven mould species (*Aspergillus* sp, *Penicillium* sp, *Fusarium* spp, *Mucor* sp, *Alternaria* sp, *Cladosporium* sp and *Rhizopus* sp) were isolated from both yellow and white garri displayed in the open market. The fungi in white garri were *Alternaria* sp. (16.7%), *Aspergillus* sp. (33.3%), *Penicillium* sp. (33.3%) and *Rhizopus* sp. (16.7%) as shown in Table 1.

In yellow garri, the distribution of occurrence were *Aspergillus* sp. (33.3%), *Cladosporium* sp. (11.1%), *Fusarium* sp. (11.1%), *Mucor* sp. (11.1%), *Penicillium* sp. (22.2) and *Rhizopus* sp. (11.1%) as shown in Table 2.

In white garri, *Aspergillus* sp. and *Penicillium* sp. had the highest occurrence in 33.3% each, while *Aspergillus* sp. was found more in yellow garri (33.3%). Both freshly processed garri yielded no fungal growth in this study. The relationship between fungi species and type of garri (white or yellow) was examined using a Chi square test

Table 1. The frequency of occurrence of mould species isolated from white garri.

Fungi	Frequency		Percentages (%)
	White garri (market)	White garri (fresh)	
<i>Alternaria</i> sp.	1	0	16.7
<i>Aspergillus</i> sp.	2	0	33.3
<i>Penicillium</i> sp.	2	0	33.3
<i>Rhizopus</i> sp.	1	0	16.7
Total	6	0	100

Table 2. The frequency of occurrence of fungi species isolated from yellow garri.

Fungi	Frequency		Percentages (%)
	Yellow garri (Market)	Yellow garri (fresh)	
<i>Aspergillus</i> sp.	3	0	33.3
<i>Cladosporium</i> sp.	1	0	11.1
<i>Fusarium</i> sp.	1	0	11.1
<i>Mucor</i> sp.	1	0	11.1
<i>Penicillium</i> sp.	2	0	22.2
<i>Rhizopus</i> sp.	1	0	11.1
Total	9	0	100

and the association was not statistically significant as the calculated value was greater than the p value of 0.05. Garri type that was exposed to unhygienic post production processes was more likely to be contaminated with fungi

DISCUSSION

In their metabolic process, moulds produce mycotoxins. These natural products, poisonous to humans and animals, are created as the result of a secondary metabolic process of fungi when grown on organic substrates. Chemical structure of these metabolites varies, however, they are largely of small molecular mass, which conditions their varied toxic characteristics. So far over 400 metabolites produced by moulds have been identified from different genus of fungi: *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. and *Trichothecium* sp. (Krzyściak et al., 2011; Bräse et al., 2009).

The mould isolated from both yellow and white garri sample were *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Mucor* sp., *Alternaria* sp., *Cladosporium* sp. and *Rhizopus* spp (Appendix). Fungi isolated from garri in this study is slightly different from isolates from other states in Nigeria. In the western part of Nigeria, an assessment of some fermented cassava products obtained from three states reported the isolation of *Aspergillus* spp., *Mucor*

spp. and *Penicillium* spp. from garri (Egbebi et al., 2012) while Ogugbue et al. (2011) isolated *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Mucor* sp. from garri stored under various storage conditions while a study carried out in Makurdi isolated similar fungi subspecies from garri (Agoura et al., 2014). In this study, *Cladosporium* sp. was found in yellow garri only and differs from the isolation of it in white garri as documented by Agoura et al. (2014) in Makurdi. Its absence in yellow garri might be due to its non exposure to an environment harbouring the mould during post production processes. The result of this study shows that garri from the open market harbor arrays of fungal contamination which conforms to reports from other parts of Nigeria but the fungi subspecies isolated from the different locations might be different due to varying post production processes.

The result of this study clearly showed no fungal growth in freshly produced yellow and white garri. The absent of moulds in freshly prepared garri may be due to the inability of these fungi to resist the frying stage during garri processing. Since, there was no fungal growth in freshly prepared garri, the mould recorded in this study may be due to contamination as a result of varying factors. These factors may include unhygienic practices involved in post processing handling such as spreading on the floor and mats after frying, displaying in open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from

production site to storage area and also the use of bare hands by different customers during the buying process. Also, buyers hand may contain fungal particle which they use to feel the garri, or sometimes put it in their mouth to test the quality of the garri before buying and the left over is put back into the selling bowl. These activities may lead to contamination due to deposition of fungal spores or mycellia on the exposed garri products and transfer of infectious agent (Ogiehor et al., 2007; Ogugbue and Obi, 2011; Ogugbue et al., 2011; Ogeihor and Ikenebomeh, 2005).

Aspergillus species and *Penicillium* species had the highest frequency of occurrence in the different garri samples, while *Aspergillus* species had the highest occurrence in yellow garri. This high frequency of occurrence of these moulds in white and yellow garri could have been as a result of the unhygienic handling of the garri or poor storage conditions of the garri in compounds where they were isolated. High moisture content favours the growth of these moulds and significant mould count has been obtained from garri samples kept in basins exposed to air (Ogugbue and obi, 2011).

Aspergillus species which had the highest occurrence in both yellow and white garri in this study are among the most abundant and widely distributed organisms on earth (Bennett and Klich, 2003). Most members of this genus being saprophytic moulds, are found in the environment without causing disease (Cheesborough, 2005). Fungi of the *Aspergillus* species are typical isogenic opportunistic moulds, which for the most part fail to trigger an infection with a healthy person; however, they constitute a threat predominantly to persons with immunity disorders (Türel, 2011).

It is one of the most commonly reported fungi isolated from foods and indoor environments with ability to produce aflatoxin as its major mycotoxins (Pitts and Hocking, 1997; Klich, 2002). *Penicillium* sp. commonly found in carpet, wall paper and in the interior may cause hypersensitivity, pneumonitis, allergic alveolitis in susceptible individuals and is also a common cause of extrinsic asthma (John et al., 1995).

Though not a common pathogens to humans, it has however, been reported as a common opportunistic pathogen causing Peniciliosis among HIV positive individuals in Southern Asia (Skoulidis et al., 2004). The presence of *Penicillium* species in food puts the consumer at risk of ingesting citrinin which had been reported to be nephrotoxic in pigs and in broilers (Ojo, 2003).

These three moulds, *Aspergillus* sp. (isolated from the compound Okori-Ama, Okulobo-Ama, Adule-Ama), *Penicillium* sp. (isolated from Wapere-Ama, Ogoun-Ama) and *Rhizopus* sp. (isolated from Ebilade-Ama) were present in yellow garri and also white garri. While in white garri, *Aspergillus* sp. was isolated from Agbedi-Ama, Goin-Ama, *Penicillium* sp. was isolated from the compounds, Bietebi-Ama, Ebitimikondei-Ama and *Rhizopus*

was isolated from Ogbopina-Ama compound. The isolation of these three moulds in both white and yellow garri may be due to poor storage of the garri sample in shops of these compounds. Also, *Alternaria* sp. was present in white garri obtained in Foro-Ama compound but absent in yellow garri, while *Fusarium* sp., *Mucor* sp. and *Cladosporium* sp. were absent in white garri in the market but present in yellow market garri samples obtained from Efeke-Ama, Sadeimo-Ama and Ayaogbo-Ama compound, respectively. The variation in moulds obtained from the different compound could be due to their different storage conditions in the market and also their relative permeability to oxygen, carbon dioxide and water vapour. Permeability characteristics and oxygen transfer rate (OTR) has been reported to be factors responsible for differences obtained in mould count progression in stored market garri (Amadi and Adebola, 2008). The growth of fungi in any food results in changes in the organoleptic, microbiological and nutritive quality which leads to spoilage (Ogeihor and Ikenebomeh, 2005) and its presence in food suggest an imminent public health danger since their metabolites (mycotoxins) if produced in food (Klich, 2002) like garri may lead to serious and devastating clinical conditions in the consumers.

Conclusion

Fungal contamination of garri is as a result of practices associated with post processing of this product. These processes are spreading on the floor to cool, poor packaging, storage conditions, displaying in open bowl or buckets in the markets during sales and some customers antics before purchase. Some of the fungi isolated in this study can cause diseases or produce mycotoxins that can have serious health effect in man; it is henceforth important to develop a strategy to properly package and store this product to reduce fungal contamination. Also, handling of this product with bare hand, displaying it in open bowl and buckets in the market during sales should be avoided to prevent the introduction of fungi spores to the product.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

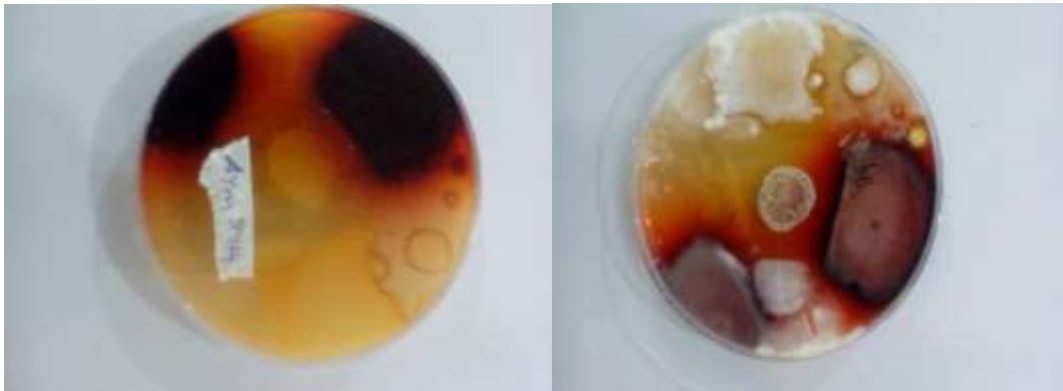
We are really grateful and appreciate the great women in the different family compounds in Amassoma community for their help in obtaining the garri samples. Without their help, this research would not have been possible.

REFERENCES

Adepoju OT, Adekola YG, Mustapha SO, Ogunola SI (2010). Effect of processing methods on nutrient retention and contribution of cassava

- to nutrient intake of Nigerian consumers. *Afr. J. Food Agric Nutr. Dev.* 10 (2):2099-2111.
- Amadi JE, Adebola MO (2008). Effect of moisture content and storage conditions on the storability of garri. *Afr. J. Biotechnol.* 7(24):4591-4594
- Asegbeloyin JN, Onyimonyi AE (2007). The effects of different processing methods on the residual cyanide of Garri. *Pak. J. Nutr.* 62:163-166.
- Bräse S, Encinas A, Keck J, Nising C (2009). Chemistry and biology of mycotoxins and related fungal metabolites. *Chemical Reviews* 109:(9)3903-3990,
- Edem DO, Ayatse JOI, Itam EH (2001). Effects of soy protein supplementation on the nutritive value of garri farina from *Manihot-esculenta*. *Food Chem.* 75:57-62.
- Efiuwewwere BJO, Isaiah AU (1998). Effects of hygienic handling in combination with potassium sorbate treatment and smoking on the microbial quality and shelf-stability of croaker (*Micropogonias furnieri*). *Ekundayo CA (1984). Microbial spoilage of packaged garri in storage. Microbiol. Letters.* 23:271-278.
- Ernesto M, Cardoso AP, Cliff J, Bradbury JH (2000). Cyanogens in cassava flour and roots and urinary thiocyanate concentration in Mozambique. *J. Food Composition and Analysis.* 13:1-12.
- Fawole M, Osho A (1995). *Laboratory methods in microbiology.* Books Ltd; Nigeria. Pp 34-35.
- Frazier WC, Westhoff DC (2000). *Food Microbiology.* McGraw-Hill Inc., New York. pp. 17-48, 441-443.
- Igbeka JC (1987). Stimulation of moisture profile in stored garri. *J. Food Agric.* 1:5-9.
- Jekayinfa SO. and Olajide JO. (2007). Analysis of energy usage in the production of three selected cassava based foods in Nigeria. *J. Food Eng.* 82:217-226.
- John TC, Heidi L, Lawrence CP (1995). *Manual of Medical Mycology,* Blackwell Sciences, Cambridge, Massachusetts pp 263
- Klich MA (2002). Identification of common *Aspergillus* species Centraalbureau voor Schimmecultures. Pp. 116. Utrecht
- Kostinek M, Specht I, Edward VA, Schillinger U, Hertel C, Holzapfel WH, Franz CM (2005). Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Garri, a traditional African food. *Syst. Appl. Microbiol.* 286:527-540.
- Krzyściak P, Skóra M, Macura AB (2011). *Atlas grzybów chorobotwórczych człowieka,* MedPharm POLSKA, ISBN 978-83-60466-80-3, Wrocław, Poland
- Ogiehor IS, Ekundayo AO, Okwu GI (2005). Shelf stability of agidi produced from maize (*Zea mays*) and the effects of sodium benzoate treatment in combination with low temperature storage. *Afr. J. Biotechnol.* 4(7):738-743.
- Ogiehor IS, Ikenebomeh, MJ. (2005). Extension of shelf life of garri by hygienic handling and sodium benzoate treatment. *Afr. J. Biotechnol.* 4(7):744-748.
- Ogiehor IS, Ikenebomeh MJ, Ekundayo A.O. (2007). The bioload and aflatoxin content of market gari from some selected states in Southern Nigeria: public health significance. *Afr. Health Sci.* 7(4):223-227.
- Ogugbue CJ, Mbakwem-Aniebo C, Akubuenyi F (2011). Assessment of microbial air contamination of post processed garri on sale in markets. *Afr. J. Food Sci.* 5(8):503-512
- Ojo MO (2003). *Food and animal borne disease.* Vol II, Dart ventures, Ibadan, Nigeria 64:77
- Oluwole OB, Olatunji OO, Odunfa SS (2004). A process technology for conversion of dried cassava chips into garri. *Nig. food J.* 22:65-73
- Pitts JI, Hocking AD (1997). *Fungi and food spoilage* 2nd ed. London Blackie Academic and Professional
- Royal Horticultural Society (RHS), (2005). *Mini colour chart,* Flower Councile Holland.
- Skoulidis F, Marina S.M, Kenneth MM (2004). *Penicillium marneffer,* a pathogen on our doorstep. *Journal of the Royal society of Medicine* 97: 394-396
- Subba Rao NS (2000). *Soil Microbiology (4th Edn).* Oxford and IBH publishing Co. PUT Ltd New Dehli. pp. 283-286.
- Türel O (2011). Newer antifungal agents. *Expert Review of Anti-Infective Therapy,* 9(3):325-338.

Appendix: Photo plates of fungi isolated



Reverse side

Obverse side

Plate 1. *Cladosporium* sp.

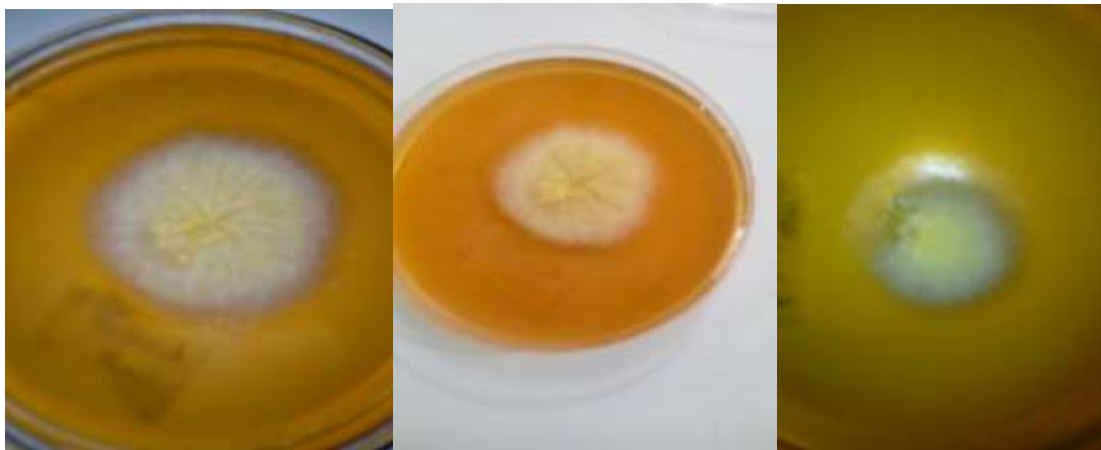


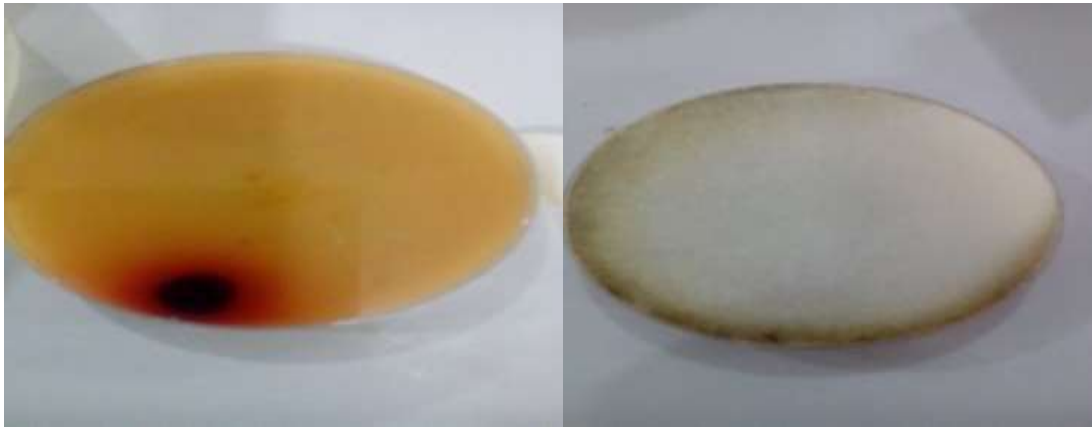
Plate 2. *Penicillium* sp.



Reverse

Obverse

Plate 3. *Rhizopus* sp. (obverse side and reverse side).



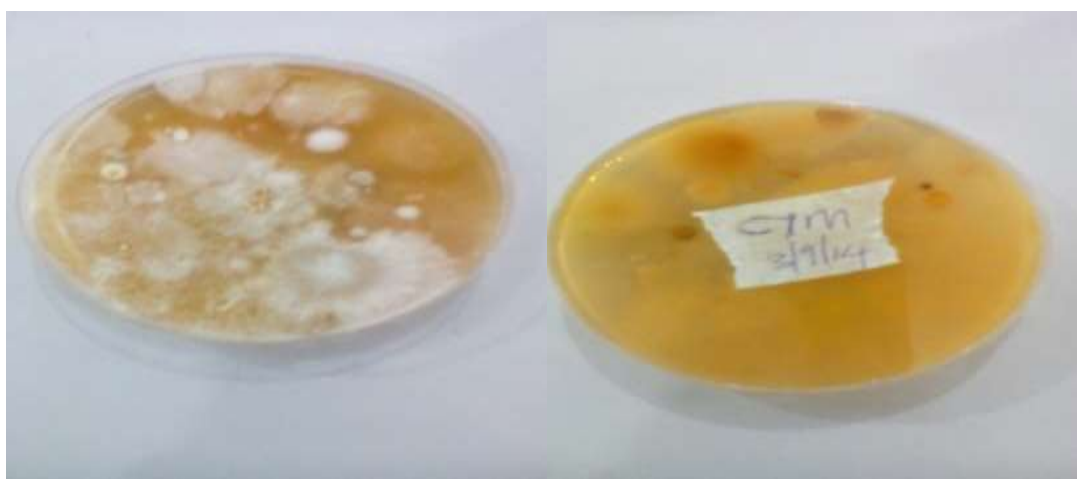
Reverse side

Obverse side

Plate 4. *Mucor* sp.



Plate 5. *Aspergillus* sp.



Obverse side

Reverse side

Plate 6. Culture of *Fusarium* sp.



Obverse side

Reverse side

Plate 7. *Alternaria* sp.

