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Effect of Honey as Partial Sugar Substitute on Pasting Properties, Consumer Preference and Shelf Stability of Cassava-Wheat Composite Bread

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ABSTRACT

The effect of partial substitution of sugar with liquid honey on the pasting properties of cooked dough made from cassava-wheat composite (10:90) flour as well as the sensory preference and shelf stability of its bread was investigated. Sucrose (S) in the bread recipe formulation was substituted with honey (H) at levels 0, 10, 20, 30, 40 and 50%, respectively to give 6 treatments, namely 0H:100S, 10H:90S, 20H:80S, 30H:70S, 40H:60S and 50H:50S. Amylograph pasting properties of the dried crumbs were determined using standard analytical procedures. Fresh bread samples were subjected to sensory evaluation and fungal count during storage (6 days). Peak, final and setback viscosities of bread crumb decreased (32.29 to 25.33, 58.54 to 43.00 and 30.96 to 23.66 RVU), respectively as the level of honey inclusion increased. Honey substitution levels used did not significantly ($p > 0.05$) affect aroma and texture of the bread samples but composite bread with 20% level of honey substitution was most preferred in terms of colour while composite bread with 30% level of honey substitution was most acceptable in terms of taste and overall acceptability. Fungal count in stored honey-cassava-wheat bread varied significantly ($p < 0.05$) from 0.6 to 4.0×10^2, 1.0 to 6.9×10^2, 2.2 to 57.0×10^2, 32.0 to 135.7×10^2, 34.0 to 140.0×10^2 and 42.0 to 159.3×10^2) cfu/ml from day 1 to day 6, respectively. From the study, it was concluded that substitution of sugar with honey in dough formulations significantly ($p < 0.05$) affects dough pasting properties, improves acceptability of the composite bread and reduces staling rate.

Keywords: Honey, sucrose sugar, cassava-wheat bread, dough, pasting properties.

Introduction

Bread is one of the most widely consumed food products in the world. Baking technology has developed gradually as new materials, equipment and processes are advancing (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).

In 2005 and 2012, the Federal Government of Nigeria mandated the use of composite cassava-wheat flour for baking by adding minimum of 10% cassava flour to wheat, to cut the expense on wheat importation and find more use for the increasingly produced cassava tubers. One of the studies conducted to understand the performance of the 10% cassava-wheat composite flour system for bread making focused on the effect of baking time and temperature on some physical properties of bread. It was reported that fresh crumb moisture, density, porosity and softness as well as the dried crumb hardness were significantly affected by both the baking temperature and time (Shittu *et al.*, 2007).

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Honey is an alternative therapy firmly rooted in ancient medical remedy and its anti microbial properties (Shultz, 2009) are well documented. 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 reported to offer functional benefits, improve water-binding capacity of dough, provide increased production volumes; and improves shelf life of baked products (Addo, 1997; Foster, 2008). Addo (1997) found that at 4 – 6% liquid or dry honey, the rheological properties of frozen dough and the freshness of bread were improved. Frank and Matthew (1999) recommended substitution level of 25% liquid honey as a partial substitute for sugar as the optimum in terms of physical and sensory qualities of fat-reduced muffin.

The substitution of a type of sugar by another had typically been studied in food products with the objective of finding a level of replacement that will improve the product's characteristics. In an earlier study, Torley *et al.*, (2004) pointed out that individual starch gelatinization characteristic differs in their response to partial or complete substitution of various sugars. The quality and stability effect of honey in 100% wheat flour bread have been reported in literature (Qunyi *et al.*, 2010); specifically, increased addition of honey in bread formulations was said to have resulted into a higher water absorption, shorter development and stability time in the dough. Reckling, (2004) reported that 5% level of substitution of pure sugar with honey in whole wheat bread formulations did not cause significant change in size related and physical properties of loaves.

