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Co-culture of Amylolytic *Lactobacillus plantarum* and *Saccharomyces cerevisiae* Starters effects on the Nutritional and Sensory Properties of Wheat-Cassava Bread

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Abstract: **This study determines the effect of amylolytic Lactobacillus plantarum (AMz5) and Saccharomyces cerevisiae (YSg2) co culture fermentation of wheat cassava flour dough on the nutritional quality and sensory acceptability of the resultant bread. Strains of the starter cultures are isolates from foods and identified based on physiological properties using API 20C AUX and API 50CHL kits (Biomerieux, France). Bread produced using 10 to 50% (w/w) wheat cassava flour inclusions were evaluated for nutritional and sensory qualities. The result showed that cassava flour contained the following proximate values: moisture content (2.54±0.35%), fat (6.51 ± 0.12%), protein (2.59±0.38%), carbohydrate (88.76±0.80%) and ash (0.60±0.08%). Comparatively, wheat flour had higher ($p<0.05$) proximate values than reported for the cassava flour. Proximate contents of the wheat-cassava composite flour bread decreased with increased cassava flour ratios except for ash and carbohydrate contents. However, the amylolytic L. plantarum AMz5 and S. cerevisiae YSg2 co culture fermented wheat-cassava composite bread exhibited higher proximate values above the conventional yeast fermented bread. An increase of 8.45% and 80.20% proteins was observed in 10% cassava-wheat bread starter with amylolytic L. plantarum and S. cerevisiae above composite bread starter with conventional baker's yeast and the wheat bread respectively. Sensory profile of the cassava-wheat composite flour bread was observed to decrease with increasing cassava flour inclusion however, overall acceptability ranged from 2.07±0.21 to 4.60±0.11. On the whole, composites bread with 10 and 20% cassava flour inclusions were most preferred and compared ($p>0.05$) with the control. The strain of amylolytic L. plantarum and S. cerevisiae co-culture increased the nutritional value and sensory acceptability of wheat-cassava composite bread when employed as starter during bread dough fermentation.**

Keywords: **Amylolytic lactic acid bacteria, organoleptic quality, proximate value, wheat/cassava bread.**

1. INTRODUCTION

In developing countries, bread consumption is continually expanding however, with increasing dependence on wheat importation. Currently, Nigeria relies heavily on the inter-national market for securing wheat supply and imports an estimated 5.5 million metric ton of wheat annually (USDA, 2018). However, continuous wheat import does not negatively impact on the gross domestic product (GDP) only, but have substantially over levied the cost of bread in Nigeria.

As a strategy, concept of composite flour technology (CFT) attracted much attention of researchers, especially in the production of bakery products and pastries (FAO, 2011). The composite flour programme initiation was to evaluate the feasibility of alternative locally available flours as a substitute for wheat flour (Moreno *et al*., 2009; Kadam *et al*., 2012). The advantages of this programme is more for developing countries such as Nigeria to increased usage of domestic grown staple crops with potentials for bread production and save foreign exchange on wheat importation (FAO, 2011). As the world largest producer of cassava, Nigeria conceived an inward policy programme to boast both production and encouraged wide consumption of cassava as staple food (FAO, 2011; Cassava Flour Feasibility Report CFFR, 2012). Accordingly, high quality cassava flour (HQCF) inclusion policy to take care of the management of large volume of cassava produced in Nigeria was enacted (CFFR, 2012). The ripple effect of this was observed in stimulated researches into inclusion of the HQCF in bread production (Ali and Mustafa, 2009; Aboaba and Obakpolar, 2010; Oluwale *et al*., 2018). Several findings reported that the High Quality Cassava Flour (HQCF) was suitable for partial substitution of wheat at 20 - 40% in bread (FAO, 2011; Nwosu *et al*., 2014; Oluwale *et al*., 2018). Wheat-cassava bread although have gained appreciable acceptability, its production still presents considerable technological difficulties due to reduced formation of gluten network resulting to poor gas holding capacity of the dough (Gallagher *et al*., 2003; Eduardo *et al.*, 2013; Nwosu *et al.*, 2014). The use of lactic acid bacteria to improve the amylolytic properties of dough in bread making is not a current approach (Amapu *et al*., 2016). Basically, the α-amylase hydrolyze damaged starch granules to fermentable sugars necessary for optimal yeast growth and gas production and then improve the bread quality (AOAC 1995; El-Okki *et al.*, 2017). Moreover, co existence of yeasts with lactic acid bacteria (IAB) in naturally fermented foods suggests possible interactions between these groups of microorganisms. Mutually, the growth of yeasts in fermented foods is favored by acidification of the environment created by lactic acid bacteria (El-Okki *et al.*, 2017). In addition, the interaction provides growth factors such as vitamins and soluble nitrogen compounds that stimulate growth of the lactic acid bacteria (Nout and Sarkar, 1999).