Full Length Research Paper

A survey on traditional fish smoking and the socio-economic status of the fish processors in Lagos State, Nigeria

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Received 10 October 2014; Accepted 21 January 2015

This study examined the socio-economic characteristics, technologies and hygiene of the processors in 20 different 'fish' processing centres in fishing communities in Lagos State. A total of 200 questionnaires were administered through purposive sampling method at 10 respondents/processing centre. Data were collected through field observation and administration of structured questionnaire. Analytical technique used was descriptive statistics. Results reveal that most of the households were relatively poor, using age and educational level of the processors and availability of household amenities as proxies for socio-economic status. Majority of the processors (55.5%) were old women, 51.5% had primary school education while 38% had post-primary school education. The study shows that majority (98.0%) of the processors practiced manual operations while 2.0% practiced mechanical operation. Every processor used eviscerating, washing, filleting and de-scaling and 99.0% of the processors operated on non-concrete floor while 1.0% were on mould floor. Majority (98%) used firewood as normal smoking fuel while only 0.5% used charcoal. Majority (77.5%) of the processors used full drum as smoker, 2.0% used half drum while 19.0% used mud oven and the rest (1.5%) used charcoal oven and 92.0% of the processors were from urban communities while 8.0% of them were from rural communities. Very few processors (1.0%) used disinfectants for their processing facilities and environment. The study concludes that fish smoking makes an important contribution to household food and financial security in all processing centres.

Key words: Fish, hygiene, processors, smoking, socio-economic, survey.

INTRODUCTION

Fish is an important dietary component of people all around the world and represents a relatively cheap and accessible source of high quality protein for poorer households

(Ikutegbe and Sikoki, 2014). Global production of fish, mussels and crab in 2010 was almost 60 million tonnes, a figure which includes production in marine waters,

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brackish water and freshwater. Aquaculture production is now about three quarters of that from ocean fish and seafood caught in the wild. In 2011 this amounted to 78.9 million tones, 15% of which was cured in one or another way (Ikutegbe and Sikoki, 2014; FAO, 2013). One third of the cured fish was smoked and about 20% of the smoked fish goes into international trade (Clucas and Ward, 1996). No other food industry has shown such growth as aquaculture in recent decades. Between 1970 and 2008 annual production worldwide increased by an average of 8.4%; much more than poultry farming and egg production, which have the second highest growth rates after aquaculture (da Silva, 2002; da Silva et al., 2008; Abolagba and Melle, 2008).

In Nigeria, fish production through aquaculture has risen steadily from a few hundred kilograms in the 1950s to over 45,000 metric tonnes in 2004 (FAO, 2007). Today, aquaculture is the fastest growing livestock production sector in Nigeria, with a growth of about 29% in 2006 alone, and with prospects of continued growth.

This is because demand for fish is on the increase with population growth, while catches from fisheries are on the decline, even globally (FAO, 2007). In Nigeria smoked fish products are the commonest form of fish product for consumption. Out of the total of 194,000 metric tons of dry fish produced in Nigeria, about 61% of it was smoked. One of the greatest problems affecting the fishing industry all over the world is fish spoilage. In high ambient temperature of the tropics, fresh fish have the tendency to spoil within 12 to 20 h (Clucas and Ward, 1996). Attempt has been made to reduce fish spoilage to the minimum through improved preservation techniques. Preservation and processing methods explore ways by which spoilage are stopped or slowed down to give product a longer shelf life. Fisheries have been the main source of livelihood for the population of fishing communities in Nigeria and a vital sector of the economy by employing more than 6 million fisher folks in Nigeria (Fish for All Summit, 2005; Fregene and Bolorunduro, 2009) in terms of fish production, processing and distribution. Entire family (men, women and children) in the fishing communities are engaged in the sector. The catch from these fisheries plays an important source of animal protein in peoples' diets.

Seasonal fluctuation in food availability and household responses to this insecurity has been observed to influence individual consumption patterns (Longhurst, 1986; Fregene and Bolorunduro, 2009). In fishing communities, during the off-fishing season, which is usually in the rainy season (July to September), fish catch is low. This is because of the increased level of turbid water and strong wind, which hinder the fishermen without outboard engine from going far out to sea. In an attempt to make a living, they have resulted to the exploitation of generally fragile environment, thereby leading to a cycle of low production, low income, and poverty and being food in-secured (National Institute of Oceanography and Marine Research, (NIOMR), 1989; Federal Office of Statistics, (FOS), 1999). As a

result of inadequate purchasing power (income), fisher folks often experience a food in-secured period (Fregene, 2002; Fregene and Bolorunduro, 2009).

Small scale fishing communities in Nigeria, as elsewhere in the world are vulnerable to exploitation due to poverty and uncertainty of their income. The seasonality of fish catch coupled with inadequate processing capacity has resulted in high post-harvest losses, which diminish benefits accruing to small-scale operators. Realizing that fisher folks are not a homogenous group of people, there is the need for a comparative study of fishing communities.

Therefore, the main objective of this study was to carry out a survey on traditional fish smoking activities in Lagos State, Nigeria in order to examine the socio-economic characteristics, role of women and expenditure pattern as they relate to variation in poverty level in among fish processors in fishing communities in Lagos State.

METHODOLOGY

Survey

The study was carried out in Lagos State, which has 22.5% of Nigeria's coastline and occupies an area of 3,577 square kilometre mass with 786.94 square kilometre or 22% of it being lagoons and creeks, in Lagos, Ikorodu, Badagry and Epe local government areas (Udo and Mamman, 1993). Purposive sampling technique was used for surveying. Structured questionnaire was administered to 200 respondents by enumerators. Data were also collected through field observation and on the spot assessment to collect information on socio-demographic and environmental health data of selected 'smoked fish' processors. Descriptive statistics used include measures of distribution, central tendency and dispersion respectively.

Sampling sites

The sampling sites for this study include Agbalata, Ajido, Asakpo, Boguru, Fvanoveh, Gberefun/Yovoyan, Gbetrome, Ilaje, Kofegameh, Pako, Afuye, Bodin Yawa, Idale, Igbodun, Ilogun, Mejona, Oluwo, Okorisan, Orita, Orogoro from two Local Government Areas (Badagry and Epe) of Lagos State.

Area of study

Using a current geopolitical map of Nigeria (Figure 1), Lagos State lies to the south-western part of Nigeria and has boundaries with Ogun State both in the north and east. It is bordered on the west by the Republic of Benin and in the south, stretches for 180 km. along the coast of the Atlantic Ocean. It therefore has 22.5% of Nigeria's coastline and occupies an area of 3,577 km² land mass with about 786.94 km² (22%) of it being lagoons and creeks. The state is endowed with marine, brackish and fresh water ecological zones with varying fish species that provide productive fishing opportunity for fishermen (Udo and Mamman, 1993). Two local government areas (Badagry and Epe Local Government) were covered because they are highly densified fish processing centres.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of processors in the 20 processing centers surveyed. Majority of the processors



Figure 1. Map of Lagos State (<http://nigerianfinder.com> accessed Jan, 2015).

Table 1. Characteristics of the 20 study smoking/processing centres.

Parameters	Number observed (%)
Age of processors	
Old women	111(55.5)
Young women	89(44.5)
Educational level of processors	
No schooling	21(10.5)
Primary school	103(51.5)
Post secondary school	76(38)
Packaging	
Basket with dry plantain leaves	186(93)
Basket without polypropylene	14(7)
Method of sewage disposal	
Pit latrine	125(62.5)
Water-carriage system	10 (5)
None	65(12.5)
Source of processing water	
Spring	30(15)
Pipe-borne	20(10)
Stream	100(50)
Bore-hole	30(15)
Rain	20(10)
Normal cooking fuel	
Firewood	196 (98)
Coal/charcoal	1(0.5)
Others	3(1.5)
Source of income	
Husband	15(7.5)
Wife	175(87.5)
Others	10(5)

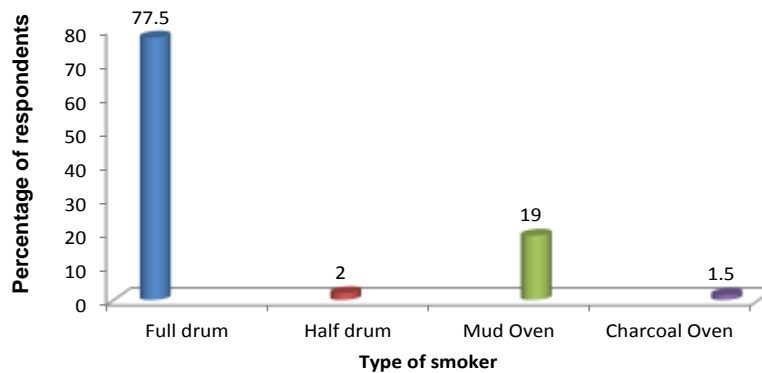


Figure 2. Distribution of type of smoker.

(55.5%) are old women, 51.5% had primary school education while 38% had post-primary school education. The relationship between household socio-economic characteristics and food processing has been amply demonstrated in the literature (Fregene and Bolorunduro, 2009). For example, using educational level of the processors and availability of household amenities as proxies for socio-economic status, it is apparent that most of the households were relatively poor. This has significant implications for fish processing in general and for fish hygiene behaviour in particular (Fregene and Bolorunduro, 2009). Education is also related to employment and income which influence access to household amenities and facilities, including those related to fish hygiene and environmental health. Result shows that 93% of the processors packaged the fish in Basket with dry plantain leaves while only 10% used Basket with polypropylene. Survey of the processing centres shows that 62.5% used pit latrine as method of waste disposal, 5% used water-carriage system and 12.5% had no means of waste disposal. Most (50%) of the processors used stream water for processing, 15% used spring and boreholes.

The processing sites in all the processing centres are mostly located in places where they remain a threat to food safety. In all the processing sites surveyed, there is no adequate drainage and waste disposal systems. The facilities provided were designed and constructed in a place closer to the processing sites which makes it having a high risk of contaminating the smoked fish. Most of the processing sites have no storage facilities for both raw materials and finished product. The processors were not applying a good quality control system into this critical aspect of the fish smoking/processing. The fresh fish are not normally inspected and sorted before processing so as to segregate fish which is evidently unfit for human consumption. Also, there supposed to be a way of protecting fresh fish from damages such as bruising which can easily initiate contamination by pests or microorganisms and enzymatic activity. Majority (98%) used firewood as normal cooking fuel while only 0.5% used charcoal. Majority (77.5%) of the processors used full drum as smoker, 2.0%

used half drum (Figure 2), while 19.0% used mud oven and the rest (1.5%) used charcoal oven. Average capacity of a full drum as smoker used by processors was 71.42 kg, half drum smoker has capacity 66.67kg, while mud oven has capacity of 82.11 kg and the charcoal oven has capacity of 100.0 kg. Majority (87.5%) of processors contributed a minimum of N1, 600.00 and maximum of 480000.00 as start-up capital for the business and the mean contribution was N51, 324.86. The other processors (12.5%) contributed a minimum of N14, 000.00 and maximum of N600, 000.00 and their mean contribution was N55, 775.26.

The study shows that fish processors are operating in rural and urban areas and the activity appears to be increasing in popularity. Fish processors operate on a range of scales and cheap and readily available smoking kilns were used. Family labour plays a critical role, but micro-enterprises which employ casual labour are also common.

For some enterprises fish processing constitutes a full-time activity. They also operate on a part-time basis, using family labour. Majority (98.0%) of the processors practiced manual operations while (2.0%) practiced mechanical operation. Every processor used eviscerating, washing, filleting and de-scaling and (99.0%) of the processors used non concrete floor while (1.0%) used mould floor. Majority (92.0%) of the processors were from urban communities (Figure 3) while (8.0%) of them were from rural communities and (17.0%) of the processors from rural communities processed Bonga shad type of fish and followed by (12.0%) of the processors processed Silver catfish. Very few processors (1.0%) used disinfectants for their processing facilities and environment (Figure 4). The facilities used for processing are not properly cleaned always and this is a threat to food safety. The cleaning system in the entire processing site is not adequate.

The materials provided for cleaning food are not adequately and suitably designed and these can easily harbor pathogenic organisms. Moreover, there is no facilities for adequate supply of potable water for cleaning. There is

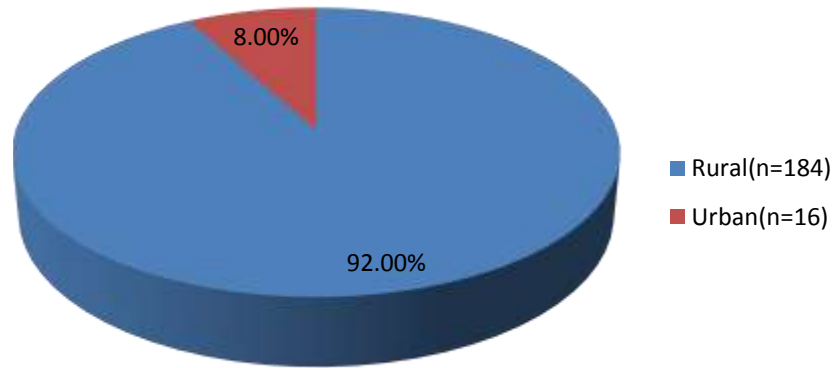


Figure 3. Distribution of processing centres/communities.

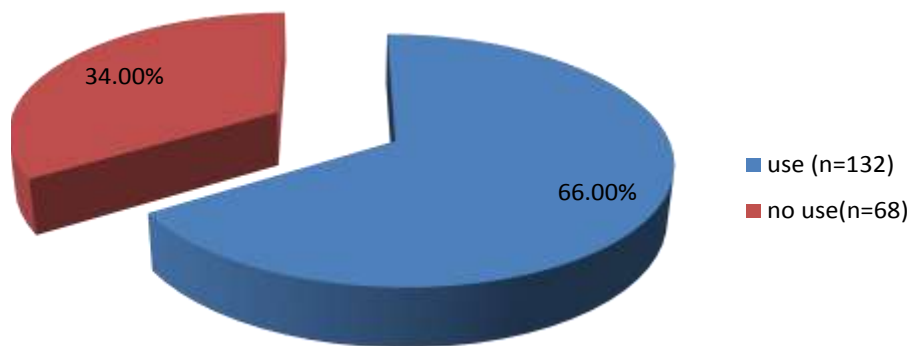


Figure 4. The use of chemicals by processors.

no adequate method of controlling the pests in all the processing sites. The containers used for processing are not always washed immediately after use, and this allows the pests free movement to operate and this can result in malicious or accidental contamination of food. Due to the traditional method of smoking fish, there are no facilities available for personal hygiene which can assist in ensuring that an appropriate degree of personnel hygiene is maintained and to avoid contaminating the fish. There was a high awareness concerning personal hygiene among the processors. People who came directly or indirectly into contact with the fish are not likely to contaminate the fish due to: maintaining of appropriate degree of personal cleanliness and behaviour and operating in an appropriate manner. Purchasing of smoked fish from market vendors poses a considerable health risk.

The reasons for this are apparent from observational data on hygiene practices in the market. Smoked fish are often displayed openly on the tray in very poor sanitary environments. The prevalence of flies at the markets and the apparent lack of facilities for food protection suggest a high potential for contamination. Smoked fish are also subjected to repeated contamination from the unwashed hands of vendors, and the materials used for wrapping, such as reusable polythene bags, waste paper and baskets, may also be a source of contamination. Figure 5 shows

that 80, 61.5 and 28% of processors respectively from year 2010, 2011 and 2012 claimed that 30 to 40% of household income was spend on food, while 14, 31 and 60% of processors respectively from year 2010, 2011 and 2012 claimed that 41 to 50% of household income was spend on food and 5.5, 7.5 and 12% of processors respectively from year 2010, 2011 and 2012 claimed that >60% of household income was spend on food. The study shows that fish smoking makes an important contribution to household security in all processing centres. As described earlier, previous understanding amongst researchers was that fish processing was predominantly a rural activity; less integrated in market systems than other agricultural pro-ducts (Fregene and Bolorunduro, 2009). The baseline survey found quite a different situation. In processing centres such as Oluwo and Agbalata, and even more remote places such as Idale, fish processors have long-standing connection to urban markets and market their produce on a relatively large scale.

Conclusion

The baseline assessment revealed that using educational level of the processors and availability of household amenities as proxies for socio-economic status, it is

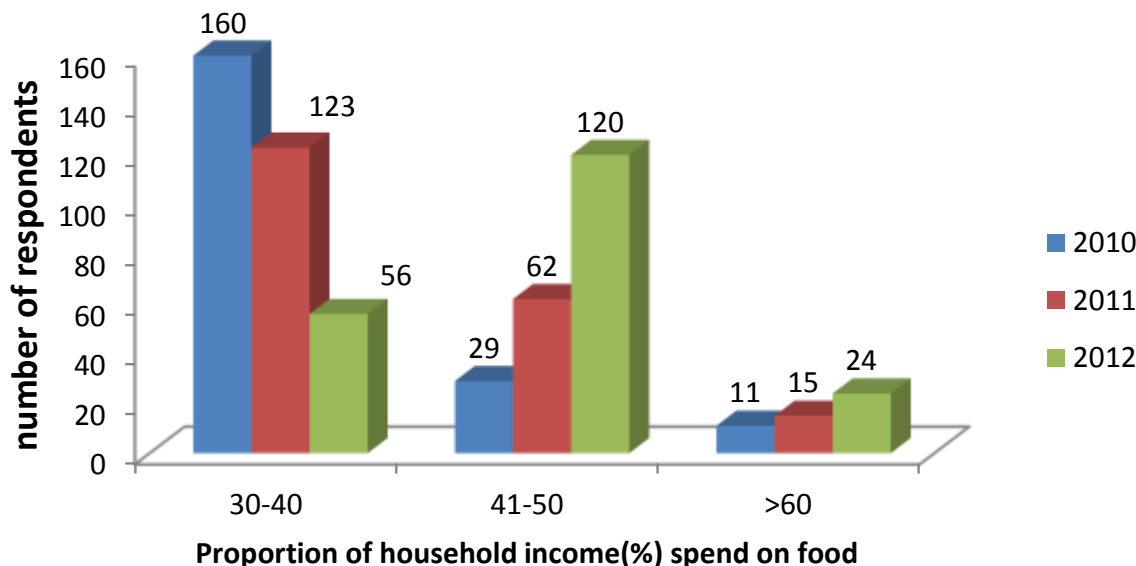


Figure 5. Proportion of household income spent on food.

apparent that most of the households were relatively poor. Majority of the processors (55.5%) are old women, 51.5% had primary school education while 38% had post-primary school education. Education is also related to employment and income which influence access to household amenities and facilities, including those related to fish hygiene and environmental health. The study shows that fish smoking makes an important contribution to household security in all processing centres. The study also found that fish processors are operating in rural and urban areas and the activity appears to be increasing in popularity. Fish processors operate on a range of scales. Family labour plays a critical role, but micro-enterprises which employ casual labour are also common. For some enterprises fish processing constitutes a full-time activity. They also operate on a part-time basis, using family labour. It was also found that the structure and condition of processing sites are below safety standard due to the following reasons: The floors are not constructed to allow adequate drainage and cleaning; the operations are made in open places without a constructed processing plant.

Conflict of interest

The authors have no conflict of interest.