In the works of Defloor *et al.*, (1993, 1994, 1995) and Khalil *et al.*, (2000), it was established that inclusion of cassava flour into wheat flour up to about 30% could give an acceptable fresh loaf depending on the flour quality and formulation. Also, 10% substitution of wheat with cassava flour gave bread with quality not significantly different from 100% wheat bread. It could be hypothetical to assume that replacement of sucrose sugar with honey in 10% cassava-wheat composite flour will experience the same effects reported for 100% wheat bread since 10% substitution of wheat with cassava flour have been reported to give bread with quality not significantly different from 100% wheat bread (Defloor, 1995). However, no study has been reported on the use of honey as ingredient in cassava-wheat composite bread. Thus, the objective of this research is to evaluate the influence of substitution of sugar with liquid honey on pasting properties of the cooked dough, consumer preference and shelf stability of cassava-wheat composite bread.

Materials and Methods

Preparation of cassava flour

Matured (12 months old) sweet (*Manihot dulcis*) variety of cassava tubers used for the production of cassava flour were obtained from the farm of Moshood Abiola Polytechnic, Abeokuta. Wheat (white) flour was donated by "Honeywell" flour mills, Lagos, Nigeria. Other ingredients used include granulated sugar (Dangote Groups Nigeria), baking yeast (Fermipan, Dordrecht-Holland) and baking fat (Pt Intibucá Sejhtera, Jakarta, Indonesia). Pure natural honey used was obtained from Institute of Vocational Beescraft, Abeokuta. Cassava flour was prepared by method described by IITA, (2005). Matured cassava roots without rot were selected. They were peeled, washed and grated in a mechanical grater. The pulp obtained was dewatered in a 'muslin' cloth placed in between a screw press. The pulverized material obtained was then spread on trays on thin layer and dried in cabinet dryer (Lukas Engineering Nig. Ltd.) at 70ºC to a constant weight to give a flour of 4% moisture. The dried pulverized material was then milled in a hammer mill and passed through sieve of 0.5 mm mesh size. The fine cassava flour obtained was stored in an airtight container.

Recipe formulation of honey cassava-wheat bread

Composite flour was obtained by mixing 10 parts of the cassava flour to 90 parts of the wheat flour by weight. Recipe used by Shittu *et al.*,(2007) was adapted for dough formulation per loaf. The formulation used for the control bread sample comprised 300 g composite flour, 145 g water, 18 g sugar, 9 g baking fat, 15 g dry baker's yeast, 0.9 g bread improver and 4.5 g fine salt. The formulation used for the other five bread samples was the same with that of the control except that sugar was replaced with honey at 10 – 50% level of substitution.

Bread baking

Formulation used in previous study (Shittu *et al.*, 2007) was adapted (table 4) and method described by Vyskocil, (2010) for production of honey whole wheat bread was used in the preparation of the dough. The ingredients (yeast, warm water, 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 then cut into uniform sizes (300 g), placed in lightly greased pan and proofed in the proving cabinet (at 30ºC and 78 – 80% RH) for 2 h before transferring into the heated oven and baked at a temperature of 220ºC for 30 min.

Rheological (RVA) study on dried honey-cassava-wheat bread crumb

Rapid Visco Analyser RVA (Model C Newport Scientific pty ltd) was used for the assessment of the amylograph pasting characteristics of the bread crumb. Method used by Shittu *et al.*, (2007) was used to prepare the samples. Each bread crumb sample was dried at 35ºC overnight in a laboratory oven (Gallenkamp pty ltd). Each dried crumb was carefully pulverized with a mild force to small grits. Approximately 3.0 g of the sample (grits) were weighed to the nearest 0.01 g into the test container to which 25.0 ml of distilled water was added and contents mixed thoroughly to avoid the formation of lumps. A paddle was then placed the canister and the canister were inserted into the RVA switched on. Results were automatically plotted into characteristics curves from which peak, trough, breakdown, final viscosity, setback, peak time were deduced.

Sensory evaluation

Multiple comparison (difference) test

In the multiple comparison test, cooled fresh samples from the experiment were served to 40 man panel of trained judges. The control sample was marked 'R' and the test samples were presented in identical containers coded with 3-digit random numbers served simultaneously.

