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

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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 on 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 × 104 cfu/g was recorded for the cassava-wheat composite bread baked with Hamba honey 2 which had significant (p ≤ 0.05) lowest total acidity (42.41 mEq/kg) and total phenolic contents (48.97 GAE/100 g) as well as significant (p ≤ 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 × 104 and 0.45 × 104 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 investi-gating 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 µL of the honey or methanolic extract was added to 125 µL of Folin-Ciocalteu reagent. The mixture was sonicated for 5 min after which 625 µL 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 ≤ 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-guage 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 ≤ 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 dependents 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).

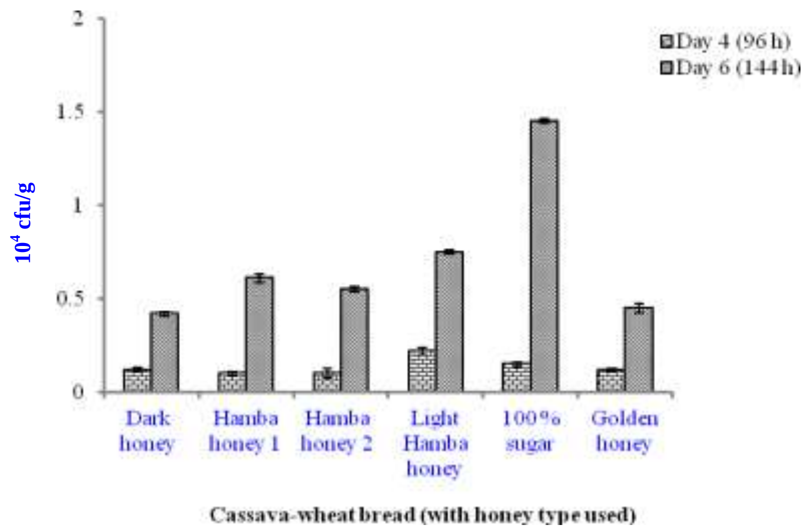

Figure 1. Microbial counts of moulds during the storage of cassava-wheat composite bread baked made with different types of pure honey for 4 and 6 days at 30°C (*n* = 3). Error bars = ± standard deviation.

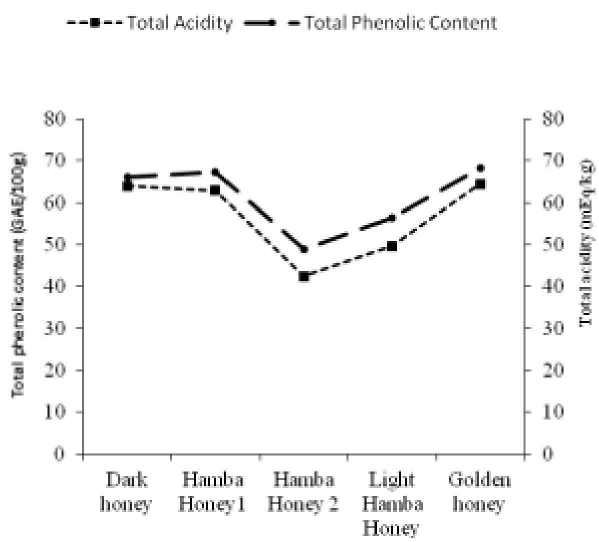

Figure 2. The positive correlation between total acidity and total phenolic content of different types of pure honey incorporated in the cassava-wheat composite bread.

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 × 10^4 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 4^{th} and 6^{th} 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.

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