REFERENCES

- Abolagba OJ, Melle OO (2008). Chemical composition and keeping qualities of a Scaly Fish Tilapia (*Oreochromis niloticus*) Smoked with two Energy Sources. *Afr. J. Gen. Agric.* 4(2):113-117.
- Clucas IJ, Ward AR (1996): Post Harvest Fisheries Development. A Guide to Handling, Preservation, Processing and Quality. Chatham Maritime, Kent ME4TB, United Kingdom. Pp. 665.
- da Silva LVA (2002) Hazard Analysis Critical Control Point (HACCP), Microbial Safety, and Shelf Life of Smoked Blue Catfish (*Ictalurus furcatus*). A Master of Science in Food Science Thesis, the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College.
- da Silva LVA, Prinyawiwatkul W, King JM, No HK, Bankston JD Jr, Ge B (2008) Effect of preservatives on microbial safety and quality of smoked blue catfish (*Ictalurus furcatus*) steaks during room-temperature storage. *Food Microbiol.* 25(8):958-963.
- FAO (2007) Fish Processing in Africa. FAO, Fish .Rep.
- FAO (2013) Post-harvest changes in fish Food and Agriculture Organization of the United Nations for a world without hunger, Fisheries and Aquaculture Department.
- Federal Office of Statistics (FOS) (1999) Poverty and Agricultural Sector in Nigeria, Federal Office of Statistics, 33 pp.
- Fregene, BT (2002) Poverty Assessment in Fishing Communities in Lagos State, Nigeria, Unpublished Ph. D. Thesis University of Ibadan, Ibadan 226p.
- Fregene BT, Bolorunduro P I (2009). Role of Women in Food Security and Seasonal Variation of Expenditure Pattern in Coastal Fishing Communities in Lagos State. *Journal of Agricultural Extension* Vol. 13 (2) December 2009 pp 21-33
- Fish for All Summit (2005). Presentation of Nigeria's Fisheries Resources Abuja, Nigeria, 22-25 August 2005.
- FDA, Department of Health and Human Services, (2001). Pathogen Growth & Toxin Formation as a Result of inadequate Drying. In *Fish & Fisheries Products Hazards & Controls Guidance: Third Ed.* Chapter 14, p.191.
- Goktepe I, Moody MW (1998). Effect of modified atmosphere package on the quality of smoked catfish. *J. Muscle Foods* 9:375-389.
- Ikutegbe V, Sikoki F (2014). Microbiological and biochemical spoilage of smoke-dried fishes sold in West African open markets. *Food Chem.* 161:332-336.
- Longhurst R (1986). Household Food strategies in Response to Seasonality and Famine, *IDS Bulletin* Vol. 17 No. 3 Institution of Development Studies, Sussex Map of various states and their local government areas. *Nigerianmuse.com*, accessed Dec. 3, 2014
- Udo RK, Mamman AB (1993). Nigeria: Giant in the Tropics Vol. 2. Gabumo Publishing Co. Ltd. Lagos Nigeria.

Full Length Research Paper

Effects of stocking density on haematological functions of juvenile African catfish (*Clarias gariepinus*) fed varying crude protein levels

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Received 5 January, 2011; Accepted 5 April 2011

This study was carried out to evaluate the growth performance and some haematological functions of *Clarias gariepinus* fed different levels of crude protein under varying stocking densities. Fish of even sizes were randomly selected and stocked in plastic circular tanks (0.05 x 0.03 x 0.03 m) at three stocking densities of 10 fish/m³ (control), 15 fish/m³ and 20 fish/m³. The fish of each stocking density were fed either a diet containing 40% Crude protein (CP) or 45% CP with a feeding rate of 3% body weight twice daily. The experiment was replicated thrice and it lasted for 12 weeks during which haematological parameters and plasma biochemistry were measured. From this study, haemoglobin (Hb) and Red Blood Cell (RBC) slightly increased in all treatments but the variation was not significant in relation to protein level in the diets. Plasma glucose increased significantly ($P < 0.05$) in relation to the stocking densities. The plasma protein showed insignificant variation in relation to the stocking density but the variation was more pronounced ($P < 0.05$) at the lowest stocking density as the protein level in the diets varied. It can be concluded that enhancing feed quality especially protein level in the fish diet may ensure faster growth, stress reduction and improve health status of the fish.

Key words: Crowding stress, haematological functions, *Clarias gariepinus*, diet quality.

INTRODUCTION

Environmental stress is an important factor responsible for limiting fish performance under aquaculture conditions (Ellis et al., 2002). When fish are subjected to adverse environmental conditions, some endocrine and physiological alterations occur, often resulting in change in ability of the fish to survive, grow and reproduce (Barton and Iwama, 1991). Overcrowding is a common chronic stressor in aquaculture that can induce a prolonged elevation of

cortisol levels, which may cause damaging consequences, and suppressed growth (Rowland et al., 2006). This effect has been attributed to factors such as decreased food consumption. The high stocking density also imposes increased energy demands that require fish to cope with metabolic adjustments, such as changes of gluconeogenic and glycolytic activities. Under such conditions, food consumption is reduced; the extra expenditure energy has

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to be met by the reserve, resulting in reduced growth (Rowland et al., 2006).

Crowding is a common aquaculture practice used to manage water usage or increase fish stocking density (Baras and Lagardere, 1995). However, the use of high stocking density as a technique to maximize water usage and thus increase stock production has also been shown to have adverse effect on growth. In many cultured fish species, growth has indirect relationship to stocking density and this observation is mainly attributed to social interactions (Holm et al., 1990; Haylor, 1991; Ma et al., 2006). Social interactions as a result of competition for food and/or space can negatively affect fish growth. On the other hand, the fish price is influenced by the market requirement such as size and production, which depends on their growth. In modern day commercial aquaculture the stocking density is an important indicator that determines the economic viability of the production system (Ellis et al., 2002).

Therefore by establishing the relationship between dietary protein level and crowding stress, monitoring of fish stocks and prediction on their haematological needs will be possible and enhanced. This study was carried out to assess the effects of crowding stress on the growth performance and some haematological functions of juvenile African catfish; *Clarias gariepinus* (a commercially important fish species in Africa) fed varying crude protein levels.

MATERIALS AND METHODS

Experimental procedure

This study was carried out in the Department of Wildlife and Fisheries Management laboratory, Faculty of Agriculture and Forestry, University of Ibadan. Four hundred *Clarias gariepinus* juveniles were purchased from a reputable farm - Rayak Fish farm Ibadan, Nigeria. The fish were transported in a well -oxygenated bag, which was half filled with fresh water to the Department of Wildlife and Fisheries Management laboratory. The average weight of the fish was $17.8 \text{ g} \pm 2.50$ and their average length was $14.4 \text{ cm} \pm 1.20$. The fish were kept to acclimatize for 2 weeks under laboratory condition in an indoor circular plastic tank. The fish were randomly distributed in 18 experimental tanks (each of $0.05 \times 0.03 \times 0.03 \text{ m}$) and the tank was filled with water at 60% capacity. The positioning of the tanks allowed a natural photoperiod of 12 h sunlight and 12 h darkness throughout the experiment. Water was changed daily to prevent fouling resulting from food residues. The source of water was the University of Ibadan Borehole Water Station. The fish were stocked into the tanks using three different stocking densities of 10 fish/m³, 15 fish/m³ and 20 fish/m³ for treatments I and II respectively. The fish with lowest stocking density and fed with 40% CP diet served as the control based on recommendation of Akinwole (2007). The fish were fed with 40% crude protein and 45% crude protein diets for 12 weeks twice daily between the hours of 8.00 and 17.00 at the rate of 3% body weight. Feeds were given in pellet form and the particle size increased periodically as fish grew. Fish were weighed initially using a weighing scale (g) before the commencement of the experiment and on a bi-weekly basis during the experiment. A calibrated measuring ruler (cm) was used to take the length of the fish before the experiment and bi-weekly during the experiment for 12 weeks.

The experimental diets were analyzed for proximate composition by using the methods prescribed by Association of Official Analytical Chemists (AOAC, 1990).

Haematological and biochemical analyses

This was carried out at the haematological laboratory of Veterinary Medicine Department University of Ibadan. Fish were starved for 24 h prior to sampling. Fish were anaesthetized with buffered MS222 (50 mg/l) and blood was collected with a hypodermic syringe from the caudal vein. Blood collection lasted less than 3 min in order to avoid stress-induced situation during sampling. The extracted blood was divided in two sets of eppendorf tubes. One set contained heparin, used as anticoagulant, for haematology (haemoglobin, hematocrit and red blood cell counting). The second set, without anticoagulant, was left to clot at 4°C and subsequently centrifuged at 5000 rpm for 10 min at room temperature. The collected serum was stored at -20°C for further assays (Stoskopf, 1993).

Physiological measurements

Red blood cells (RBCs, cells/ μl) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline solution. White blood cells were counted by using diluting fluids (3% aqueous solution of acetic acid plus 1% aqueous solution of gentian violet up to 1%). A 1:20 dilution was made and charged into Neubauer hemocytometer chamber for counting. Glucose was determined colorimetrically using glucose kits according to Trinder (1969). Total protein content in blood plasma was determined colorimetrically according to Henry (1964).

Statistical analysis

The data obtained were subjected to two-way ANOVA test and the differences between means were at the 5% probability level using Duncan's new multiple range test (DMRT). The software SPSS, version 10 (SPSS, Richmond USA) was used as described by Dytham (1999).

RESULTS

Gross composition of experimental diets at 40% and 45% CP is presented in Table 1 while Table 2 shows the haematological parameters of the test fish at various stocking density fed varying crude protein diets. Result from this study showed that there is no variation in the RBCs level with increasing stocking density; the RBCs level however increased significantly ($P < 0.05$) with increase in diet protein level. Also the Hb and Hct followed the same pattern as observed for the RBC in relation to the stocking density but however did not show any appreciable reduction with increase in diet protein level.

The plasma protein was significantly influenced by the crude protein elevation in the diets while high stocking density gave no significant variation in the plasma protein level. Generally, the plasma glucose level ranged from 90.00 to 126.28 mg/dl with lowest value recorded at lowest stocking density in the fish fed 40% crude protein diet. The plasma glucose level significantly increased

Table 1. Gross composition and chemical analysis of experimental diets using 40% and 45% crude protein.

Ingredient	(40% CP)	(45% CP)
Fish Meal	8.38	9.95
Groundnut Cake	25.14	29.85
Soybean Meal	33.52	39.81
Wheat offal	10.49	6.30
Yellow Maize	20.97	12.59
Salt	0.5	0.5
Vitamin Premix	0.25	0.25
Vegetable Oil	0.5	0.5
Mineral Premix	0.25	0.25
Total (kilogram) 100	100	
Chemical analysis (%)		
Moisture	8.61±0.02	8.52±0.03
Crude Protein	41.28±1.01	45.43±0.57
Crude Fat	4.79±0.002	5.86±0.02
Crude Fiber	2.97±0.02	2.84±0.02
Ash	14.88±0.001	15.34±0.01
Dry Matter	91.36±0.02	91.46±0.01
Nitrogen Free Extract	27.44±0.02	27.98±0.01

Vitamin premix contains; Vit. A, 20000000 I.U; D₃, 2000000 I.U; E, 200000 mg; K₃, 8000 mg; B₂, 30000 mg; B₁, 30000; B₆, 12000mg; B₁₂, 50 mg; C, 500000 mg; Niacin, 150000; pantothenic acid, 50000 mg; folic acid, 4000 mg; Biotin H12, 800 mg; choline chloride, 600000 mg; cobalt, 2000mg; copper, 4000 mg; iodine, 5000 mg; inositol, 200000 mg; iron, 40000 mg; manganese, 30000 mg; selenium (organic), 200 mg; zinc, 40000 mg; lysine, 100000 mg; methionine, 100000 mg and Antioxidant, 100000 mg.

Table 2. Haematological parameters of African catfish (*C. gariepinus*) fed different protein levels at two stocking densities.

Parameter	Treatment					
	40%CP			45% CP		
	SD1 (10 fish/tank)	SD2 (15 fish/tank)	SD3 (20 fish/tank)	SD1 (10 fish/tank)	SD2 (15 fish/tank)	SD3 (20 fish/tank)
RBCs (10 ⁶ /mm ³)	1.68 ^a ± 1.05	1.82 ^a ± 0.04	1.94 ^a ± 0.04	1.74 ^a ± 0.13	1.88 ^a ± 0.14	2.05 ^a ± 0.05
Hb (g/dl)	7.02 ^a ± 0.82	7.50 ^a ± 0.54	7.62 ^a ± 0.54	7.90 ^a ± 0.42	8.22 ^a ± 0.20	8.90 ^a ± 0.45
Glucose (mg/dl)	90.00 ^c ± 8.25	102.24 ^b ± 6.25	112 ^b ± 3.42	122.40 ^a ± 10.00	125.20 ^a ± 8.22	126.28 ^a ± 10.25
Protein (g/dl)	3.84 ^d ± 0.20	4.10 ^c ± 0.05	4.25 ^c ± 0.45	4.90 ^c ± 0.32	5.10 ^b ± 0.58	6.05 ^a ± 0.35

The same letter in the same row is not significantly different at P<0.05. Means with the same superscript along the same row are not significantly different (P>0.05).

(P<0.05) in relation to stocking densities at 40% CP. A significant increase (P< 0.05) was however observed in the plasma glucose level between the two protein diets with higher values recorded in the fish fed diet containing 45% CP. There was significant elevation (P< 0.05) in the plasma glucose level in relation to stocking density as it increases with the increasing stocking density.

DISCUSSION

The use of haematological parameters in assessing the

stress level of teleost fish has been widely adopted in recent times (Barton and Iwama, 1991; Ajani, 2008). The use of these immune system parameters in the determination of alterations in fishes as a result of stress relating issues and the renewed interest in understanding the fish body defense mechanism comes from the need to develop a good healthy management tools toward the sustenance of the rapidly growing aquaculture industry (Mehdi et al., 2010). The RBCs, Hb, and Hct levels in the *C. gariepinus* increased as the stocking density increased. This may be linked to the issue of length of

study, space and water volume used in this study, which resulted in low level of fish activity during the experiment. This observation agreed with the report of McFarlane et al. (2004) and Akungur et al. (2007) who confirmed that any chronic stress induced by high fish density may be limited, but if the fish density increased for a long time or the fish growth was faster, the stress effect may be higher. It was observed that under longer stress time, growth reduction would be recorded especially at higher fish density, as fish will spend more energy on swimming activity at the expense of growth. Mehdi et al. (2010) reported that length of time may be a factor affecting the physiological functions as a result of crowding-related social stress due to increase in biomass with time.

Cortisol and glucose are two of the most common stress indicators but due to their high variability, they must be complemented with other stress indicators in order to have a more complete profile about the stress status of any fish (Martinez-Porchas et al., 2009) and its levels are enhanced under adverse situations (Grutter and Pankhurst, 2000). The plasma glucose of the fish increases with increase in stocking density. This increase may be attributed to the mobilization of glucose by the fish in response to crowding stress; this is generally used as a means of providing extra energy resources so as to enable the fish to overcome the disturbance (Arends et al., 1999; Carragher and Rees, 1994). Alterations in glucose metabolism are a common response to stress (Barton and Iwama, 1991; Braley and Andersson, 1992). On the other hand, the direct effect of the higher metabolic energy demands during stress is driven by an increase in oxygen transport efficiency by elevation of the haematocrit and red blood cell numbers (Acerete et al., 2004). Farghaly et al. (1973) and Wedemeyer and Yasutake (1977) observed that plasma proteins contribute significantly to the maintenance of the blood volume and the water content of the fluid in tissue. The colloidal plasma protein cannot diffuse through capillary membranes to the surrounding relatively protein-free tissues. In this way, they exert an osmotic pressure thus allowing a minimum liquid volume to be maintained in the capillary blood vessels (Verdegem et al., 1997). Plasma protein in this study reflected the protein feeding levels. Schippers et al. (1994) in their works with freshwater-reared rainbow trout did not find significant differences in total plasma protein levels in fish fed different protein levels.

Therefore from the results of this study, it can be concluded that African catfish can easily adapt to high stocking density without any serious effect on the health status or big response of the fish if the correct management procedures are adopted.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- Acerete L, Balasch JC, Espinosa E, Josa A, Tort L (2004). Physiological responses in Eurasian perch (*Perca fluviatilis* L.) subjected to stress by transport and handling. *Aquaculture* 237:167-178.
- Ajani F (2008). Hormonal and hematological responses of *Clarias gariepinus* (Burchell 1822) to ammonia toxicity. *Afr. J. Biotechnol.* 7 (19):3466-3471.
- Akinwale A.O. and Faturoti E.O. (2007): Biological performance of African catfish cultured in recirculating system in Ibadan. *Aquaculture Engineering* 36 (1):18-23.
- Akungur N, Akusungur M, Akbuhit B, Kutlu I (2007). Effects of stocking density on growth performance, survival and food conversion ratio of Turbot (*Psetta maxima*) in the net cages on the southeastern coast of the black sea. *Turk. J. Fish. Aquat. Sci.* 7:147-152
- AOAC (1990). Official Methods of Analysis. 15th edition. K. Helrich (Ed.). Association of Official Analytical Chemists Inc., Arlington, VA.
- Arends RJ, Mancera JM, Munoz JL, Wendelaar Bonga SE (1999). The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. *J. Endocrin.* 163:149-157.
- Baras E, Lagardere JP (1995). Fish telemetry in aquaculture: review and perspectives. *Aquac. Int.* 3:77-102.
- Barton BA, Iwama GK (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish Dis.* 10:3-26.
- Braley H, Andersson TA (1992). Changes in blood metabolite concentrations in response to repeated capture, anesthesia and blood sampling in the Golden perch, *Macquaria ambigua*. *Comp. Biochem. Physiol.* 103A:445-450.
- Carragher JF, Rees CM (1994). Primary and secondary stress responses in golden perch, *Macquaria ambigua*. *Comp. Biochem. Physiol.* 107A:49-56
- Dytham C (1999). Choosing and using statistics: A Biologist's guide. Blackwell Science Ltd., London, United Kingdom, p. 147.
- Ellis T, Ellis B, North AP, Scott NR, Bromage M, Porter M, Gold D (2002). The relationship between stocking density and welfare in farmed Rainbow trout. *J. Fish Biol.* 61:493-531.
- Farghaly AM, Ezzat AA, Shabana MB (1973). Effect of temperature and salinity changes on the blood characteristics of *Tilapia zillii* G. in Egyptian littoral lakes. *Comp. Biochem. Physiol.* 46A:183-193.
- Grutter AS, Pankhurst NW (2000). The effects of capture, handling and ectoparasites load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. *J. Fish Biol.* 57: 391-401.
- Haylor GS (1991). Controlled hatchery production of *Clarias gariepinus* (Burchell 1922): growth and survival of fry at high stocking density. *Aquacult. Fish. Manag.* 22:405-422.
- Henry RJ (1964). Colorimetric determination of total protein. In: *Clinical Chemistry*. Harper and Row Publ., New York, p. 181.
- Holm JC, Reifstie T, Bo S (1990). The effect of fish density and feeding regimes on individual growth rate and mortal rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 89:3-4.
- Ma A, Chen C, Lei J, Chen S, Zhuang Z, Wang Y (2006). Turbot *Scophthalmus maximus*: stocking density on growth, pigmentation and feed conversion. (Abstract) *Chin. J. Oceanol. Limnol.* 24(3):307-312.
- Martinez-Porchas M, Martinez-Cordova LR, Ramos-Enriquez R (2009). Cortisol and glucose: Reliable indicators of stress? *Pan Am. J. Aquat. Sci.* 4:158-178.
- McFarlane WJ, Cubitt KF, Williams H, Rowsell D, Moccia R, Gosine R, McKinley RS (2004). Can feeding status and stress level be assessed by analyzing patterns of muscle activity in free-swimming rainbow trout (*Oncorhynchus mykiss* Walbaum)? *Aquaculture* 239: 467-484.
- Mehdi N, Masood A, Behzan NH, Ali A, Mosa ZD, Hamid FL (2010). Physiological stress responses in Kutum *Rutilus frisii kutum* subjected to captivity. *Int. Aquat. Res.* 2:55-60.
- Rowland SJ, Mifsud C, Nixon M, Boyd P (2006). Effects of stocking density on the performance of the Australian freshwater silver perch (*Bidyanus bidyanus*) in cages. *Aquaculture* 253:301-308.
- Schippers C, Ulloa Rojas JB, Booms GHR, Machiels MAM, Verdegem MCJ, Boon JH (1994). A dietary effect on some cellular and humoral

- parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Fish. Manag.* 25:659-659.
- Stoskopf MK (1993). Clinical pathology. In Stoskopf M.K. (Ed.) *Fish Medicine*. Philadelphia, Pa: WB Saunders. pp. 113-131.
- Trinder P (1969). Determination of glucose concentration in the blood. *Ann. Clin. Biochem.* 6:24.
- Verdegem MCJ, Hilbrands AD, Boon JH (1997). Influence of salinity and dietary composition on blood parameter values of hybrid red tilapia, *Oreochromis niloticus* (Linnaeus) x *O. mossambicus* (Peters). *Aquac. Res.* 28:453-459.
- Wedemeyer GA, Yasutake WT (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Technical Paper 89, U.S. Department of the Interior, Fish and Wildlife Service, Washington DC.