The panelist were asked to compare each test sample with the reference sample and tick the expression that best described their judgment using the questionnaire provided. Necessary precautions were taken to prevent carry over flavor during the tasting by ensuring that panelists rinsed their mouth with water after each test of the sensory evaluation. After the evaluation, numerical scores were assigned to the expressions of the panelist with 1 as extremely better than, 5 as equal to 'R' and 9 as extremely poorer than 'R'. The data obtained were then analyzed for variance and degree of difference (Iwe, 2002).

Acceptability (preference) test

In the acceptability test conducted simultaneously with the same panelist using ranking test; the control sample was coded with a different set of 3-digit random numbers alongside other samples. All the samples were simultaneously presented in identical containers to the panelists in a random order. Panelists were asked to evaluate the entire samples and mark the degree of their acceptability of each sample using the questionnaire provided with a scale of 1 (most acceptable) to lowest score of 6 as (most unacceptable). The data obtained were then analysed for variance (Iwe, 2002).

Assessment of microbial shelf stability

Freshly produced samples were allowed to cool for 3 h. Each loaf was put in polythene bag and placed on a stainless steel shelf free from insects and rodents and at room temperature (27 ± 3°C) (Shuttleworth, 2008). The samples were assessed for total plate count and mould count for six (6) storage days.

Microbiological analyses
Method described by Harley (2002) was used for the microbiological analyses. Conical flasks and test tube containing sterile distilled water, sterile pipettes, sterile Petri dishes, non absorbent cotton wool, foil paper, autoclave, incubator, sterile forceps, spirit lamp, inoculation loop, canister, bread sample treated with honey and media (for the cultivation of the samples) and universal bottles.

Preparation of the media
Two media were prepared for the growth of bacteria and fungi respectively, and they are nutrient agar and sabonrad agar. Nutrient agar was prepared by weighing 28 grams of the powder and dissolved in 1litre of distilled water by heat boiling. It was dispensed into universal/McCartney bottles 15 – 20 ml and sterilized using an autoclave at 121ºC for 15 min. Sixty two (62) grams of powder sabonrad agar was weighed into a conical flask, 1litre of distilled water was added and allowed to stay for 10 minutes before stirring to dissolve. It was sterilized using an autoclave at 121ºC for 15 min.

Ninety milliliters (90 ml) distilled water was measured with measuring cylinder into a conical flask. 9 ml of distilled water was measured into test tubes. These were covered with plug of non-absorbent cotton wool, and aluminum foil paper and sterilized using an autoclave at 121°C for 1 min. The 90 ml sterile water was used as diluents while the 9 ml were kept for serial dilution. 10 grams of the bread samples were added to each diluent using sterile forceps and were taken as stock solution. From the stock solution, sterile dilutions were prepared using the 9 ml sterile distilled water in the test tubes. 1 ml was withdrawn from the stock solution into 9 ml sterile distill water in the test tube to give a dilution of 10 – 1 = 1: 10 using a new sterile pipette. 1ml was transferred to the next dilution blank. This was done on the bread samples. From each tube number, using sterile pipette, 1ml was transferred to the sterile Petri dishes each (i.e. in duplicate). Molten nutrient agar in universal bottles was poured into the dishes. The dishes were moved gently six times clockwise, six times anti-clockwise, six times back and forth repeated with to and fro movement. The medium was allowed to set inverted and incubate at 37ºC for 24 – 48hr. This was also done to the sabonrad agar.

Colony counting
After incubation at 37 ºC for 24 – 48 h, the colonies were counted for total viable and mould count respectively. The average of the countable colonies was multiplied by the reciprocal of the dilution factor and reported as colony count per ml.

Statistical analysis
Data generated in all analyses were subjected to analysis of variance (ANOVA) using statistical package for social sciences (SPSS Inc. USA 17.0 version). The calculated mean values were separated using Duncan's multiple range test with significance level of $p < 0.05$.