Full Length Research Paper

Effect of different types of honey on the microbial shelf stability of cassava-wheat composite bread

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Received 31 August 2014; Accepted 12 January, 2015

The effect of physicochemical properties (moisture content, total acidity, total phenolic content and viscosity) of honey on the microbial shelf stability of cassava-wheat composite bread was investigated. The percentage weights of the sugar (sucrose) required in the formulated recipe was substituted with different types of honey at the same level (70% sucrose: 30% honey). Freshly baked and cooled cassava-wheat composite bread loaves were stored or shelf at ambient temperature and the total aerobic bacteria and mould counts were determined after 4 and 6 days. The physicochemical analyses revealed that the total acidity, total phenolic content, moisture and viscosity of the different types of honey used varied. The physicochemical properties of the various honey used influenced the microbial shelf stability of the cassava-wheat composite bread. Highest total aerobic bacteria counts of 0.25×10^4 cfu/g was recorded for the cassava-wheat composite bread baked with Hamba honey 2 which had significant ($p \leq 0.05$) lowest total acidity (42.41 mEq/kg) and total phenolic contents (48.97 GAE/100 g) as well as significant ($p \leq 0.05$) highest % moisture after 4 days of storage. However, the incorporation of dark and golden honey with higher total acidity and total phenolic contents compared with other types of honey used, resulted in the least mould counts of 0.42×10^4 and 0.45×10^4 cfu/g, respectively, after 6 days of storage. The physicochemical properties of honey could enhance the microbial shelf stability of baked bread if the minimum inhibitory concentration of the honey after baking is employed.

Key words: Honey, cassava-wheat composite bread, microbial shelf stability.

INTRODUCTION

Bread has become the second most widely consumed non-indigenous food products in Nigeria. To cut the nation's expense on wheat importation and find wider utilization for the increasingly produced cassava roots in Nigeria, the Federal Government mandated the use of composite cassava wheat flour for baking by adding

minimum of 10% cassava flour to wheat for a start (Shittu et al., 2007). According to Shittu et al. (2007), fresh crumb moisture, density, porosity and softness as well as the dried crumb hardness were significantly affected by both the baking temperature and time. The studies of Defloor et al. (1993, 1994, 1995) and Khalil et al. (2000)

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established that 10% substitution of wheat with cassava flour gave bread with quality not significantly different from 100% wheat bread. Comparative studies have shown that honey has less impact on blood sugar level because it offers low glycemic index (GI) response (Foster, 2008). Beyond many health claims and ability to mask any taste deficiency that may have resulted from ingredient interactions, inclusion of honey into bread formulation is said to offer functional benefits (Foster, 2008). Baking technology has been evolving continuously as new materials; equipment and processes are being developed (Selomulyo and Zhou, 2007). The impacts of various ingredients on sensory and nutritional quality of bread have been widely studied (Barcenas and Rosell, 2005; Plessas et al., 2005).

Pure honey has been shown to be bactericidal to many pathogenic microorganisms and the antimicrobial activity has been reported to be as a result of the presence of osmotic effect, acidity and hydrogen peroxide (Radwan et al., 1984; Jeddar, et al., 1985). The pH of honey is reported to be low enough to slow down or prevent the growth of many species of bacteria. The high sugar content of honey makes the water unavailable for microorganisms: no bacteria or fungi can grow in fully ripened honey, but the more diluted honey becomes, the more species can grow in it (Molan, 1992). It was also reported that glucose oxidase enzyme activated by dilutions of honey generates hydrogen peroxide which generally is the major antibacterial factor in honey. This enzyme is inactivated by heating honey, and by exposure to light in some honeys which contain a sensitizing factor. Some honeys also contain substances which destroy the hydrogen peroxide generated by the enzyme (Molan, 1992).

According to Beatriz et al. (2011), the water content is important for honey stability, while the acidity content of honey is a function of honey fermentation. Studies have shown that honeys from the tropics with high water content tend to ferment readily and the free acidity is increased (Sibel et al., 2010; Beatriz et al., 2011) and CA (2001) prescribes a maximum value of 50 milliequivalents of free acids per kilogram of honey. Antimicrobial effects of honey against microorganism associated with disease or infection, food spoilage, including foodborne pathogens and yeasts, have been reported and honey biological activity has been attributed not only to the high sugar concentration and hydrogen peroxide production but also to different compounds such as acids, phenolics, proteins and carbohydrates (Gheldof et al., 2002; Olasupo et al., 2003; Mundo et al., 2004; Guerrini et al., 2009; Gomes et al., 2010). However, the findings of Olaitan et al. (2007) revealed that microorganisms that survive in honey are those that withstand the concentrated sugar, acidity and other antimicrobial characteristics of honey. Stefan et al. (2008) reported that honey from different sources differs in appearance, sensory perception and composition. This suggests that their antimicrobial properties/potential may vary. Although, information is still

scarce on utilization of honey in bread formulation developed from cassava wheat composite flour, previous study on the influence of a single type of honey on microbiological shelf stability of cassava-wheat composite bread revealed that the honey used extended the shelf life of the baked bread loaves (Adeboye et al., 2013). Therefore, this current study therefore aims at investigating the microbial shelf life stability of cassava-wheat composite bread baked with different types of honeys obtained from different locations in South Western Nigeria.

MATERIALS AND METHODS

The sweet cassava variety (*Manihot esculenta Crantz*) tubers used for the production of cassava flour in this study was harvested from the farm of Moshood Abiola Polytechnic, Abeokuta, Nigeria and the white wheat flour was obtained from honey well flour mill, Lagos, Nigeria. Other ingredients used were granulated sucrose sugar (Dangote groups (Nig) Ltd. Nigeria), Fermipon baking yeast (DSM bakery ingredient, Dordrecht -Holland) and baking fat (Pt Intibuca Sehtera, Jakarta, Indonesia). The various honey used were obtained from local bee keeping and honey production farms (Ibadan, Nigeria). The composite flour was made by mixing 10 part cassava flour with 90 part wheat flour.

Determination of the physicochemical properties of honey types

Total phenolic content

The procedure for the determination of total phenolic content was adapted from Zalibera et al. (2008) with some modifications. 50 μ L of the honey or methanolic extract was added to 125 μ L of Folin-Ciocalteu reagent. The mixture was sonicated for 5 min after which 625 μ L of sodium carbonate was added and the absorbance was determined after 2 h at 760 nm. The results were expressed as milligram of gallic acid equivalents per kilogram of honey (mg GAE/kg).

Moisture content, total acidity and viscosity

Moisture content, total acidity and viscosity of the honey types were determined according to methods described by the AOAC (2000) (Official Methods 979.20, 969.38, 962.19).

Preparation of cassava flour

The matured cassava tubers were peeled, washed and grated in a mechanical grater and the pulp obtained was dewatered in a 'muslin' cloth placed in between a screw press. The pulverized cassava mash obtained was then dried in cabinet dryer (Lukas Engineering Nig. Ltd) at 70°C to a constant weight to give 4% moisture content. The dried cassava mash was milled in a hammer mill (Lukas Engr. Ltd. Lagos) and sieved with a mesh of 0.5 mm pore size and fine cassava flour obtained was stored in an airtight container.

Preparation of the recipe

The calculated weight of the sugar (sucrose) in the recipe used was substituted with the different honey types at the optimum

Table 1. The physicochemical properties of the different types of honey used for the baking of the cassava-wheat composite bread.

Sample	Total acidity (mEq/kg)	Total phenolic content (GAE/100 g)	Moisture (%)	Viscosity (cP)
Dark honey	63.99 ^d (0.02)	66.03 ^c (0.007)	16.03 ^a (0.028)	1.75 ^a (0.014)
Hamba Honey1	62.93 ^c (0.02)	67.25 ^d (0.007)	16.26 ^a (0.03)	3.55 ^a (0.014)
Hamba Honey 2	42.41 ^a (0.02)	48.97 ^a (0.127)	22.60 ^c (0.007)	5.00 ^b (0.14)
Light Hamba Honey	49.75 ^b (0.01)	56.40 ^b (0.084)	20.41 ^e (0.014)	3.40 ^{ab} (0.021)
Golden honey	64.41 ^e (0.01)	68.29 ^e (0.021)	19.86 ^b (0.014)	2.50 ^a (0.021)

Values are the means and standard deviations of three replicate experiments (n=3). Means with different superscripts in the same column are significantly different ($P \leq 0.05$).

substitution ratio of 30:70 of honey (H) to sugar (S). The six bread samples were one control with 100% sugar and five with different honey types. The ratio of honey and sugar was 7.56: 17.64 g in all samples.

Production and storage of honey-cassava wheat composite bread

The honey-cassava wheat bread was prepared using the procedure described by Shittu et al. (2007) with slight modification. The ingredients (yeast, water (at 40°C), honey and butter) were combined in a large liquid measuring cup and stirred until the yeast has dissolved and the baking fat has melted. The sugar, composite flour and salt were dry mixed in a large bowl. The yeast mixture was thoroughly incorporated into the mixture of dry ingredients. The dough obtained was then transferred into a lightly floured work surface of the kneading machine and kneaded for about 15-20 min to form smooth and elastic dough. The dough was cut into uniform sizes (300 g), placed in lightly greased pan and proofed at 30°C and 78-80% RH for 2 h then baked in an oven at 220°C for 30 min. Samples from each honey-cassava wheat bread were stored at ambient temperature in nylon 6-gauge of polythene bag for 6 days during which the total viable bacteria and mould growth for each sample type were monitored

Microbiological analysis

The microbial profile of the total aerobic bacteria and yeasts and moulds of the composite bread was determined using nutrient agar (NA) (Oxoid, Basingstoke, Hampshire, England, UK) and potatoes dextrose agar (PDA) (Merck, Darmstadt, Germany) (supplemented with 50 mg/litre of streptomycin), respectively. Ten gram (10 g) of each of the baked bread sample was aseptically homogenized with 90 mL sterile 0.1% buffered peptone water (BPW) (Merck) solution. After serial dilutions of all the samples, the appropriate dilution was spread plated and the NA (Oxoid) agar plates were incubated at 37°C for 24 h while yeast and mould plates were incubated at 25°C for 3-5 days. Samples were analysed after 4 and 6 days of storage.

Statistical analysis

Analysis of variance (ANOVA) was used to determine if physicochemical properties of honey affected the microbial shelf stability of honey cassava-wheat bread significantly at 95% confidence levels. Each experiment was repeated in triplicate and data were analysed using the Statistical Package for the Social

Sciences (SPSS) 21.0 (IBM SPSS Inc., Chicago, IL) and mean separation was carried out with Duncan's new multiple range test.

RESULTS AND DISCUSSION

Physico-chemical properties of honey

There was a significant difference ($P \leq 0.05$) in all the physicochemical properties of the honey samples. The total acidity of the honey types used for the cassava-wheat composite bread ranged between 42.41 and 64.41 mEq/kg with the golden honey having the highest mean value while the lowest value was recorded for the Hamba honey 2 (Table 1). Similarly, Golden honey had the highest total phenolic content while Hamba honey 2 had the least total phenolic content of 68.29 mEq/kg and 48.97 GAE/100 g, respectively.

The variation in phenolic contents of the different types of honey used in this study is in accordance with the findings of Estevinho et al. (2003) and Alzahrani et al. (2012) who reported that the differences between honey samples in terms of antibacterial and antioxidant compounds could be attributed to the natural variations in floral sources of nectar and the different locations. Studies have also shown that honey phenolic compounds composition and consequently antioxidant capacity depends on their floral sources used to collect the honey predominance of which depends on seasonal and environmental factors (Al-Mamary et al., 2002; Yao et al., 2003).

There was a positive correlation (Figure 2) between total acidity and total phenolic content of honey types used in this study. Moisture and viscosity of the Dark, Hamba 1, Hamba 2, Light hamba and Golden honey were; 63.99, 62.93, 42.41, 49.75 and 64.41 mEqkg⁻¹; 66.03, 67.25, 48.97, 56.40 and 68.29 GAE/100 g, 16.03, 16.26, 22.60, 20.41 and 19.86%; 1.75, 3.55, 5.00, 3.40 and 2.50. Hamba honey 2 had the highest moisture and viscosity value (22.60% and 5.00P) while Dark honey had the least moisture and viscosity value (16.03% and 1.75P).

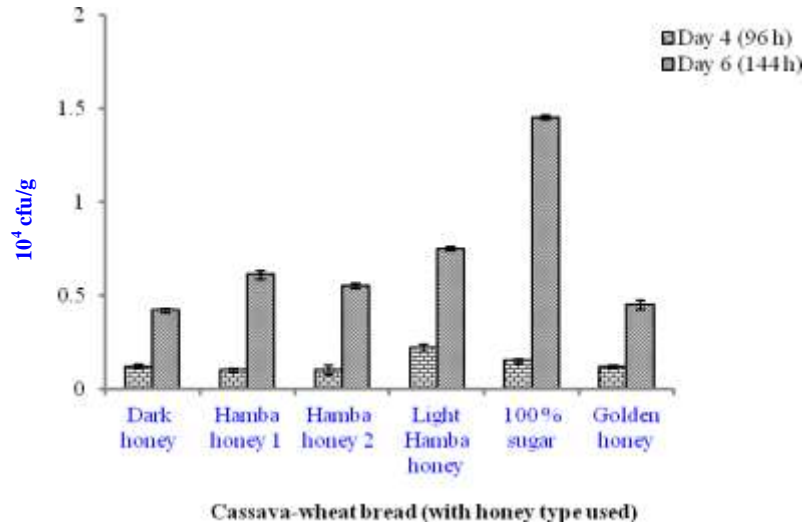


Figure 1. Microbial counts of moulds during the storage of cassava-wheat composite bread baked made with different types of pure honey for 4 and 6 days at 30°C ($n = 3$). Error bars = \pm standard deviation.

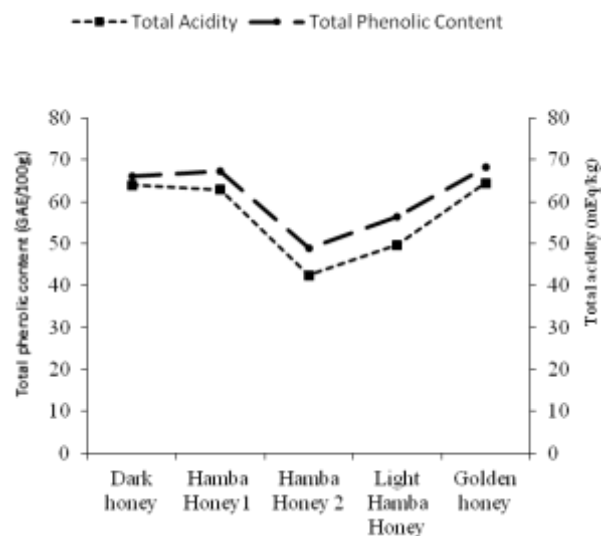


Figure 2. The positive correlation between total acidity and total phenolic content of different types of pure honey incorporated in the cassava-wheat composite bread.

Microbial shelf stability

The addition of the different types of pure honey used had a significant ($p \leq 0.05$) effect on the total aerobic bacteria and mould counts in all the cassava-wheat composite bread. Bacteria and moulds were not detected within the first 3 days of storage at 30°C. This can be attributed to the antimicrobial potency of the physico-chemical properties of honey used for baking against the bacteria and moulds growth. Studies have reported that

most bacteria and other microbes are dormant in honey and therefore cannot grow due to the antibacterial activity of honey (Al Somai et al., 1994; Olaitan et al., 2007). However, total aerobic counts (TAC) were detected after 4 days of storage with highest significant ($p \leq 0.05$) counts of 0.25×10^4 cfu/g in the cassava-wheat bread incorporated with Hamba honey 2 (Table 2) while the cassava-wheat composite bread baked with Light hamba honey has highest mould counts (Figure 1). The level of TAC and moulds in the cassava-wheat bread incorporated

Table 2. Total viable bacteria counts in cassava-wheat composite bread baked with different types of pure honey after 4 and 6 days of storage at 30°C.

Sample	Day 4 (96 h) h (10 ⁴) cfu/g	Day 6 (144 h) h (10 ⁴) cfu/g
Golden Honey	0.1 ^a (0.0)	0.65 ^a (0.0)
Dark Honey	0.1 ^a (0.0)	0.65 ^a (0.0)
Hamba Honey1	0.1 ^a (0.0)	0.80 ^a (0.0)
Hamba Honey 2	0.25 ^b (0.01)	0.13 ^a (0.01)
Light Hamba Honey	0.1 ^a (0.0)	0.65 ^a (0.0)
0H:100S	0.15 ^a (0.0)	1.15 ^b (0.0)

Values are the means and standard deviations of three replicate experiments (n =3); Means with different superscripts in the same column are significantly different (P ≤ 0.05); H = Honey, S = Sugar (Sucrose); 0H:100S = Cassava-wheat composite bread baked with 100% honey (Control); Dark honey = Cassava-wheat composite bread baked with 30% Dark honey; Hamba honey 1 = Cassava-wheat composite bread baked with 30% Hamba honey 1; Hamba honey 2 = Cassava-wheat composite bread baked with 30% Hamba honey 2; Light Hamba honey = Cassava-wheat composite bread baked with 30% Light Hamba honey; Golden honey = Cassava-wheat composite bread baked with 30% Golden honey.

with Hamba honey 2 and Light Hamba honey could be attributed to the significant (p ≤ 0.05) lower total acidity and total phenolic contents in these two honey types in comparison with Dark, Hamba honey 1 and Golden honey used for the baking of the cassava-wheat bread (Table 1). This is in accordance with the findings of Weston et al. (1999) who revealed that antimicrobial activity of honey depends on the aromatic acids or phenolic contents derived from the honey. Furthermore, the significant (P ≤ 0.05) higher percentage moisture content in the Hamba honey 2 and Light hamba honey could have possibly influenced the water activity of the cassava-wheat composite bread and consequently enhanced the bacteria and moulds growth during storage at ambient temperature (Cooper et al., 1999). There was no significant (p > 0.05) difference in the TAC in all the cassava-wheat bread baked with different types of honey after 6 days of storage despite the significant (P ≤ 0.05) variations in their physicochemical properties (Table 2). This could possibly be as a result of the baking temperature which might have resulted in a reduction in the antimicrobial potency of the different types of honey used against some species of bacteria. Molan (1992) reported reduction in all the antimicrobial activities of honey such as total acidity and phenolic contents after exposure to 100°C for 15 min. Furthermore, exposure of honey to 100°C for 10 min has been reported to cause a complete loss of activity against different species of bacteria, but only partial loss of activity against *Bacillus pumilus* and *Streptomyces*, and no loss of activity against *Bacillus subtilis* and *Sarcina lute* (Molan 1992). Our previous study also reported a significant reduction for mould and total viable bacteria counts in the cassava-

wheat composite bread baked with a single type of honey ((Adeboye et al., 2013). However, in this current study, lowest mould counts of 0.42 and 0.45 × 10⁴ cfu/g were recorded for the cassava-wheat composite bread baked with the Dark honey and Golden honey, respectively, after 6 days of storage. This can be attributed to the higher significant (p ≤ 0.05) level of total acidity and phenolic contents in the Dark and Golden honey which resulted in partial inhibitory effect on the moulds. Studies have also shown that the phenolic compounds in honey have growth inhibition on a wide range of Gram-positive and negative bacteria (Davidson et al., 2005; Estevinho et al., 2008). However, the TAC and mould counts were significantly (P ≤ 0.05) higher in the cassava-wheat composite bread baked with sugar (control sample) than the in the cassava-wheat composited bread baked with different types of honey after 6 days of storage. This could be as a result of the fact that the physicochemical properties of honey which are responsible for its antimicrobial potency were not completely loss during baking thereby enhancing partial inhibition of the bacteria and moulds. Partial loss of antibacterial properties of honey after heating has been reported (Molan, 1992). Furthermore, the increase in the TAC and mould counts in all the cassava-wheat composite bread between 4th and 6th day of storage could possibly be attributed to the concentration of the honey incorporated into the bread which was not up to the minimum inhibitory concentration needed after baking to prevent the growth of bacteria and moulds. Study has shown that the antimicrobial potency of honey depends on the minimum inhibitory concentration (MIC) (Cooper et al., 1999) and the MIC of honey has been found to be eight times higher after exposure of honey to 55°C for 8 h (Wooton et al., 1978).