Results and Discussion
Pasting properties of honey-cassava-wheat bread crumb
Pasting properties of bread crumb samples are presented in Table 2. The result indicated that there were significant differences ($p < 0.05$) in all the pasting parameters among all the samples. According to Shittu *et al.*, (2007), it could be established that baking temperature and time have significant effects on viscosity parameters of cassava-wheat composite bread crumb. In this present study, honey concentration was the only variable; baking temperature and time were kept constant for all formulated composite dough. Peak viscosity is the ability of starch to swell freely before their physical breakdown (Sanni *et al.*, (2004). The peak viscosity of the samples in this study were 32.29, 32.29, 31.04, 22.04, 28.21 and 24.33 RVU for 0, 10, 20, 30, 40 and 50% honey substituted dried

Table 1: Ingredient formulation used for honey-cassava-wheat bread

Material	Honey : Sucrose sugar					
	0%H: 100%S	**10%H: 90%S**	**20%H: 80%S**	**30%H:70%S**	**40%H:60%S**	**50%H : 50%S**
Wheat Flour	270.0 g	270.0 g	270.0 g	270.0 g	270.0 g	270.0 g
Cassava Flour	30.0 g	30.0 g	30.0 g	30.0 g	30.0 g	30.0 g
Salt	4.5 g (1.5%)	4.5 g (1.5%)	4.5 g (1.5%)	4.5 g (1.5%)	4.5 g (1.5%)	4.5 g (1.5%)
Honey	-	1.8 g (0.6%)	3.6 g (1.2%)	5.4 g (1.8%)	7.2 g (2.4%)	9 g (3.0%)
Sugar	18 g (6.0%)	16.2 g (5.4%)	14.4 g (4.8%)	12.6 g (4.2%)	10.8 g (3.6%)	9 g (3.0%)
Yeast	15 g (5.0%)	15 g (5.0%)	15 g (5.0%)	15g (5.0%)	15 g (5.0%)	15 g (5.0%)
Vegetable Oil	9 g (3.0%)	9 g (3.0%)	9 g (3.0%)	9 g (3.0%)	9 g (3.0%)	9 g (3.0%)
EDC	0.9 g (0.3%)	0.9 g (0.3%)	0.9 g (0.3%)	0.9 g (0.3%)	0.9 g (0.3%)	0.9 g (0.3%)
Water	145 g	145 g	145 g	145 g	145 g	145 g

Adapted from Shittu *et al.*, (2007) S = Sucrose sugar, H = Honey. % values are based on the total flour weight (300 g). Values in parenthesis denotes percentage ingredient.

Table 2: Pasting characteristics of honey-cassava-wheat bread crumb

Sample	Peak (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)	Pasting Time (min)	Peak Temp (ºC)
0H:100S	$32.29^{d} \pm 1.96$	$27.58^{d} \pm 1.25$	4.71ab ± 0.71	$58.54^{c} \pm 4.04$	$30.96^{b} \pm 2.79$	$5.87^{ab} \pm 0.13$	$79.55^{b} \pm 0.05$
10H: 90S	$32.29^{d} \pm 0.29$	$27.33^{d} \pm 0.25$	4.96b ± 0.04	$56.84^{c} \pm 0.58$	$29.50^{b} \pm 0.83$	$5.90^{ab} \pm 0.03$	$79.48^{b} \pm 0.02$
20H: 80S	$31.04^{d} \pm 0.21$	$25.92^{cd} \pm 0.16$	$5.13^{a} \pm 0.37$	$56.75^{c} \pm 1.08$	$30.83^{b} \pm 1.25$	$5.75^{ab} \pm 0.08$	$79.52^{b} \pm 0.02$
30H:70S	$22.04^{a} \pm 0.04$	$17.13^{a} \pm 0.05$	$4.92^{a} \pm 0.0$	$39.16^{a} \pm 0.42$	$22.04^{a} \pm 0.46$	$5.74^{ab} \pm 0.03$	$79.33^{a} \pm 0.02$
40H:60S	$28.21^{c} \pm 0.04$	$25.08^{c} \pm 0.25$	$3.13^{a} \pm 0.29$	$46.50^{b} \pm 0.5$	$21.42^{a} \pm 0.75$	$6.07^{b} \pm 0.11$	$79.55^{b} \pm 0.15$
50H:50S	$25.33^{b} \pm 0.08$	$19.34^{b} \pm 0.6$	$6.00^{b} \pm 0.75$	$43.00^{ab} \pm 0.92$	$23.66^{a} \pm 1.58$	$5.63^{a} \pm 0.14$	$79.48^{b} \pm 0.02$