Conclusions

This study has demonstrated that the physicochemical properties of honey could influence the microbial shelf stability of cassava-wheat composite bread. However, it may be necessary to increase the ratio of partial substitution with concentration of the honey used in this current study in order to determine the minimum inhibitory concentration of honey in bread and to circumvent the effect of the baking temperature on the antimicrobial potential of the honeys.

REFERENCES

- Adeboye AS, Babajide JM, Shittu TA, Omemu, AM, Oluwatola OJ (2013). Effect of honey as partial sugar substitute on pasting properties, consumer preference and shelf stability of cassava-wheat composite bread. NIFOJ 31(1):13-22.
- Al Somai N, Coley KE, Molan PC, Hancock BM (1994). Susceptibility of *Helicobacter pylori* to the antibacterial activity of Manuka honey. J. Royal Soc. Medicine. 87:9-12.
- Al-Mamary M, Al-Meer A, Al-Habori M (2002). Antioxidant activities and total phenolics of different types of honey. Nutrition

- Research. 22:1041-1047.
- Alzahrani HA, Alsabehi R, Boukraâ L, Abdellah F, Bellik Y, Bakhotmah BA (2012). Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules*. 17:10540-10549.
- AOAC (2000). Association of Official Analytical Chemist. Methods 979.20, 969.38, 962.19, approved methods for nutraceutical analyses. Official methods of analysis of AOAC International, (17th ed.). Gaithersburg, MD, USA: AOAC.
- Barcenas ME, Rosell CM (2005). Effect of HPMC on the microstructure, quality and aging of wheat bread. *Fd Hydrocolloids*. 19:1037-1043.
- Beatriz A, Sandra M, Montserrat H, Eduardo C (2011). Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *J.Fd. Sc.* 71: 1
- CA (2001). Codex Alimentarius Commission: Codex standard 12, revised Codex standard for honey standards and standard methods, Vol. 11. *Sperimentale per la Zoologia*, Rome. Available from: <http://www.codexalimentarius.net/> Accessed July 15, 2013.
- Cooper RA, Molan PC, Harding KG (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J.of the Royal Soc. Med.* 92: 283-285.
- Davidson PM, Sofos JN, Brenem AL (2005). *Antimicrobials in foods*, third ed. Marcel Dekker Inc., New York. pp. 291-306.
- Defloor I, De Geest C, Schellekens M, Martens A, Delcour JA (1994). Impact of genotype and cropage on the breadmaking and physicochemical properties of flour produced from cassava (*Manihot esculenta Crantz*) planted in the dry season. *J. Sc. Fd Agr.* 66:193-202.
- Defloor I, Leijskens R, Bokanga M, Delcour JA (1995). Impact of genotype, crop age and planting season on the breadmaking and gelatinization properties of flour produced from cassava (*Manihot esculenta Crantz*). *J. Sc. Fd Agr.* 68: 167-174.
- Defloor I, Nys M, Delcour JA (1993). Wheat starch, cassava starch, and cassava flour impairment of the bread making potential of wheat flour. *Cereal Chem.* 78 :525-530
- Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food Chem. Toxicol.* 46:3774-3779.
- Foster RJ (2008). Fortification on the rise. *Bulletin of Food Product Design*, official media site of supply side innovative ingredient international. Retrieved on 22/05/10 7:00am from <http://www.foodproductdesign.com>
- Gheldof N, Wang X, Engeseth NJ (2002). Identification and quantification of antioxidant components of honey from various floral sources. *J Agric Food Chem* 50:5870-5877.
- Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L (2010). Physicochemical, microbiological and antimicrobial properties of commercial honey from Portugal. *Food Chem. Toxicol.* 48:544-548.
- Guerrini A, Bruni R, Poli F, Paganetto G, Muzzoli M, Scalvenzi L, Sacchetti G (2009). Ecuadorian stingless bee (*Meliponinae*) honey: a chemical and functional profile of an ancient health product. *Food Chem.* 114:1413-1420.
- Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Haffejee IE, Moosa A (1985). The antibacterial action of honey. An in vitro study. *SA Med. J.* 67(7):257-258.
- Khalil AH, Mansour EH, Dawood FM (2000). Influence of malt on rheological and baking properties of wheat-cassava composite flours. *J. Fd Sc. Tech.* 33: 159-164.
- Molan PC (1992). The antibacterial activity of honey. The nature of the antibacterial activity. *Bee World* 73(1):5-28.
- Mundo MA, Padilla-Zakour OI, Worobo RW (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int. J. Food Microbiol.* 97:1-8.
- Olaitan PB, Adeleke OE, Iyabo OO (2007). Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *J. Afr. Health Sc.* 7(3):159-165.
- Olasupo NA, Fitzgerald DJ, Gasson MJ, Narbad A (2003). Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Lett App Micro.* 37 (6): 446-451.
- Radwan SS, El-Essawy AA, Sarhan MM (1984). Experimental evidence for the occurrence in honey of specific substances active against microorganisms. *Zentralblattfür Mikrobiologie.* 139(4): 249-255.
- Selomulyo VO, Zhou WB (2007). Frozen bread dough: Effects of freezing storage and dough improvers. *J. Cereal Sc.* 45:1-17.
- Shittu TA, Raji AO, Sanni LO (2007). Bread from composite cassava-wheat flour: Effect of baking time and temperature on some physical properties of bread loaf. *Fd. Res. Int.* 40:280-290.
- Sibel S, Osman S, Lutfiye E (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry* 121:238243.
- Stefan B, Tomislav J, Robert S, and Peter G (2008). Honey for Nutrition and Health: a Review. *American J. Coll. of Nutr.* 27:677-689.
- Weston RJ, Mitchell KR, Allen KL (1999). Antibacterial phenolic components of New Zealand manuka honey. *Food chemistry*, 64:295-301.
- Wooton M, Edwards RA, Rows EA (1978): Antibacterial properties of some Australian honeys. *Food Technology in Australia (May)*: 175-176.
- Yao L, Datta N, Tomás-Barberán FA, Ferreres F, Martos I, Singanusong R (2003). Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys. *Food Chemistry*, 81:159-168.
- Zalibera M, Stasko A, Slobodavá A, Jancovicová V, Cermáková T, Brezová V (2008). Antioxidant and radical-scavenging activities of Slovak honeys: an electron paramagnetic resonance study. *Food Chem.* 110:512-521.

Full Length Research Paper

Particulating broiler finisher feeds into forms and diameters for nutritional and economic benefits (part 2)

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Received 22 October, 2015; Accepted 15 January, 2015

A study was conducted to examine the nutritional and economic effects of using pellet diets at various pellet diameters of 2, 4 and 6 mm pellet for broiler finisher birds in an experiment using Arbor Acre broilers chickens. Two hundred and forty Arbor Acre birds at the finisher phase (28-56 days) of broiler production were used in a 2 x 4 factorial experiment using completely randomized design. Diets in this study had same quantities of ingredients with identical nutrients composition. The study was conducted under similar environmental conditions and management practices. Data on growth performance, nitrogen utilization, carcass and visceral organs characteristics, haematology and serum biochemistry were collected and evaluated. Economic analyses were carried out. Birds on 4 mm pellet diameter diet had the highest body weight gain of 59.11 ± 0.54 g/bird/day and the lowest feed conversion ratio (FCR) of 2.11 ± 0.03 . Measured carcass and visceral organs were higher ($P < 0.05$) for birds on the pellet diets. Most haematological parameters were higher for birds on the pellet diets. Birds on balanced 4 mm pellet diets had better feed intake, increased growth rate and better feed efficiency when fed with broiler finisher birds. The 4 mm pellet diets also enhanced most carcass and organs characteristics. Haematological and blood biochemistry indices of experimental birds on 4 mm pellet diets were not adversely affected and were better in some cases than values obtained from existing literature. There was an overall better net return per bird for birds on 4 mm pellet diameter diets in broiler finisher diets.

Key words: Nutritional benefit, cost and benefit analyses, feed pelleting.

INTRODUCTION

Grinding and pelleting have been identified to have greatest influence on feed quality of all feed processing operations (Lahaye et al., 2004). The feed processing operations can affect, either positively or negatively, the feed quality and subsequent bird performance (Ravindran and Amerah, 2008).

Broilers are known to make better gains on pelleted feed than a mash ration. However, Hoffman (1963), posited that valuing benefit and costs of pellet and mash

feed is important because conserving and investing in analyzing the costs and benefits of each feed form showed that mash was the cheapest method of feeding compounded rations when considered in terms of price per tonne.

Offering feed to poultry in pellet form enhances the economics of production by improving feed efficiency and growth performance in broilers (Behnke and Beyer, 2002). The improvement in feed efficiency and ultimately

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the growth performance can be attributed to the decreased feed wastage, higher bulk and nutrient density, zero selective feeding, decreased time and energy spent for eating, decreased ingredient segregation, destruction of feed-borne pathogens, thermal modification of starch and protein, improved palatability and inactivation of enzyme inhibitors (Behnke, 1994; Jensen, 2000; Peisker, 2006).

The present study was undertaken to determine the nutritional advantages with reference to the cost and benefit analyses of pelleting feeds for broiler finisher production.

MATERIALS AND METHODS

Experimental site

The two studies were carried out at the Poultry Research Unit of the Teaching and Research Farm of Ekiti State University, Ado-Ekiti, Nigeria.

Site preparation

The experimental site was properly cleaned and disinfected. The experimental site was partitioned into twelve separate pens of equal sizes (90 x 80 cm) using wooden poles and wire nets. The floor of the pens was covered with dry litter (wood shaving) up to about 5 cm deep.

Experimental design

The experiment was a 2 x 4 factorial arrangement in a completely randomized design achieved by feeding two forms of diets (mash and pellet) with a mash diet form and three varying diet sizes (2, 4 and 6 mm), respectively. There were 4 dietary treatments in which diet 1 (mash feed) was the control diet while diets 2, 3 and 4 were the pelleted diets with varying pellet diameters of 2, 4 and 6 mm, respectively. Each treatment was replicated thrice with each replicate having 20 birds at the commencement of experiment making a total of 240 birds.

Experimental animals and management

A total of 240 agile birds were randomly selected from the initial population at 28 days of age of birds. They were redistributed into 4 treatments ensuring equal weight and sex per treatment. The birds were raised on conventional deep litter system, where they were supplied with feed and clean with water *ad-libitum*. All the pens were located in one house to have similar environmental condition. Natural ventilation was ensured in each pen throughout the period of the experiment.

Experimental diets

Three pellet diets of different diameters (2, 4 and 6 mm) were used as experimental diets 2, 3 and 4. The control diet (diet 1) in this experiment was a mash feed (Table 1). Particle sized screen was achieved by using a hammer mill with small diameters of screen openings. Manufactured experimental feeds were made into pellets using diesel flat die feed pellet mill with customized diameters of

2.0/3.0/4.0/6.0/8.0 mm. Power rating is 8-55 HP and has a capacity of 70-1000 kg/h.

Data collection

The weights of the experimental birds were taken every 3 days to record the weight gain. Feed consumption was recorded on daily basis. Nitrogen digestibility trial was carried out for each study to determine the nitrogen retention and utilization. Carcass and relative organs characteristics were recorded at dissection after slaughter when the experiment was terminated. Haematological parameters such as haemoglobin concentration, HBC; packed cell volume, PCV; red blood cell, RBC; erythrocyte sedimentation rates, ESR; mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH and mean corpuscular haemoglobin concentration, MCHC were determined from blood samples collected from experimental birds when slaughtered at the termination of experiment. Biochemical components such as total serum protein, albumin, globulin and albumin/globulin ratio were also determined. Cost benefit analysis was carried out.

Carcass, muscle and organ measurements

After slaughtering, the carcasses were scalded at 75°C in a water bath for about 30 s before defeathering. The dressed chicks were later eviscerated. The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, drumstick, shank, chest, back, neck, wing, belly fat and head) were taken before dissecting out the organs. The organs measured were the liver, kidneys, lungs, pancreas, heart, spleen, bursa of fabricus and gizzard.

Estimation of nitrogen retention, nitrogen digestibility and protein efficiency ratio

Total droppings (faeces and urine) voided during the last 5 days were collected, weighed, dried at 65-70°C in an air circulating oven for 72 h and preserved while the corresponding feed consumed was also recorded for nitrogen studies. The nitrogen contents of the samples were determined by the method of AOAC (2010).

Blood sampling

At the end of the feeding trial, a male chick per replicate was randomly selected, weighed and scarified by severing the jugular vein and blood allowed to flow freely into labeled bottles one of which contained a speck of EDTA while the other without EDTA was processed for serum. The serum was kept deep frozen prior to analysis. The packed cell volume (PCV%) was estimated in heparinized capillary tubes in an haematocrit microcentrifuge for 5 min while the total RBC count was determined using normal saline as the diluting fluid. The haemoglobin concentration (Hbc) was estimated using cyanomethaemoglobin method while the MCHC, moglobin MCH and the MCV were calculated.

Economic analysis

The cost of birds, feed and medication incurred during the two phases of the experiment were collected from the income and expenditure statements. Revenue generated from the sale of the birds was recorded. The cost per kilogram gain, total profit and returns to naira invested were also calculated.

Table 1. Composition of the experimental diets (28-56 days).

Ingredients	Dietary treatments			
	1	2	3	4
	Mash	Pellet diameters		
		2 mm	4 mm	6 mm
Maize (9%)	43.0	43.0	43.0	43.0
Soya bean meal (45% CP)	32.6	32.6	32.6	32.6
Maize offals	18.0	18.0	18.0	18.0
Fish meal (68% CP)	2.0	2.0	2.0	2.0
Bone meal	2.50	2.50	2.5	2.5
Oyster shell	0.50	0.50	0.5	0.5
Salt (NaCl)	0.50	0.50	0.5	0.5
DL-methionine	0.20	0.20	0.2	0.2
L-lysine	0.20	0.20	0.2	0.2
Premix	0.50	0.50	0.5	0.5
Total Calculated:	100	100	100	100
Crude protein,%	21.2	21.2	21.2	21.2
Me(kcal/kg)	2809.7	2809.7	2809.7	2809.7
Crude fibre,%	3.3	3.3	3.3	3.3
Ether extract,% Analysed (as fed):	8.1	8.1	8.1	8.1
Ash,%	8.8	8.3	8.3	8.3
Moisture content,%	14.8	16.2	16.2	16.2
Crude protein,%	21.6	21.8	20.7	20.7
Ether extract,%	3.7	3.7	3.7	3.7
Crude fibre,%	3.7	3.7	3.7	3.7

% CP, Percentage crude protein; broilers vitamin premix supplied the following vitamins and trace elements per kg of diet: Vit A 7812.50IU; Vit D 1562.50IU; Vit E 25mg; Vit K 1.25mg; Vit B1 1.88mg; Vit B2 3.44mg; niacin 34.38mg; calcium pantothenate 7.19 mg; Vit B 36.13 mg; Vit B 102.016 mg; Choline chloride 312.50 mg; Folic acid 0.62 mg; Biotin 0.05; Mn 75mg; Fe 62.5mg; Zn 50mg; Cu 5.31 mg; Iodine 0.94 mg; Co 0.19 mg; Se 0.07mg and Antioxidant 75 mg.

Statistical analysis

All recorded and calculated data were statistically analyzed with the standard procedures of analysis of variance (One way ANOVA) technique by a computer using Minitab statistical computer software package (2005 version). Results were expressed as mean \pm standard deviation of two measurements.

RESULTS

Average growth performance

Average growth performance indices are shown in Table 2. The average daily feed intake (FI) value was lowest at 113.4 \pm 0.18 g/bird/day for birds on mash diet (diet 1). Birds on 2 mm pellet diet (diet 2) had the highest FI at 119.2 \pm 0.04 g/bird/day but similar ($P>0.05$) to the FI value obtained for birds on 6 mm pellet diet (diet 4) at 118.9 \pm 0.04 g/bird/day. The average daily weight gain (WG) value obtained for birds fed 4 mm pellets (diet 3) was highest at 59.1 \pm 0.54 g/bird/day but similar ($P>0.05$) to average daily weight gain obtained for birds on 2 mm pellets (diet 2) and 6 mm pellets (diet 4) at 54.6 \pm 2.87 and 57.3 \pm 1.77 g/bird/day, respectively. The birds on mash

(diet 1) had the lowest WG value at 47.8 \pm 4.19 g/bird/day, but also similar ($P>0.05$) to the WG values obtained for birds fed 2 mm pellet diets (diet 2) and 6 mm pellet diets (diet 4). Birds fed 4 mm pellet diets (diet 3) had the best feed conversion ratio at 2.11 \pm 0.03 but similar ($P>0.05$) to the value of birds fed 6 mm pellet diets (diet 4) at 2.35 \pm 0.07. The birds fed mash in the control diet (diet 1) had the highest FCR value at 2.71 \pm 0.13 which indicated low feed conversion ratio and it was similar ($P>0.05$) to the value obtained for the birds fed 2 mm (diet 2) pelleted feed form at 2.48 \pm 0.05. The protein efficiency ratio (PER = gain in body weight/protein intake, g) seemed to increase from birds on the mash diets (control diet) at 1.94 \pm 0.16 to 2.08 \pm 0.11 for birds on 2 mm pellet diets (diet 2) and finally peaked at 2.50 \pm 0.04 for birds on 4 mm pellet diet (diet 3) before a decline for birds on 6 mm pellet diet (diet 4) at 2.36 \pm 0.06.

Nitrogen utilization

Nitrogen utilization for broiler finisher phase is shown in Table 3. Birds on 2 mm pellet diet (diet 2) had the highest nitrogen retention value of 2.65 \pm 0.45 gN/bird/day.

Table 2. Average growth performance of broilers fed various feed forms (28–56 days).

Parameters	Dietary Treatments			
	1	2	3	4
	Control/mash	Diameters of pelleted feed form		
		2 mm	4 mm	6 mm
Ave. Daily Feed Intake (g/bird/day)	113.4 ^a ±0.18	119.2 ^b ±0.04	118.7 ^c ±0.09	118.9 ^{bc} ±0.04
Ave. Daily Weight Gain (g/bird/day)	47.8 ^a ±4.19	54.6 ^{ab} ±2.87	59.1 ^b ±0.54	57.3 ^{ab} ±1.77
Feed Conversion Ratio (FCR)	2.71 ^a ±0.13	2.48 ^{ac} ±0.05	2.11 ^b ±0.03	2.35 ^{bc} ±0.01
Protein Efficiency Ratio (PER)	1.94 ^a ±0.16	2.08 ^a ±0.11	2.50 ^b ±0.04	2.36 ^{ab} ±0.06

Means within a row with different superscript are significantly different ($P < 0.05$).

Table 3. Nitrogen utilization of broilers fed various feed forms (28–56 days).

Parameters	Dietary Treatments			
	1	2	3	4
	Control/mash	Diameters of pellet feed form		
		2 mm	4 mm	6 mm
Nitrogen Intake (g/day)	4.19±0.75	4.26±0.50	3.84±0.61	3.92±0.72
Faecal Nitrogen (g/day)	2.23±0.41	1.61±0.63	1.23±0.52	1.41±0.76
Nitrogen Retention (g/day)	1.96±0.73	2.65±0.45	2.61±0.73	2.52±0.55
Apparent nitrogen digestibility (%)	46.70 ^a ±0.08	62.13 ^b ±0.06	68.01 ^c ±0.10	64.18 ^d ±0.05

Means within a row with different superscript are significantly different ($P < 0.05$).

However, this value was similar ($P > 0.05$) to the nitrogen retention value obtained for birds on 6 mm pellet diet (diet 4) and 4 mm pellet diet (diet 3) at 2.52 ± 0.55 and 2.61 ± 0.73 gN/bird/day, respectively. The significantly lowest ($P > 0.05$) value of 1.96 ± 0.73 gN/bird/day was obtained for those birds fed mash diet in the control diet (diet 1).

The apparent nitrogen digestibility (AND) value obtained for birds on 4 mm pellet diets (diet 3) was the highest at $68.01 \pm 0.10\%$ followed by 6 mm pellet diets (diet 4) at $64.18 \pm 0.05\%$, 2 mm pellet diet (diet 2) at $62.13 \pm 0.06\%$ and mash (control diet, diet 1) at $46.70 \pm 0.08\%$ in that order. There were significant differences ($P < 0.05$) among the AND values obtained for all experimental birds. There was a significantly better apparent nitrogen digestibility (AND) for broiler finisher birds on 4 mm pellet diets over the other feed form and diameters.

Carcass characteristics and organ weights

Carcass characteristics of broiler fed various feed forms are presented in Table 4. All live weight values were statistically similar ($P < 0.05$) for experimental birds on mash (diet 1), 2 mm pellet diets (diet 2), 4 mm pellet diets (diet 3) and 6 mm pellet diets (diet 4) at 2534.0 ± 12.5 , 2540.0 ± 10.7 , 2632.0 ± 9.7 and 2557.0 ± 10.5 g, respectively. The average value of dressed weights for birds on mash

(diet 1), 2 mm pellet diets (diet 2), 4 mm pellet diets (diet 3) and 6 mm pellet diets (diet 4) were also similar ($P > 0.05$).

Eviscerated weight obtained for birds on 4 mm pellet diets (diet 3) was the highest at 2197.0 ± 10.2 g. While the lowest eviscerated weight value was obtained for birds on the mash (diet 1) at 1915.0 ± 10.5 g.

The value obtained for carcass weight revealed that the average weight of the carcasses of birds placed on mash diet at 1541.0 ± 10.7 g was significantly different ($P < 0.05$) from 4 mm pellet diets (diet 2) at 1858 ± 7.7 g, but was similar ($P > 0.05$) to the average carcass weights obtained for birds on 2 mm pellet diets (diet 2) and 6 mm pellet diets (diet 4).

The birds fed 4 mm pellet diet (diet 3) had the highest dressing percentage at $70.59 \pm 0.61\%$ which was significantly different ($P < 0.05$) from the dressing percentages birds on other diets. Other carcass cuts had either similar weights or better weight values for cuts obtained from birds on 2 mm pellet diet (diet 4).

Table 5 shows the visceral organs characteristics of birds fed various pellet diameters of experimental feeds. There were no significant differences ($P > 0.05$) in the values of gizzard weights obtained for birds fed mash (diet 1), 2 mm (diet 2), 4 mm (diet 3) and 6 mm (diet 4) pellet feeds at 39.0 ± 0.53 , 38.0 ± 0.33 , 37.0 ± 0.41 and 37.0 ± 0.68 g, respectively.

Most visceral organs examined had similar ($P > 0.05$)

Table 4. Carcass characteristics of broilers fed various feed forms (28-56 days).

Parameters	Dietary treatments			
	1	2	3	4
	Control/mash	Diameters of pelleted feed form		
		2 mm	4 mm	6 mm
Live weight (g)	2534.0±12.5	2540.0±10.7	2632.0±9.7	2557.0±10.5
Dressed weight (g)	2265.0±12.7	2230.0±10.2	2392.0±10.7	2245.0±9.6
Eviscerated weight (g)	1915.0 ^a ±10.5	1985.0 ^{ab} ±11.8	2197.0 ^b ±10.2	1932.5 ^{ab} ±7.7
Carcass weight (g)	1541.0 ^a ±10.7	1604.0 ^{ab} ±6.3	1858.0 ^b ±7.7	1637.0 ^{ab} ±6.5
Dressing percentage (%)	60.81 ^a ±0.51	63.15 ^{ac} ±0.58	70.59 ^b ±0.61	64.02 ^c ±0.71
Carcass Cuts (g)				
Head	48.2 ^a ±0.50	54.0 ^{bc} ±0.52	53.0 ^c ±0.64	66.0 ^d ±0.71
Neck	109.0 ^a ±0.56	107.0 ^a ±0.51	133.0 ^b ±0.44	112.0 ^c ±0.77
Wing	213.0 ^a ±0.55	202.0 ^b ±0.74	227.0 ^c ±0.67	189.0 ^d ±0.75
Thighs	221.0 ^a ±0.41	229.0 ^b ±0.73	285.0 ^c ±0.66	252.0 ^d ±0.72
Drumstick	264.0 ^a ±0.67	240.0 ^b ±0.71	268.0 ^c ±0.51	259.0 ^d ±0.73
Breast	564.0 ^a ±0.42	608.0 ^b ±0.71	793.0 ^c ±0.62	598.0 ^d ±0.55
Back	234.0 ^a ±0.71	234.0 ^a ±0.66	261.0 ^b ±0.71	268.0 ^c ±0.45
Shank	83.0 ^c ±0.72	74.0 ^b ±0.61	87.0 ^a ±0.42	82.0 ^c ±0.52

Means within a row with different superscript are significantly different (P<0.05).

Table 5. Relative organs weights broiler chicks fed various feed forms (28-56 days).

Parameters	Dietary treatments			
	1	2	3	4
	Control/mash	Diameters of pelleted feed form		
		2 mm	4 mm	6 mm
Gizzard	39.0±0.53	38.0±0.33	37.0±0.41	37.0±0.68
Liver	46.0 ^a ±0.50	43.0 ^b ±0.71	47.0 ^c ±0.63	45.0 ^{ab} ±0.87
Heart	7.0 ^a ±0.52	11.0 ^b ±0.79	11.0 ^b ±0.61	8.0 ^a ±0.74
Kidney	9.0 ^a ±0.60	11.0 ^{ab} ±0.51	13.0 ^b ±0.56	13.0 ^b ±0.72
Spleen	4.0±0.55	3.0±0.71	2.0±0.43	3.0±0.56
Lungs	19.0 ^a ±0.76	11.0 ^b ±0.67	15.0 ^c ±0.53	13.0 ^{bc} ±0.64
Proventriculus	9.0±0.44	7.0±0.65	7.0±0.80	9.0±0.61
Crop	25.0 ^a ±0.54	52.0 ^b ±0.83	44.0 ^c ±0.64	18.0 ^d ±0.73
Intestine	21.0 ^a ±0.58	96.0 ^b ±0.67	141.0 ^c ±0.72	120.0 ^c ±0.76
Bursa of fabricius	2.0±0.64	1.0±0.60	2.0±0.71	1.0±0.58
Pancreas	4.0±0.74	4.0±0.55	4.0±0.67	5.0±0.73

Means within a row with different superscript are significantly different (P<0.05).

values or significant values (P<0.05) for organ dissected from birds on 4 mm pellet diets (diet 4). Most visceral organs such as liver, heart, kidney, lungs, proventriculus, crop, intestine and pancreas showed similarity of growth at broiler starter and finisher phases of the indicating similar organ development.

Haematological and serum biochemical parameters

Haematology and serum biochemistry are presented in the Table 6. Except for PCV, ESRs and MCHC, all other parameters examined were either similar (P>0.05) or had

better values for the pellet diets. The albumin/globulin ratio values obtained for birds fed mash, 2, 4 and 6 mm were similar (P>0.05) at 1.38±0.70, 0.76±0.54, 1.45±0.57 and 2.12±0.42, respectively. The significant improvement in the Hbc, PCV and RBC contents of the birds on 2, 4 and 6 mm pellet diets could have been an indication of an increment in the oxygen carrying capacity of the animal's blood.

Economic analysis (cost benefit analysis)

The economic analysis of feeding various feed forms to

Table 6. Haematological and biochemical profile of broilers fed various feed forms (28– 56 days).

Parameters	Dietary treatments			
	1	2	3	4
	Control/mash	Diameters of pelleted feed form		
		2 mm	4 mm	6 mm
Hbc (g/dl)	6.57±0.45	9.04±0.67	8.21±0.78	8.21±0.53
PCV (%)	15.00 ^a ±0.57	25.00 ^b ±0.73	25.00 ^b ±0.62	23.00 ^b ±0.81
RBC x10 ⁶ (mm ³)	1.49±0.52	1.99±0.43	1.95±0.68	1.78±0.56
ESRs (mm ³ /l)	6.00 ^a ±0.64	3.00 ^b ±0.50	4.00 ^{ab} ±0.71	3.00 ^b ±0.61
MCV x10 ⁻⁶ (µl)	0.10±0.77	0.13±0.62	0.13±0.80	0.13±0.53
MCH x10 ⁻⁶ (µg)	4.41±0.32	4.54±0.55	4.28±0.64	4.61±0.78
MCHC (g/dl)	43.80 ^a ±0.70	36.16 ^b ±0.51	32.84 ^c ±0.73	36.69 ^b ±0.84
Serum biochemical parameters (g/100 ml)				
Total serum protein	18.44 ^a ±0.73	34.81 ^b ±0.78	34.51 ^b ±0.65	27.34 ^c ±0.87
Albumin	10.69 ^a ±0.81	15.06 ^b ±0.75	20.40 ^c ±0.63	18.57 ^c ±0.82
Globulin	7.750 ^a ±0.72	19.75 ^b ±0.42	14.11 ^c ±0.50	8.77 ^d ±0.76
Albumin/globulin ratio	1.38±0.70	0.76±0.54	1.45±0.57	2.12±0.42

Means within a row with different superscript are significantly different (P<0.05).

Table 7. Economic analysis of the broilers fed various feed forms (28–56 day of age).

Parameters	Dietary treatments			
	1	2	3	4
	Control/mash	Diameters of pelleted feed form		
		2 mm	4 mm	6 mm
Total feed intake (kg/bird)	3.06	3.22	3.20	3.21
Feed cost/kg of diet (₦/kg)	116	137	133	133
Cost of feed intake/bird (₦/bird)	355.23	440.84	426.12	426.78
Cost of starter broiler (₦/bird)	500	500	500	500
Total cost of production/bird (₦/bird)	855.23	940.84	926.12	926.78
Ave. body wt. at 56 th day of age(kg/bird)	2.37	2.45	2.61	2.50
Cost of 1kg of poultry meat (₦/kg)	750	750	750	750
Total revenue/bird (₦/bird)	1,777.5	1,837.5	1,957.5	1,875.0
Total net returns/bird (₦/bird)	922.27	896.66	1,031.38	948.22

Naira is Nigeria currency; 1 Nigerian Naira equals 0.0060 US Dollar (\$). Labour and miscellaneous expenses are not included.

broilers is shown in Table 7. Expectedly, the cost of pellet feeds were higher than for mash diet as a result of the additional cost of processes leading to the pellet feeds. However, birds fed 4 mm pellet feeds (diet 3) had the best total net returns of ₦1031.38.

DISCUSSION

Average growth performance

Feed intake was observed to be higher in the pelleted diets 2, 4 and 6 mm as compared to mash. Pelleted diets increased feed intake in broilers (Moran, 1990; Bertechini et al., 1992; Behnke, 1994; Engber et al., 2002). In few

instances where no differences were found in the feed intake between mash and pelleted feed, it was attributed to a low pellet quality (Moran, 1990).

The considerably higher feed intakes among finisher phase birds on pellet diets agreed with previous finding that older birds desire a feed in particulate form in order to conform to the changes in dimension of the oral cavity and the gut (Moran, 1990). Banerjee (1987) reported that feed intake is stimulated by granulation of the feed.

Bolton (1960) reported that pelleting improved weight gain and feed efficiency, but digestibility of nutrients was not affected. Relatively, recent research studies showed that poor feed forms had significant negative effects on body weight and feed efficiency of broilers fed maize-

based diets (Cutlip et al., 2008; Kenny, 2008; Corzo et al., 2011) and wheat-based diets (Kenny, 2008). Cutlip et al. (2008) showed that broilers fed a maize-soy pelleted diet had a 433 g greater final body weight (39 days) and a decreased feed per gain (10 points) as compared to those fed the same diet as unprocessed mash.

Nitrogen utilization

Although earlier report on digestibility of nutrients of pelleted feeds (Bolton, 1960) indicated that pelleting improved weight gain and feed efficiency but failed to affect digestibility of nutrients; the present study indicated a significant improvement in the digestibility of nitrogen and subsequently, a better nitrogen retention by birds on pelleted diets. The thermal processing during the pelleting of feed must have improved the nutrient value of broiler diets which usually results in beneficial effects on performance (McCracken, 2002).

It had also been postulated that pellet diets in reducing maintenance energy expenditure would allow for an increase in productive energy value of the diet, thus providing more calories for protein and lipid synthesis in growing birds (Greenwood and Beyer, 2003). Heat increment and the energy from each unit of feed utilized by broiler birds were also reported to be better affected by feed form (Latshaw and Moritz, 2009) and utilized more for productive purposes than those fed mash.

Carcass characteristics and organ weights

Although most birds on pellet diets had carcass characteristics and organ weights similar to the values obtained for birds on mash diet, birds on 4 mm pellet diets significantly manifested better values for carcass characteristics and organ weights. Feed forms and diameters have been reported to cause significant effects on the digestive tract development and its morphology. Choi et al. (1986) reported that feeding pelleted diet during the finisher period (28-56 days) reduced weights of the digestive tract and gizzard as compared to those fed the mash diet.

These results suggested that birds may not fully develop their digestive tract when highly processed feeds are offered. The similarity in the gizzard weights of all experimental birds disagree with previous work (Munt et al., 1995) that reported a greater gizzard weight in broilers fed mash diets over pellet-fed birds. Nir et al. (1994) also reported that pelleting reduced the relative weight of the gizzard, as well as length of jejunum and ileum. A more recent similar study (Engberg et al., 2002) showed that pellet-fed birds had lower gizzard and pancreas weights than mash-fed birds. The increase in gizzard weight appeared to be due to a more developed muscular wall. The high but inconsistent values of the weights of crop and intestine of birds on diets 2 and 3 (2

and 4mm diameter pellets, respectively) may be as a result of the prolonged distention of these chambers from constant ingestion of pellet feeds. Mash feed can easily fill the crop and intestine in a more orderly manner. The uniformity in the growth rate and muscle development of most organs investigated compared favourably with previous standard growth pattern and muscle development of birds of the same age and strain (Oluyemi and Roberts, 1979; Rodehutscord et al., 2004).

Haematological and serum biochemical parameters

Increase in the haemoglobin may be accompanied by a rise in the RBC and packed cell volume (haematocrit) indicating absence of anaemia (Moss, 1999; Waugh et al., 2001). The ESRs of broiler starter and finisher birds were similar and compared with ESRs obtained for healthy birds in literature (Oluyemi and Roberts, 1979; Rodehutscord et al., 2004) indicating that the pellet diets did not predispose the birds to any known general infections, or malformation of any kind.

Economic analysis (cost benefit analysis)

It is imperative that birds fed 4 mm pellet diets at the broiler finisher phase generated more total net returns per bird and would be more profitable for the purpose of commercial broiler production. It is generally accepted that pelleting enhances the economics of production by improving growth and feed efficiency in broilers (Behnke and Beyer, 2002).

CONCLUSIONS AND RECOMMENDATIONS

The present study revealed that pelleting of broiler poultry feed had both nutritional and economic benefits as it attracted better feed intake, increased growth rate and better feed efficiency when a balanced diet of 4 mm pellet diets were fed to experimental birds for broiler finisher birds. The 4 mm balanced pellet diets also promoted a better uniform growth rate and carcass conformation. Haematological and blood biochemistry indices were not adversely affected and in some cases were found to be optimally enhanced when broiler finisher birds were fed 4 mm pellet diets. A better total net return per bird was achieved for birds reared on the 4 mm pellet diameter diets.

REFERENCES

- Amerah AM, Ravindran V, Lentle RG, Thomas DG (2008). Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. *Poultry Science* 87:2320-2328.
- AOAC (2010). Association of official analytical chemists. Official methods of analysis 6th ed. Washington D.C.

- Banerjee GC (1987). Animal husbandry, 8th Edition, Oxford and IBN Publishers, Oxford.
- Behnke KC (1994). Factors affecting pellet quality. Maryland Nutrition Conference, University of Maryland, College Park, MD. pp: 44-54.
- Behnke KC, Beyer RS (2002). Effect of feed processing on broiler performance. VIII International Seminar on Poultry Production and Pathology, Santiago, Chile.
- Bernardin JE, Kasarda DD (1973). Hydrated protein fibrils from wheat endosperm. *Cereal Chemistry* 50:529-536.
- Bertechini AG, Rostagno HS, Oliveira AIG (1992). Effect of nutrient concentration on performance of broilers. *Poultry Abstract* 18:2965.
- Bolton W (1960). The digestibility of mash and pellets by chicks. *J. Agr. Sciences* 55:141-142.
- Choi JH, So BS, Ryu KS, Kang SL (1986). Effects of pelleted or crumbled diets on the performance and the development of the digestive organs of broilers. *Poultry Science* 65:594-597.
- Corzo A, Mejia L, Loar II RE (2011). Effect of pellet quality on various broiler production parameters. *J. Appl. Poultry Res.* 20:68-74.
- Cutlip SE, Hott JM, Buchanan NP, Rack AL, Latshaw JD, Moritz JS (2008). The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. *J. Appl. Poultry Res.* 17:249-261.
- Engberg RM, Hedemann MS, Jensen BB (2002). The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, 43:569-579.
- Greenwood MW, Beyer RS (2003). Effect of feed manufacturing practices on nutrient availability and feed quality. Proceedings of the 30th Annual Carolina Poultry Nutrition Conference, Raleigh, NC. pp: 7-16.
- Hoffman C (1963). *Poultry Nutrition*, 5th Edition LaRoche Inc Nutley, New Jersey.
- Kenny M (2008). Broilers perform well on pellet rations. *World Poultry* 24(7):18-19.
- Lahaye L, Ganier P, Thibault JN, and Seve B (2004). Technological processes of feed manufacturing affect protein endogenous losses and amino acids availability for body protein deposition in pigs. *Animal Feed Science and Technology* 113:141-156.
- Latshaw JD, Moritz JS (2009). The partitioning of metabolisable energy by broiler chickens. *Poultry Science* 88:98-105.
- McCracken KJ (2002). Effects of physical processing on the nutritive value of poultry diets, in: MCNAB, J.M. & BOORMAN, K.N. (Eds) *Poultry Feeds, Supply, Composition and Nutritive Value*, pp: 301-316 (New York, CAB International).
- Minitab Computer Software Package (2005). One-way Analysis of Variance (ANOVA) (2005). Version Developed by Minitab Inc. (www.minitab.com)
- Moran Jr ET (1990). Effect of pellet quality on the performance of meat birds. *Poultry Abstract* 16: 2875.
- Moss PP (1999). *Blood banking: Concepts and Applications*. W.B. Saunders Co. Philadelphia, pp: 12-34.
- Munt RHC, Dingle JG, Sumpa MG (1995). Growth, carcass composition and profitability of meat chickens given pellets, mash and free choice diet. *British Poultry Science* 36: 277- 284.
- Nir I, Twina Y, Grossman E, Nitsan Z (1994). Quantitative effects of pelleting on performance, gastrointestinal tract and behaviour of meat-type chickens. *British Poultry Science* 35:589-602.
- Oluyemi JA, Roberts FA (1979). *Poultry production in warm wet climates*. Macmillan Tropical Agriculture, Horticulture and Applied Ecology Series.
- Rodehutsord M, Kapcius M, Timmler R, Dieckmann A (2004). Linear regression approach to study amino acid digestibility in broiler chickens. *British Poultry Science* (45):85-92.
- Waugh A, Grant A (2001). *Anatomy and Physiology in Health and Illness*. 9th ed. Churchill Livingstone, an Imprint of Elsevier Science Limited, pp: 59-70.

Full Length Research Paper

Rheological properties of wheat-maize dough and their relationship with the quality of bread treated with ascorbic acid and Malzperle Classic® bread improver

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Received 24 November, 2014; Accepted 16 January, 2015

The aim of this work was to investigate the effect of ascorbic acid and a commercial bread improver on the physical quality of wheat-maize bread, and establish correlations between the physical properties of the bread and rheological properties of the dough. Wheat flour was substituted with 10, 20 or 30% maize flour and the farinograph and extensograph properties of the dough were evaluated. Farinograph water absorption, dough development time, dough stability and farinograph quality number decreased whereas the degree of softening increased with increasing substitution of wheat flour with maize flour. Extensograph dough energy, resistance to extension, extensibility and maximum resistance decreased with increasing substitution of wheat flour with maize flour. Ascorbic acid and commercial bread improver improved bread specific volume and form ratio; decreased crumb firmness, resilience and chewiness; and increased crumb springiness and cohesiveness. Farinograph water absorption and degree of softening; and extensograph energy, extensibility, maximum resistance and ratio number showed the highest number of significant correlations ($P \leq 0.01$ or $P \leq 0.05$) with the physical properties of wheat-maize bread.