0H:100S = Cassava-wheat composite bread baked with 0% honey (Control), 10H:90S = Cassava-wheat composite bread baked with 10% honey, 20H:80S = Cassava-wheat composite bread baked with 20% honey, 30H:70S = Cassava-wheat composite bread baked with 30% honey, 40H:60S = Cassava-wheat composite bread baked with 40% honey, 50H:50S = Cassava-wheat composite bread baked with 50% honey.

Mean ± S*tandard deviation of triplicate reading. Mean values followed by different superscript within column are significantly different* ($P < 0.05$)

bread crumb sample respectively. The values are close to those (30.05, 31.58, 32.50, 33.17, 34.08 and 29.58 RVU obtained with composite cassava-wheat dried crumb bread samples in the work of Shittu *et al.*, (2007) in which the temperature and time combination were varied. There was no direct trend in the manner in which the concentration of honey affects the peak viscosity of the cassava-wheat composite starch used. This is in agreement with the report of Torley *et al.*, (2004) that changes in honey concentration may not particularly manifest a definite pattern on the viscosity parameters of starch. It can however be noted that peak viscosity increased and decreased alternatively up till 40% substitution. This could be attributed to the fact that the alternate decrease of sucrose sugar with corresponding increase of honey in the partial substitution levels have significant manifestation that depends on which source of sugar is dominating. This could further be substantiated by the fact established by Torley and Molen, (2005) that different sugars differ in their effect on starch gelatinization, and their mixtures have an effect that is largely proportional to the concentration of the two sugar sources present.

The trough is the minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of paste to withstand breakdown during cooling. Although the values obtained are less than those obtained with cassava-wheat composite bread crumb in the work of Shittu *et al.*, (2007) in which the temperature and time combination were varied; the trend in the peak viscosity of samples in this present study is in agreement with report of Torley and Molen, (2005) that different sugars differ in their effect on starch gelatinization, and their mixtures have an effect that is largely proportional to the concentration of the two sugars present.

The breakdown viscosity value is an index of the stability of starch (Fernandez and Berry, 1989). The values 4.71, 4.96, 5.13, 4.92, 3.13, and 6.00 RVU obtained for 0, 10, 20, 30, 40 and 50% honey substitution respectively are higher than those (1.17, 1.92, 0.75, 0.42, 1.33, 1.75) obtained in the work of Shittu *et al.*, (2007). The final viscosity is the change in the viscosity after holding cooked starch at 50°C and it is the most commonly used parameter to define the quality of a particular starch-based sample. It is also regarded as an indicator of the resistance of the paste to shear forces (Adeyemi and Idowu, 1990). The set back value is a measure of recrystalization of gelatinizing flour (retrogradation) and is a function of amylose and amylopectin configuration (Okoli, 1998). The lower setback values of the honey substituted samples suggests that they would be more stable to retrogradation (staling) than the control sample.