Key words: Bread, maize, wheat, rheology, texture profile analysis.

INTRODUCTION

The unique dough-forming and breadmaking property of wheat is ascribed to gluten protein, which is formed when wheat flour is hydrated and subjected to mechanical shear. Substitution of wheat flour with non-wheat flour reduces the bread making potential of wheat flour due to dilution and disruption of the rheological and mechanical

properties of gluten (Schoenlechner et al., 2013; Ribotta et al., 2005). Changes in the water absorption capacity and rheological properties of dough made from wheat and non-wheat flours cannot be generalized but seem to be influenced by the botanical origin, physicochemical nature and quantity of non-wheat flour (Shittu et al.,

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2009; Ribotta et al., 2005; Hung and Morita, 2005). Instrumentally measured bread characteristics show declining sensory properties such as decreased bread specific volume; increased crumb firmness and chewiness; and decreased crumb cohesiveness and springiness (Charoenthaikij et al., 2012; Mcwatters et al., 2004).

Wheat production in sub-Saharan Africa remains insignificant relative to demand because of the unsuitable production environment (UNECA, 1998). By contrast, maize grows well in diverse agro-ecological zones and is an important source of macro- and micronutrients to millions of people in sub-Saharan Africa. Although maize is principally used to make stiff or thin porridge (Onyango, 2014), potential exists to further increase its utilisation in processed foods such as bread (UNECA, 1998).

Additives and processing aids (also known as bread improvers) are widely used in the breadmaking industry to improve dough handling properties and bread quality. Bread improvers can be added as separate materials during dough preparation although they are also available as ready-to-use mixtures. The premixes commonly consist of a carrier agent, such as enzyme-active soybean flour, to which optimized amounts of several materials such as malt flour, emulsifiers, enzymes, hydrocolloids, vital gluten, oxidizing and reducing agents, sugar and fat have been added (Sluimer, 2005). Amongst these additives, ascorbic acid is the most frequently used in the breadmaking industry because it hinders the detrimental effect of glutathione on gluten proteins (Joye et al., 2009) thereby contributing to improved dough strength, reduced dough stickiness, and improved bread crumb and crust characteristics (Aamodt et al., 2003).

Several bread improvers, such as vital gluten (Mohamed et al., 2010), hydrocolloids (Shittu et al., 2009), emulsifiers (Schoenlechner et al., 2013; Alasino et al., 2011; Yamsaengsung et al., 2010) and enzymes (Schoenlechner et al., 2013) are also recommended for the production of bread from wheat and non-wheat flours. Nonetheless, identifying effective and inexpensive generally recognized as safe (GRAS) additives for the production of bread made from wheat and diverse non-wheat flours remains a challenge. For instance, Mohamed et al. (2010) reported that vital gluten improves the specific volume of wheat-banana bread but Conforti and Davis (2006) reported that it did not improve the specific volume of wheat-soya-flaxseed bread. Furthermore, although Mohamed et al. (2010) reported that 25% vital gluten could make wheat-banana bread with a specific volume that is comparable to wheat bread, practical application of this work may be limited because of the large amount of vital gluten that would be required. Health implications should also be considered in the choice of the bread improver used to make bread from wheat and non-wheat flours. For instance, Alasino et al. (2011) recommended sodium stearoyl lactylate and azodicarbonamide for the production of wheat-pea bread but azodicarbonamide is banned in several countries

because it is converted to biurea which is partly transformed to semicarbazide - a compound that has mutagenic and carcinogenic effects (Joye et al., 2009).

The purpose of this work was to evaluate the effect of a commercial bread improver on the physical quality of wheat-maize bread. The commercial bread improver (Malzperle Classic®, IREKS GmbH, Kulmbach, Germany) contained sugars, malt flour, hydrocolloid, ascorbic acid, pH-regulator, enzymes and emulsifier. A further objective was to relate the physical quality parameters of the wheat-maize bread with the rheological properties of the dough.

MATERIALS AND METHODS

Raw materials

Wheat flour (Type 550, not treated with any additive) was purchased from Bremer Rolandmühle Erling GmbH & Co. KG, Germany. Its physicochemical properties were: moisture 11.7% (ICC Standard No. 110/1), protein 12.4% (ICC Standard No. 167), fat 1.2% (ICC Standard No. 136), ash 0.6% (ICC Standard No. 104/1), total dietary fiber 4.5% (AOAC, 2012, Method 991.43), Zeleny sedimentation value 31.7 ml (ICC Standard No. 116/1); wet gluten 27.5% (ICC Standard Method 137) and Hagberg Falling Number 379 s (ICC Standard No. 107/1). Yellow maize flour (Star dust brand) was donated by Cornexo GmbH, Freimersheim, Germany. Its proximate composition was: moisture 11.7% (ICC Standard No. 110/1), protein 7.4% (ICC Standard No. 167), fat 2.8% (ICC Standard No. 136), ash 0.8% (ICC Standard No. 104/1) and total dietary fiber 5.0% (AOAC Method 991.43).

Rheological properties of wheat-maize dough

The wheat and maize flours were blended in the ratios of 100:0, 90:10, 80:20 and 70:30 in a Diosna Laboratory Hubknetter S120a (Diosna Dierks & Söhne GmbH, Osnabrück, Germany) at speed level 2 for 10 min. Farinograph properties of the doughs were evaluated using a Brabender Farinograph-AT (Brabender GmbH & Co. KG, Duisburg, Germany) according to ICC Standard No. 115/1 (ICC, 1992). Extensograph properties of doughs containing 2% sodium chloride, 20 ppm ascorbic acid and distilled water were evaluated using a Brabender Extensograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) according to ICC Standard No. 114/1 (ICC, 1992).

Physical quality of wheat-maize bread

Baking studies were made using wheat-maize flour blends prepared in ratios of 100:0, 90:10, 80:20 and 70:30. The other ingredients, weighed on flour-weight-basis were 10% sugar, 10% compressed yeast (Uniferm GmbH and Co. KG, Werne, Germany), 3% baker's fat (CSM Deutschland GmbH, Bremen, Germany), and 1.5% salt. The amount of chilled water (8°C) used to prepare the dough was determined from the farinogram water absorption (Table 1). Baking tests were made: (i) without additive, (ii) with 20 ppm ascorbic acid and, (iii) with 20 ppm ascorbic acid and 3% Malzperle Classic® bread improver (composition: wheat malt flour, sugar, guar gum, barley malt extract, mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids, disodium diphosphate, monocalcium phosphate, glucose, ascorbic acid and

Table 1. Farinograph properties of wheat-maize dough.

Wheat : maize flour	WA (%)	DDT (min)	DS (min)	FQN	DOS (BU)
100:0 ^a	60.8±0.2 ^c	2.76±0.60 ^b	8.58±1.25 ^b	93±14 ^b	62±12 ^a
90:10	60.9±0.4 ^c	1.62±0.36 ^a	3.34±1.84 ^a	43±21 ^a	90±15 ^b
80:20	59.7±0.1 ^b	1.26±0.08 ^a	3.59±0.55 ^a	42±9 ^a	100±4 ^{bc}
70:30	58.9±0.1 ^a	1.46±0.09 ^a	3.05±1.20 ^a	39±9 ^a	119±8 ^c

WA: water absorption; DDT: dough development time; DS: dough stability; FQN: Farinograph Quality Number; DOS: degree of softening Mean ± standard deviation. Means sharing the same letters in each column are not significantly different from each other (Tukey's HSD test, $P \leq 0.05$).

enzyme bread improver) from IREKS GmbH (Kulmbach, Germany).

The ingredients and additives were mixed for 1 min and then kneaded for 5 min in a Diosna spiral mixer (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). The dough was allowed to rest for 10 min in a proofing chamber (Manz Backtechnik GmbH, Creglingen, Germany) at 32°C and 80% RH. It was then divided into 750 g pieces and rounded before being allowed to rest for 15 min while covered with a porous plastic sheet. The dough was shaped (Frilado Bäckereimaschinen Fabrik, Dortmund, Germany) and placed in oiled baking tins (290 mm long x 130 mm wide x 100 mm deep). It was proofed for 60 min at 32°C and 80% relative humidity. The tins were loaded into preheated Matador multi-deck oven (Werner und Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany) at 200°C for 40 min with steam injection for ca. 10 s immediately after loading. After baking, the loaves were depanned and left to cool for 2 h. Thereafter, they were packed in unperforated low density polythene bags (BÄKO Marken und Service eG, Bonn, Germany), closed with a twist tie and stored for 22 h at 25°C. The experiments were set-up as single-factor completely randomized designs and the baking tests were made in triplicate.

The breads were weighed on a 3-decimal digital weighing scale. Bread volume (cm^3) was determined by rapeseed displacement and specific volume was calculated from the bread weight and volume. The loaves were sliced into 10 mm thick slices using an electric bread slicer (Wabäma GmbH, Haan, Germany). A slice was taken from the center of the bread and the height (mm) and width (mm) used to calculate the form ratio (height/width) (Aamodt et al., 2003). Two slices were taken from the centre of the bread and 30 mm diameter rings punched out. Texture profile analysis of the bread crumbs (20 mm thick) was measured using a 50 mm diameter aluminum cylinder probe (P/50) attached to a TA-XT2i Texture Analyzer with a 5 kg load cell (Stable Micro Systems, Surrey, UK). The TPA settings were: height calibrated at 30 mm, pre-test speed 1 mms^{-1} , test speed 5 mms^{-1} , post-test speed 5 mms^{-1} , target mode distance, distance 10 mm (50% compression), trigger type auto force 0.05 N, data acquisition rate 200 pps. The waiting time between the first and second compression cycle was 5 s. The texture profile analysis properties (firmness, cohesiveness, springiness, resilience and chewiness) were calculated from the obtained graph using EXPONENT Texture Analysis software version 6.1.5.0 (Stable Micro Systems, Surrey, UK). The measurements were made in triplicate and the results reported as mean ± standard deviation.

Statistical analysis

The data was analyzed using one-way analysis of variance and differences in treatment means evaluated using Tukey's test at 5% with SPSS software v.13.0 (SPSS, Chicago, USA). Pearson correlation coefficients (r) between wheat-maize dough rheological properties and physical properties of the bread were evaluated using SPSS software v.13.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Rheological properties of wheat-maize dough

The water absorption of dough prepared from 100% wheat flour was not significantly different ($P > 0.05$) from that of dough prepared from 90% wheat flour and 10% maize flour (Table 1). The water absorption of doughs made from wheat-maize flours tended to decrease with increasing substitution of wheat flour with maize flour. The chemical composition of the non-wheat flour has a strong influence on the water absorption of capacity of the composite dough. Protein-rich flours (Mashayekh et al., 2008; Ribotta et al., 2005) and fibre-rich flours (Mohamed et al., 2010; Mariotti et al., 2006; Koca and Anil, 2007) increase water absorption of dough made from composite flours whereas non-wheat flours with low protein or fibre contents decrease water absorption (Hung and Morita, 2005; Miyazaki and Morita, 2005). Dough development time, dough stability and farinograph quality number decreased by about 40, 60 and 50%, respectively, when wheat flour was substituted with 10% maize flour but did not change significantly ($P > 0.05$) on further substitution of wheat flour with maize flour (Table 1). The degree of softening tended to increase with increasing substitution of wheat flour with maize flour. These changes in dough rheological properties on increasing substitution of wheat flour with maize flour indicate that the baking quality of the dough was declining due to dilution and disruption of the gluten macromolecular network (Ribotta et al., 2005). Low quality breadmaking flour has a short dough development time and rapidly becomes unstable on prolonged mixing whereas high quality breadmaking flour requires more time to attain maximum consistency and is more stable on prolonged mixing (Sluimer, 2005; Mailhot and Patton, 1988). The decline in dough rheological properties has also been reported when wheat flour was partially substituted with other non-wheat flours such as maize (Miyazaki and Morita, 2005), barley (Finocchiaro et al., 2012), soybean (Mashayekh et al., 2008; Ribotta et al., 2005) and flaxseed (Koca and Anil, 2007).

The dough energy, resistance to extension, extensibility and maximum resistance tended to decrease with increasing substitution of wheat flour with maize flour at

Table 2. Extensograph properties of wheat-maize dough.

Wheat : maize flour	Time (min)	Energy (cm ²)	R ₅₀ (EU)	E (mm)	MR (EU)	R ₅₀ /E	MR/E
100:0**		117±3.5 ^d	419±58 ^a	149±9.9 ^c	596±72.8 ^b	2.8±0.6 ^a	4.1±0.8 ^a
90:10	45	87±3.5 ^c	392±2.8 ^a	132±3.5 ^{bc}	482±0.0 ^{ab}	3.0±0.1 ^{ab}	3.7±0.1 ^a
80:20		71±1.4 ^b	391±4.9 ^a	119±3.5 ^b	439±0.7 ^a	3.3±0.1 ^b	3.7±0.1 ^a
70:30		51±4.9 ^a	391±7.1 ^a	90±5.7 ^a	393±7.8 ^a	4.4±0.1 ^b	4.4±0.1 ^a
100:0		140±8.5 ^c	612±65.1 ^a	135±12.0 ^c	818±36.1 ^b	4.7±0.9 ^a	6.2±0.8 ^a
90:10	90	100±8.5 ^b	548±65.1 ^a	119±4.2 ^{bc}	650±71.4 ^a	4.7±0.6 ^a	5.5±0.7 ^a
80:20		74±0.7 ^a	549±9.9 ^a	99±0.7 ^{ab}	566±8.5 ^a	5.6±0.1 ^a	5.8±0.2 ^a
70:30		53±0.7 ^a	511±0.7 ^a	78±1.4 ^a	512±1.4 ^a	6.6±0.1 ^a	6.6±0.1 ^a
100:0		144±9.2 ^c	695±65.8 ^b	127±10.6 ^c	902±23.3 ^c	5.5±1.0 ^a	7.2±0.8 ^a
90:10	135	105±6.4 ^b	614±18.4 ^{ab}	114±2.1 ^{bc}	721±35.4 ^b	5.4±0.1 ^a	6.4±0.2 ^a
80:20		77±2.8 ^a	590±28.3 ^{ab}	97±2.1 ^{ab}	615±32.5 ^{ab}	6.1±0.4 ^a	6.4±0.4 ^a
70:30		53±2.8 ^a	506±36.8 ^a	79±1.4 ^a	507±37.5 ^a	6.4±0.6 ^a	6.4±0.6 ^a

R₅₀ (EU): Resistance to extension at 50 mm (extensograph units); E: extensibility; MR: maximum resistance (extensograph units); R₅₀/E: ratio number; MR/E: ratio number maximum. *Mean ± standard deviation; Means sharing the same letters in each column, for each time, are not significantly different from each other (Tukey's HSD test, P ≤ 0.05).

all measurement times (Table 2). The ratio number and maximum ratio number did not show any major changes with increasing wheat flour substitution at all measurement times. Dough rigidity tended to increase with increasing incubation time as was evidenced by the increasing dough energy, resistance to extension, maximum resistance, ratio number and ratio number maximum; and decreasing dough extensibility (Table 2). The extensograph properties of dough with good breadmaking quality include high resistance to extension, high energy and long extensibility (Sluimer, 2005). The extensograph properties of dough made from wheat and non-wheat flours is dependent on the botanical origin of the non-wheat flour and the modification it has been subjected to. It is for this reason that these results may not fully agree, for instance, with those of Ribotta et al. (2005) or Koca and Anil (2007) who reported on the extensograph properties of wheat-soy and wheat-flaxseed dough, respectively.

Physical quality of wheat-maize bread

Table 3 shows the specific volume, form ratio and texture profile analysis of wheat-maize breads prepared from untreated flour, flour treated with ascorbic acid, and flour treated with ascorbic acid and Malzperle Classic® bread improver. The specific volume of breads decreased with increasing substitution of wheat flour with maize flour, irrespective of the treatment. Non-wheat flour reduces the specific volume of bread due to dilution of gluten and disruption of its rheological and mechanical properties (Schoenlechner et al., 2013; Ribotta et al., 2005). Although ascorbic acid improved the specific volume of

wheat-maize breads, a greater effect was achieved when ascorbic acid was supplemented with Malzperle Classic® bread improver. The specific volume of wheat-maize breads treated with ascorbic acid and Malzperle Classic® bread improver were 31-47% higher than for those not containing any additive. By contrast, the specific volumes of wheat-maize breads treated with ascorbic acid only were 5-10% higher than for those not treated with any additive. Hydrocolloids and enzymes present in the Malzperle Classic® bread improver were most likely responsible for the higher specific volume of the wheat-maize breads. Malzperle Classic® bread improver contained guar gum, a hydrocolloid, which improves the specific volume of bread by increasing dough viscosity thereby conferring greater stability to gluten-starch network (Koca and Anil, 2007; Shittu et al., 2009). Polysaccharide-degrading enzymes, proteases and cross-linking enzymes have also been reported to improve the specific volume of bread (Caballero et al., 2007; Schoenlechner et al., 2013).

The form ratio (height to width ratio) is an important quality index that influences consumer acceptance of pan bread. The form ratio of wheat-maize breads tended to decrease with increasing substitution of wheat flour with maize flour, irrespective of the treatment (Table 3). Ascorbic acid did not increase the form ratio of the wheat-maize breads but supplementation of ascorbic acid with Malzperle Classic® bread improver increased the form ratio of wheat-maize breads by 17-33% as compared to those treated with ascorbic acid only or without any additive. The effect of ascorbic acid and Malzperle Classic® bread improver on the form ratio of wheat-maize breads was also evident in their general appearance (Figure 1). The improved form ratio and general

Table 3. Physical properties of wheat-maize bread.

Treatment	Wheat : maize flour	Specific volume (cm ³ /g)	Form ratio	Texture Profile Analysis				
				Firmness (N)	Springiness*	Cohesiveness*	Resilience*	Chewiness (N)
Without additive	100:0*	4.8±0.0 ^{**d}	1.3±0.0 ^c	1.27±0.14 ^a	2.279±0.363 ^a	0.836±0.003 ^d	0.443±0.005 ^c	2.44±0.65 ^a
	90:10	4.4±0.1 ^c	1.2±0.0 ^{bc}	1.52±0.12 ^a	2.096±1.065 ^a	0.803±0.005 ^c	0.414±0.004 ^b	2.53±1.22 ^a
	80:20	4.0±0.2 ^b	1.1±0.1 ^b	2.68±0.49 ^b	1.183±0.313 ^a	0.748±0.018 ^b	0.393±0.013 ^b	2.43±1.07 ^a
	70:30	3.0±0.1 ^a	0.9±0.0 ^a	7.62±0.30 ^c	0.956±0.034 ^a	0.638±0.013 ^a	0.337±0.010 ^a	4.64±0.23 ^a
Ascorbic acid	100:0	5.1±0.2 ^c	1.3±0.0 ^c	1.29±0.05 ^a	3.256±0.121 ^b	0.837±0.008 ^c	0.419±0.006 ^c	3.52±0.04 ^a
	90:10	4.6±0.1 ^b	1.2±0.0 ^{bc}	1.62±0.10 ^{ab}	2.342±0.903 ^{ab}	0.805±0.009 ^c	0.404±0.009 ^{bc}	3.06±1.22 ^a
	80:20	4.4±0.1 ^b	1.1±0.0 ^b	2.25±0.34 ^b	1.543±0.614 ^a	0.758±0.012 ^b	0.385±0.011 ^b	2.71±1.47 ^a
	70:30	3.3±0.1 ^a	0.9±0.0 ^a	5.24±0.48 ^c	1.000±0.003 ^a	0.667±0.023 ^a	0.339±0.012 ^a	3.49±0.25 ^a
Ascorbic acid and Malzperle Classic® bread improver	100:0	6.3±0.1 ^d	1.6±0.1 ^b	0.91±0.07 ^a	2.845±0.553 ^b	0.834±0.009 ^c	0.392±0.010 ^c	2.15±0.31 ^a
	90:10	5.8±0.2 ^c	1.4±0.0 ^b	0.98±0.05 ^a	2.815±0.268 ^b	0.806±0.007 ^c	0.379±0.001 ^{bc}	2.22±0.09 ^a
	80:20	5.3±0.1 ^b	1.4±0.1 ^b	1.09±0.16 ^a	2.126±0.562 ^b	0.773±0.014 ^b	0.358±0.017 ^b	1.78±0.46 ^a
	70:30	4.4±0.0 ^a	1.2±0.0 ^a	2.20±0.11 ^b	1.000±0.006 ^a	0.683±0.014 ^a	0.319±0.005 ^a	1.50±0.05 ^a

*Dimensionless. **Mean ± standard deviation. Means sharing the same letters in each column, for each treatment, are not significantly different from each other (Tukey's HSD test, $P \leq 0.05$).

appearance of the wheat-maize breads can be attributed to the action of enzyme(s) in Malzperle Classic® bread improver. Different enzyme combinations, such as transglutaminase and xylanase, amylase and protease or glucose oxidase and protease increase the form ratio of breads (Caballero et al., 2007).