Peak time is defined as a measure of the cooking time (Adebowale *et al.*, 2005). The peak time values (5.63 – 6.07 min) points to the fact that all the cassava-wheat dough got cooked within the baking time used. Pasting temperature gives an indication of the gelatinization time during processing. It is the temperature at which the first detectable increase in viscosity is measured and is an index characterized by the initial change due to the swelling of starch (Emiola and Delarosa, 1981). Pasting temperature has been reported to relate to water binding capacity. A higher pasting temperature implies higher water binding capacity, higher gelatinization and lower swelling property of starch due to a high degree of association between starch granules (Emiola and Delarosa, 1981; Numfor *et al.*, 1996). Sugars are an important ingredient in many starch based foods, affecting properties such as sweetness, texture, colour, staling and starch gelatinization (Torley and Molen, 2005). Although sample with 30% honey substitution (30H:70S) was significantly ($p < 0.05$) lower than all others in pasting temperature while others were not significantly ($p > 0.05$) different from each other and the control; the results of the pasting temperature (79.33 – 79.55°C) is an indication that honey concentration used in this study did not largely affect the response of the mixture of starches to heat treatment.

Sensory evaluation of honey-cassava-wheat bread

Tables 3 and 4 show the mean sensory scores of honey-cassava-wheat breads. Although in the multiple comparisons of the honey-cassava-wheat bread samples with the control sample as reference, there was no significant difference ($p > 0.05$) in the aroma (3.33 – 3.80) and texture 3.56 – 4.15) of bread from all the honey substitution levels, 20H:80S (20% honey substituted cassava-wheat bread) sample was most preferred in terms of aroma while 30H:70S (30% honey substituted cassava-wheat bread sample) was most preferred in terms of texture in the overall acceptability test. There was significant difference ($p < 0.05$) in the taste, crumb colour and overall acceptability of the honey substituted cassava-wheat bread samples (3.02 – 4.13), (3.43 – 4.18), and (1.58 – 3.05), respectively. 30H:70S (30% honey substituted cassava-wheat bread) sample was most preferred in terms of taste and overall acceptability while 20H:80S (20% honey substituted cassava-wheat bread) sample was most preferred in terms of colour. Sensory properties are more important for optimizing consumer acceptability (Shittu *et al.*, 2007). There was no significant difference ($p > 0.05$) in the aroma and fluffiness of samples from all the substitution levels, which precludes that the honey substitution in this experiment did not affect aroma and fluffiness of cassava-wheat bread.

Table 3: Mean sensory scores of honey-cassava-wheat bread (Multiple Comparison)

Samples	Taste	Colour	Aroma	Texture
10H:90S	3.53^{ab}	3.50^{ab}	3.55^{a}	3.98^{a}
20H:80S	3.08^{a}	3.43^{a}	3.33^{a}	3.96^{a}
30H:70S	3.02^{a}	4.18^{c}	3.55^{a}	3.56^{a}
40H:60S	4.13^{b}	4.03^{bc}	3.80^{a}	4.15^{a}
50H:50S	3.85^{b}	3.80^{abc}	3.53^{a}	3.78^{a}

0H:100S = Cassava-wheat composite bread baked with 0% honey (Control), 10H:90S = Cassava-wheat composite bread baked with 10% honey, 20H:80S = Cassava-wheat composite bread baked with 20% honey, 30H:70S = Cassava-wheat composite bread baked with 30% honey, 40H:60S = Cassava-wheat composite bread baked with 40% honey, 50H:50S = Cassava-wheat composite bread baked with 50% honey. Mean values followed by different superscript within column are significantly different ($p < 0.05$).

Microbiological shelf stability of honey-cassava-wheat bread

Figure 1 shows the result of total plate count in stored (6 days) samples. At day 0, there was no significant difference ($p > 0.05$) in the total viable count of all the cassava-wheat bread samples. The count however varied significantly ($p < 0.05$) from 1.5 to 7.0×10^2, 1.8 to 9.86×10^2, 1.8 to 76.0×10^2, 49.0 to 155.7×10^2, 58.0 to 160.0×10^2 and 62.0 to 245.0×10^2 cfu/ml) at day 1 to day 6, respectively. Total count increased progressively with storage days but the rate of increase in the honey substituted samples was lower than the control cassava-wheat bread. Cassava-wheat bread at 40% level of honey substitution (40H:60S) had the least count up to day 3 while cassava-wheat bread at 30% level of honey substitution (30H:70S) had the least count at days 4 and 5. Cassava-wheat bread at 40% level of honey substitution had the least count at the end of the storage period.