The texture profile analysis property of crumb firmness, which is equivalent to the force of mastication during eating (Guiné and Barroca, 2012), tended to increase with increasing wheat flour substitution, irrespective of the treatment (Table 3). The hydration of glutenin and gliadin fractions in wheat flour enables them to form gluten with three-dimensional viscoelastic network, which stretch and rise as it traps carbon dioxide produced by yeast. When the dough is baked, it yields light, evenly structured bread with a soft crumb. Substitution of wheat flour with non-wheat flour dilutes and disrupts formation of this viscoelastic network and hinders entrapment of carbon dioxide leading to increased crumb firmness.

Crumb firmness of the wheat-maize breads tended to decrease on addition of ascorbic acid and Malzperle Classic® bread improver (Table 3). Crumb firmness ranged between 1.27-7.62 N in wheat-maize breads not treated with any additive and declined to 1.29-5.24 N in wheat-maize breads treated with ascorbic acid only and further to 0.91-2.20 N in wheat-maize breads treated with ascorbic acid and Malzperle Classic® bread improver. The decrease in crumb firmness can be attributed to the presence of enzymes, emulsifiers and hydrocolloids in Malzperle Classic® bread improver. This finding is consistent with previous results that show the crumb-softening effects of these additives in breads made from

wheat and non-wheat flours (Schoenlechner et al., 2013; Yamsaensung et al., 2010). Emulsifiers enhance crumb softness by forming complexes with amylose and amylopectin, and by reducing starch swelling and solubilisation during gelatinization (Goesaert et al., 2005). Enzymes that are capable of decreasing crumb firmness include amylase-protease (Caballero et al., 2007) and xylanase-transglutaminase mixtures (Schoenlechner et al., 2013). Hydrocolloids are also capable of decreasing crumb firmness by inhibiting starch-gluten interactions or the development of macromolecular entanglements (Shittu et al., 2009; Davidou et al., 1996).

Crumb springiness, resilience and cohesiveness tended to decrease with increasing wheat flour substitution, irrespective of the treatment (Table 3). Crumb springiness and resilience measure recovery of food structure after compression during mastication (Guiné and Barroca, 2012) whereas crumb cohesiveness measures internal cohesion of the material (Bourne, 2002). Thus, decrease in crumb springiness and resilience characterizes loss of crumb elasticity whereas decrease in crumb cohesiveness reflects increased susceptibility of the crumb to crumble. The average increase of crumb springiness of wheat-maize breads was 22% when it was treated with ascorbic acid and 36% when it was treated with ascorbic acid and Malzperle Classic® bread improver. By contrast, crumb resilience decreased marginally by about 2% when wheat-maize bread was treated with ascorbic acid and 8% when it was treated with ascorbic acid and Malzperle Classic® bread improver. Crumb cohesiveness of wheat-maize bread not treated with any additive ranged between 0.638-0.836

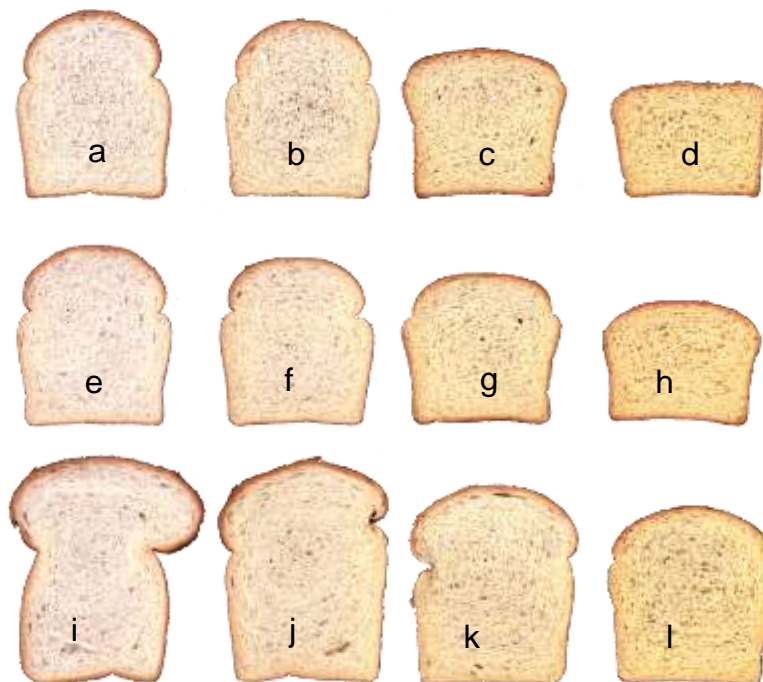


Figure 1. Cross-sections of wheat-maize breads. Breads in the first row do not contain any additives; breads in the second row contain ascorbic acid; breads in the third row contain ascorbic acid and Malzperle Classic® bread improver. Letters a, e, and i represent bread made from 100 parts wheat flour; b, f and j represent bread made from 90 parts wheat flour and 10 parts maize flour; c, g and k represent bread made from 80 parts wheat flour and 20 parts maize flour; d, h and l represent bread made from 70 parts wheat flour and 30 parts maize flour.

and increased marginally to 0.667-0.837 on addition of ascorbic acid, and further to 0.683-0.834 on addition of ascorbic acid and Malzperle Classic® bread improver. The combined interpretation of these three texture profile analysis terms imply that substitution of wheat flour with maize flour decreased the overall bread crumb quality but this was reversed by the addition of ascorbic acid and Malzperle Classic® bread improver.

Crumb chewiness, which is a product of crumb firmness, springiness and cohesiveness (Bourne, 2002), was not significantly affected ($P > 0.05$) by increasing substitution of wheat flour with maize flour, irrespective of the treatment (Table 3). Wheat-maize breads treated with ascorbic acid and Malzperle Classic® bread improver were less chewy (1.50-2.22 N) than those not treated with any additive (2.43-4.64 N) or those treated with ascorbic acid only (2.71-3.52 N).

Correlation between wheat-maize dough and bread quality

The rheological quality of dough is an important predictor of its baking performance and the final quality of bread. Correlation coefficients are used to relate dough

properties to baking performance but the results cannot be generalized because they are dependent on the type of rheological test, composition of material and sample size (Stojceska and Butler, 2012; Ktenioudaki et al., 2010). The Pearson correlation coefficients (r) between the farinograph and extensograph (at 90 min) properties of wheat-maize dough (70:30 ratio) versus the physical properties of bread are shown in Table 4. Among the extensograph data, we have displayed only the 90 min subset because this incubation time closely corresponded to the total dough resting and proofing time (85 min). Farinograph properties of water absorption and degree of softening showed the highest number of significant correlations ($P \leq 0.01$ or $P \leq 0.05$) with the physical properties of wheat-maize bread (Table 4). Water absorption was negatively correlated ($P \leq 0.01$) with crumb firmness and positively correlated ($P \leq 0.01$ or $P \leq 0.05$) with most of the other physical properties of wheat-maize bread. The degree of softening was negatively correlated ($P \leq 0.01$ or $P \leq 0.05$) with almost all physical properties of wheat-maize bread. The dough development time, dough stability and farinograph quality number showed few significant correlations ($P \leq 0.01$ or $P \leq 0.05$) with the physical properties of wheat-maize bread.

Table 4. Pearson correlation coefficients between rheological[§] quality of wheat-maize^{§§} dough and physical quality of bread.

Treatment	CC	1	2	3	4	5	6	7	8	9	10	11
Without additive	SV	0.910**	0.604*	0.635*	-0.866**	0.628*	0.912**	0.689	0.941**	0.859**	-0.847**	-0.433
	FR	0.874**	0.471	0.543	-0.798**	0.537	0.811*	0.507	0.891**	0.708*	-0.874**	-0.602
	FM	-0.877**	-0.415	-0.456	0.759**	-0.456	-0.807*	-0.605	-0.880**	-0.739*	0.841**	0.540
	CO	0.931**	0.564	0.585*	-0.840**	0.576*	0.883**	0.640	0.937**	0.819*	-0.872**	-0.495
	SP	0.828**	0.526	0.338	-0.469	0.329	0.655	0.678	0.597	0.690	-0.459	-0.053
	RE	0.899**	0.617*	0.641*	-0.851**	0.623*	0.892**	0.683	0.928**	0.841**	-0.838**	-0.441
	CH	-0.519	-0.235	-0.416	0.673*	-0.430	-0.612	-0.329	-0.711*	-0.523	0.711*	0.509
Ascorbic acid	SV	0.877**	0.565	0.629*	-0.863**	0.634*	0.935**	0.639	0.964**	0.861**	-0.877**	-0.457
	FR	0.857**	0.568	0.604*	-0.817**	0.608*	0.889**	0.647	0.912**	0.831*	-0.818*	-0.418
	FM	-0.862**	-0.410	-0.444	0.753**	-0.440	-0.779*	-0.574	-0.863**	-0.705	0.838**	0.572
	CO	0.919**	0.610*	0.571	-0.836**	0.576*	0.896**	0.631	0.943**	0.839**	-0.867**	-0.451
	SP	0.701*	0.808**	0.781**	-0.891**	0.796**	0.840**	0.491	0.832*	0.795*	-0.713*	-0.238
	RE	0.880**	0.601*	0.549	-0.814**	0.557	0.848**	0.589	0.903**	0.794*	-0.834*	-0.438
	CH	-0.085	0.481	0.420	-0.270	0.451	0.082	-0.140	0.001	0.090	0.076	0.276
Ascorbic acid and Malzperle Classic® bread improver	SV	0.883**	0.645*	0.705*	-0.914**	0.710**	0.961**	0.688	0.969**	0.908**	-0.850**	-0.371
	FR	0.783**	0.729**	0.676*	-0.816**	0.661*	0.819*	0.765*	0.798*	0.831*	-0.633	-0.178
	FM	-0.832**	-0.385	-0.417	0.704*	-0.408	-0.726*	-0.572	-0.810*	-0.662	0.782*	0.538
	CO	0.919**	0.578*	0.552	-0.798**	0.552	0.885**	0.671	0.924**	0.837**	-0.837**	-0.425
	SP	0.863**	0.544	0.421	-0.677*	0.422	0.720*	0.590	0.779*	0.703	-0.718*	-0.365
	RE	0.895**	0.604*	0.553	-0.796**	0.562	0.886**	0.620	0.929**	0.833*	-0.848**	-0.411
	CH	0.769**	0.530	0.332	-0.549	0.353	0.643	0.494	0.680	0.646	-0.618	-0.230

§The extensograph results are for wheat-maize dough incubated for 90 min. §§Dough formulated from 70 parts wheat flour and 30 parts maize flour. CC: Correlation coefficient; 1: water absorption (%); 2: Dough development time (min); 3: Dough stability (min); 4: Degree of softening (Brabender Units); 5: farinograph quality number; 6: energy (cm²); 7: resistance to extension (extensograph units); 8: extensibility (mm); 9: maximum resistance (extensograph Units); 10: ratio number; 11: ratio number maximum; SV: specific volume (cm³/g); FR: form ratio; FM: firmness (N); CO: cohesiveness (dimensionless); SP: springiness (dimensionless); RE: resilience (dimensionless); CH: chewiness (N). *Significant correlation at P ≤ 0.05 (2-tailed). **Significant correlation at P ≤ 0.01 (2-tailed).

Extensograph properties of dough energy, extensibility, maximum resistance and ratio number showed the highest number of significant correlations (P ≤ 0.01 or P ≤ 0.05) with the physical properties of wheat-maize bread. Dough energy and extensibility were negatively correlated (P ≤ 0.01 or P ≤ 0.05) with crumb firm-

ness and positively correlated (P ≤ 0.01 or P ≤ 0.05) with almost all the other physical properties of wheat-maize. Maximum resistance was positively correlated (P ≤ 0.01 or P ≤ 0.05) with most of the physical properties of wheat-maize bread while the ratio number was positively correlated (P ≤ 0.01 or P ≤ 0.05) with crumb

firmness and negatively correlated (P ≤ 0.01 or P ≤ 0.05) with most of the other physical properties of wheat-maize bread. Resistance to extension and ratio number maximum showed no significant relationships (P > 0.05) with the physical properties of wheat-maize bread, except for the correlation (P ≤ 0.05) between crumb firmness of wheat-

maze bread treated and ascorbic acid, and Malzperle Classic® bread improver and resistance to extension.

Conclusion

Substitution of wheat flour with maize flour decreased water absorption and weakened rheological properties of the dough by disrupting and diluting the gluten network. The changes in dough rheology were reflected in the physical properties of the wheat-maize breads, which declined with increasing substitution of wheat flour with maize flour. The physical properties of the wheat-maize breads were improved when ascorbic acid was supplemented with Malzperle Classic® bread improver. Wheat-maize breads treated with ascorbic acid and Malzperle Classic® bread improver were softer, springier, and more cohesive; but less chewy and resilient than breads not treated with any additive or treated with ascorbic acid only.

Conflict of interest

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

Calvin Onyango wishes to thank Alexander von Humboldt Stiftung (Bonn, Germany) for the research fellowship. The authors thank Cornexo GmbH (Freimersheim, Germany) and IREKS GmbH (Kulmbach, Germany) for donating maize and Malzperle Classic® bread improver, respectively.

REFERENCES

- Aamodt A, Magnus EM, Fægestad EM (2003). Effect of flour quality, ascorbic acid, and DATEM on dough rheological parameters and hearth loaves characteristics. *J Food Sci.* 68:2201-2210.
- Alasino MC, Osella CA, De La Torre MA, Sanchez HD (2011). Use of sodium stearoyl lactylate and azodicarbonamide in wheat flour breads with added pea flour. *Int. J. Food Sci. Nutr.* 62:385-391.
- AOAC (2012). AOAC Method 991.43. Rockville, MD, USA: AOAC International.
- Bourne MC (2002). *Food Texture and Viscosity*. 427 pp. London, UK: Academic Press.
- Caballero PA, Gomez M, Rosell CM (2007). Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *J. Food Eng.* 81:42-53.
- Charoenthaikij P, Jangchud K, Jangchud A, Prinyawiwatkul W, No HK (2012). Composite wheat-germinated brown rice flours: selected physicochemical properties and bread application. *Int. J. Food Sci. Technol.* 47:75-82.
- Conforti FD, Davis SF (2006). The effect of soya flour and flaxseed as a partial replacement for bread flour in yeast bread. *Int. J. Food Sci. Technol.* 41:95-101.
- Davidou S, Meste LM, Debever E, Bekaert D (1996). A contribution to the study of staling of white bread: effect of water and hydrocolloid. *Food Hydrocol.* 10:375-383.
- Finocchiaro F, Ferrari B, Gianinetti A, Scazzina F, Pellegrini N, Caramanico R, Salati C, Shirvanian V, Stanca AM (2012). Effects of barley β -glucan-enriched flour fractions on the glycaemic index of bread. *Int. J. Food Sci. Nutr.* 63:23-29.
- Goesaert H, Brijs K, Veraverbeke WS, Courtin CM, Gebruers K, Delcour JA (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends Food Sci. Technol.* 16:12-30.
- Guiné RPF, Barroca MJ (2012). Effect of drying treatments on texture and colour of vegetables (Pumpkin and green pepper). *Food Bioproducts Processing*, 90:58-63.
- Hung PV, Morita N (2005). Thermal and rheological properties of dough and bread as affected by various cross-linked cornstarch substitutions. *Starch / Stärke*, 57:540-546.
- ICC (1992). ICC Standard No. 104/1, 107/1, 110/1, 113, 114/1 and 115/1, 116/1, 136, 137, 167. Vienna, Austria: International Association for Cereal Science and Technology.
- Joye IS, Lagrain B, Delcour JA (2009). Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking – A review. *J. Cereal Sci.* 50, 11-21.
- Koca AF, Anil M (2007). Effect of flaxseed and wheat flour blends on dough rheology and bread quality. *J. Sci. Food Agric.* 87:1172-1175.
- Ktenioudaki A, Butler F, Gallagher E (2010). Rheological properties and baking quality of wheat varieties from various geographical regions. *J. Cereal Sci.* 51:402-408.
- Mailhot WC, Patton JC (1988). Criteria of flour quality. In: *Wheat: Chemistry and technology* (edited by Y. Pomeranz). Vol. II, Pp. 69–90. St. Paul, MN, USA: American Association of Cereal Chemists.
- Mariotti M, Lucisano M, Pagani A (2006). Development of a baking procedure for the production of oat-supplemented wheat bread. *Int. J. Food Sci. Technol.* 41:151-157.
- Mashayekh M, Mahmoodi MR, Entezari MH (2008). Effect of fortification of defatted soy flour on sensory and rheological properties of wheat bread. *Int. J. Food Sci. Technol.* 43:1693-1698.
- Mcwatters KH, Phillips RD, Walker SL, McCullough SE, Mensa-Wilmot Y, Saalia FK, Hung Y.-C, Patterson SP (2004). Baking performance and consumer acceptability of raw and extruded cowpea flour breads. *J. Food Quality*, 27:337-351.
- Miyazaki M, Morita N (2005). Effect of heat-moisture treated maize starch on the properties of dough and bread. *Food Res. Int.* 38:369-376.
- Mohamed A, Xu J, Singh M (2010). Yeast-leavened banana-bread: Formulation, processing, colour and texture analysis. *Food Chem.* 118:620-626.
- Onyango C (2014). Physical properties of dry-milled maize meals and their relationship with the texture of stiff and thin porridge. *Afr. J. Food Sci.* 8(8), 435-443.
- Ribotta PD, Arnulphi SA, León AE, Añón MC (2005). Effect of soybean addition on the rheological properties and breadmaking quality of wheat flour. *J. Sci. Food Agric.* 85:1889-1896.
- Schoenlechner R, Szatmari M, Bagdi A, Tömösközi S (2013). Optimisation of bread quality produced from wheat and proso millet (*Panicum miliaceum* L.) by adding emulsifiers, transglutaminase and xylanase. *LWT – Food Sci. Technol.* 51:361-366.
- Shittu TA, Aminu RA, Abulude EO (2009). Functional effects of xanthan gum on composite cassava-wheat dough and bread. *Food Hydrocol.* 23:2254-2260.
- Sluimer P (2005). *Principles of Breadmaking. Functionality of Raw Materials and Process Steps*. 212 pp. St. Paul, MN., USA: American Association of Cereal Chemists.
- Stojceska V, Butler F (2012). Investigation of reported correlation between rheological properties of wheat bread doughs and baking performance of the corresponding wheat flours. *Trends Food Sci. Technol.* 24:13-18.
- UNECA (1998). *Technical Handbook on Composite Flours. Processing of Tropical Flours into Bread*. 173 pp. Addis Ababa, Ethiopia: United Nations Economic Commission for Africa.
- Yamsaengsung R, Schoenlechner R, Berghofer E (2010). The effects of chickpea on the functional properties of white and whole wheat bread. *Int. J. Food. Sci. Technol.* 45:610-620.

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