Table 4: Mean sensory scores of honey-cassava-wheat bread (Overall Acceptability)

Samples	0H:100S	10H:90S	20H:80S	30H:70S	40H:60S	50H:50S
Score	3.05^{c}	2.03^{bc}	1.93^{b}	1.58^{a}	1.95^{b}	1.85^{ab}

0H:100S = Cassava-wheat composite bread baked with 0% honey (Control), 10H:90S = Cassava-wheat composite bread baked with 10% honey, 20H:80S = Cassava-wheat composite bread baked with 20% honey, 30H:70S = Cassava-wheat composite bread baked with 30% honey, 40H:60S = Cassava-wheat composite bread baked with 40% honey, 50H:50S = Cassava-wheat composite bread baked with 50% honey. Mean values followed by different superscript across the row are significantly different ($p < 0.05$).

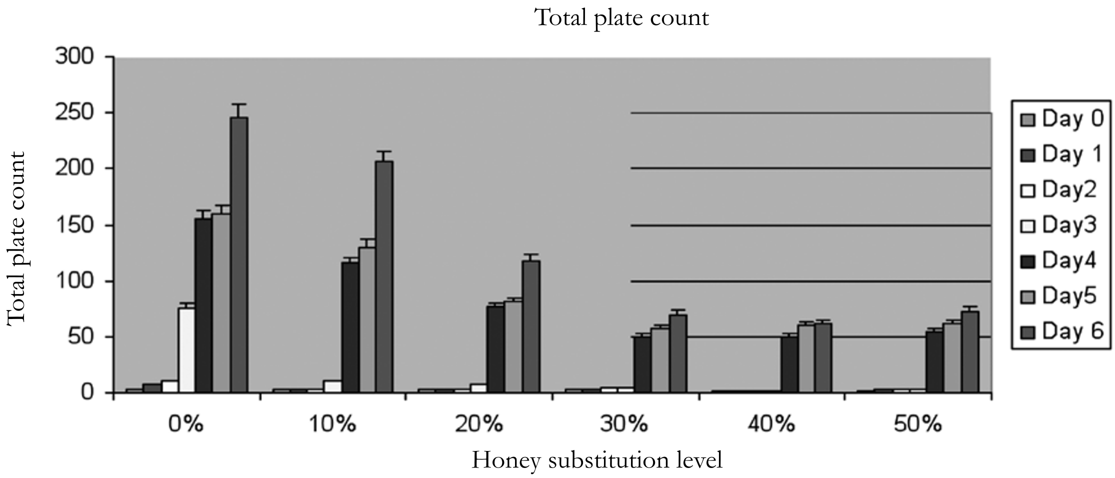

Fig. 1: Graph of 'total plate count' of stored honey-cassava-wheat based

Table 5 shows the result of fungal count in stored (6 days) samples. In previous investigation, addition of honey powder at 5 to 10% level in bread was reported to have improved the baking quality by retarding staling and increasing shelf life (Qunyi *et al.*, 2010). The fact that there was no significant difference ($p > 0.05$) in the microbial count of all the cassava-wheat bread samples at day 0 is as expected due to the lethal effect of baking temperature on microorganisms. The significant ($p < 0.05$) variation in the count from 1.5 to 7.0×10^2, 1.8 to 9.86×10^2, 1.8 to 76.0×10^2, 49.0 to 155.7×10^2, 58.0 to 160.0×10^2 and 62.0 to 245.0×10^2 cfu/ml) in day 1 to day 6, respectively presumably indicates variations in the microbiological status of stored samples. Although microbial count increased progressively with storage days in all samples but the fact that the rate of increase in the honey substituted samples was lower than the control cassava-wheat bread exemplified the antimicrobial property of honey as noted in similar study (Qunyi *et al.*, 2010). Cassava-wheat bread 40H:60S (at 40% level of honey substitution) had the least count at the end of the storage period.

Table 5: Fungal count in stored honey-cassava-wheat bread

SAMPLE	Day 0 CFU/ml $\times 10^2$	Day 1 CFU/ml $\times 10^2$	Day 2 CFU/ml $\times 10^2$	Day 3 CFU/ml $\times 10^2$	Day 4 CFU/ml $\times 10^2$	Day 5 CFU/ml $\times 10^2$
0H:100S	$1.5^a \pm 0.0$	$4.0^d \pm 0.2$	$6.9^d \pm 0.0$	$57.0^e \pm 1.4$	$135.7^f \pm 3.7$	$140.0^f \pm 0.7$
10H: 90S	$1.3^a \pm 0.0$	$4.0^d \pm 0.0$	$2.3^c \pm 0.0$	$5.6^d \pm 1.6$	$95.4^e \pm 1.5$	$100.4^e \pm 2.0$
20H: 80S	$1.5^a \pm 0.0$	$1.5^c \pm 0.4$	$1.7^b \pm 1.8$	$4.4^c \pm 1.4$	$46.4^d \pm 1.1$	$76.7^d \pm 1.4$
30H:70S	$1.5^a \pm 0.0$	$1.5^c \pm 0.0$	$1.9^b \pm 0.0$	$2.8^b \pm 2.3$	$36.0^{ab} \pm 1.5$	$39.0^c \pm 0.0$
40H:60S	$0.6^a \pm 0.0$	$0.6^a \pm 1.0$	$1.0^a \pm 0.0$	$2.2^a \pm 0.0$	$32.0^a \pm 3.2$	$34.0^a \pm 0.0$
50H:50S	$0.8^a \pm 0.0$	$1.8^b \pm 1.0$	$2.0^b \pm 2.0$	$2.4^{ab} \pm 2.2$	$40.0^c \pm 5.6$	$51.0^b \pm 1.4$

0H:100S = Cassava-wheat composite bread baked with 0% honey (Control), 10H:90S = Cassava-wheat composite bread baked with 10% honey, 20H:80S = Cassava-wheat composite bread baked with 20% honey, 30H:70S = Cassava-wheat composite bread baked with 30% honey, 40H:60S = Cassava-wheat composite bread baked with 40% honey, 50H:50S = Cassava-wheat composite bread baked with 50% honey. *Mean + Standard deviation of two replicates. Mean values followed by different superscript within column are significantly different ($p < 0.05$).*

The observation that the trend in fungal count is similar to that of the total viable count is in line with microbiological claim that most microbiological spoilage of baked products especially bread are attributed to fungal growth (Cauvain and Young, 2007; James, 2000). According to the International Commission on Microbiological Specification for Foods (ICMSF, 2010), ready-to-eat foods with standard total plate count below 10^4 CFU/g are still considered safe for human consumption. It is presumptive therefore that all the samples in this study are still microbiologically safe within six days of storage.

Conclusion

The effect of honey concentration on the pasting properties of honey substituted cassava-wheat composite bread did not follow a linear pattern. Microbial load in stored honey-cassava-wheat bread samples were lower than that in cassava-wheat bread sample without honey. The antimicrobial activity of honey on cassava-wheat bread is not infinite; honey substitution beyond 40% may compromise the activity. Honey substitution (10 – 50%) in cassava-wheat bread formulations have significant ($p < 0.05$) improvement on taste, colour and overall acceptability. Honey substitution level of 30% in cassava-wheat bread was the most preferred in terms of taste and overall acceptability while honey substitution level of 20% was the most preferred in terms of colour.

Liquid honey as partial substitute for sucrose at 20% and 30% level of substitution could be recommended for cassava-wheat composite bread making. Investigation into the chemistry of the gelatinization of cassava-wheat composite starch in mixed sugar systems (sucrose-honey) would assist in substantiating the irregular trend observed in some pasting properties of samples in this study.

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