A major impediment for extensive utilization of cassava is the fact that tuber crops are very low in proteins content and their products will necessitate protein supplementation. Protein enrichment of carbohydrate rich foods using amylolytic lactic acid bacteria (ALAB) and baker's yeast co-culture fermentation had earlier been reported (Day and Morawicki, 2018). Basically, increasing protein content of high carbohydrate foods is usually carried out by direct application of the microbial biomass as a protein supplement, fermentation byproduct or concentration of protein already in the substrate as carbohydrates are consumed (Day and Morawicki, 2018). Many reports have also demonstrated the effectiveness of lactic acid bacteria consortium in modest improvement in proximate content and palatability of fermented foods (Sohail *et al*., 2005; Veluppillai *et al*., 2010; Alloysius. and Ositadinma, 2017). The association of *L. plantarum*, *Pediococcus acidilactici* and *L. delbrueckii* and yeast have contributed to organoleptic quality of fermented products (Kamda *et al*., 2015). Moreover, improving bread quality with microbial α-amylase also conferred baked products unique aroma, taste, flavour and texture (Guyot *et al*., 2001; Sohail *et al*., 2005; Veluppillai *et al*., 2010). However, there is limited information on the proximate composition of wheat-cassava flours composite bread leavened with amylolytic *L. plantarum* and *S. cerevisiae* consortium.Therefore, this research evaluated the effect of amylolytic *Lactobacillus plantarum* and *Saccharomyces cerevisiae* strains co-cultures on the nutritional and organoleptic qualities of wheat-cassava composite bread.

2. MATERIALS AND METHODS

Sample Collection

A total of 20 kg each of high quality cassava flour (HQCF) was purchased from Federal Institute of Industrial Research Oshodi (FIIRO) and industrially wheat flour was purchased from Samaru market, Zaria. The sample obtained was transported to Food and Industrial Laboratory, Department of Microbiology Ahmadu Bello University Zaria-Nigeria for processing.

Sample Preparation

The flour obtained were screened through a 0.25 sieve and packed in low density polythene bag. The prepared samples were then stored dried at room condition (27±5^{o}C) until used.

Collection of Starter Cultures

Stains of amylolytic *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) were stock cultures previously identified using physiological characteristics (API 50 CHL and API 20 C AUX kit BIOMERIUX) and selected based on their amylolytic and dough leavening potential (Amapu *et al*., 2016). Culture of the amylolytic *Lactobacillus plantarum* (AMz5) obtained was activated in de Man, Rogosa-Sharpe (MRS) broth (Difco™, Becton, Dickinson and Co, Le Point de Croix,France) and incubated anaerobically at 37ºC for 24h. The isolate was then subcultured on MRS agar (Merck, Darmstadt, Germany) plates and incubated anaerobically at 37ºC for 48h. Distinct colonies were sub-cultured in 10 ml MRS broth and kept frozen at 4ºC in the presence of 20% glycerol. Pure culture of *Saccharomyces cerevisiae* YSg2 was however maintained on solidified Potato Dextrose Agar slant supplemented with 0.025g of chloramphenical at 4°C (Amapu *et al*., 2008).

Cultivation of Starter Cultures

Stock culture of *L. plantarum* AMz5 was grown in de Man, Rogosa-Sharpe (MRS) broth at 30°C for 24 h. The bacterial cells were then harvested by centrifugation at 12,000 ×g at 4°C for 10min and washed three times with sterile peptone solution (0.1% w/v). The cell concentrations was then adjusted to 10^7cfu/ml using same diluents and checked as viable count on MRS agar.

Propagation of *S. cerevisiae* YSg2 was carried out following the procedure of Ameh and Umaru (2000). The yeast culture was grown on a basal medium consisting of 2%(w/v)glucose,0.5%(w/v)yeast extract,1%(w/v)peptone,0.1%(w/v) ammonium sulphate and 0.1%(w/v) magnesium sulphate at pH 5.6. The culture was then harvested by centrifugation at 4000 x g for 30 min. The resultant yeast pellets were then rinsed twice with sterile distilled water, filtered on 45µm millipore filter membrane and stored at 4°C.

Preparation of Composite Flours

A modified method of Aboaba and Obakpolor (2010) was adopted for composite flour formulation. Cassava flour inclusion proportions (%) of 10, 20, 30, 40 and 50 hard wheat flour was carried out.

Bread Dough Preparation

Dough was prepared by mixing various proportions of the composite flour (400g) with 32ml each of standardised *S. cerevisiae* YSg2 (10^6 cells/ml) and *L. plantarum* AMz5 (10^8cells/ml), sugar (16 g), salt (4 g) and water (258ml) as adopted by Eddy *et al*. (2007). Controls samples were produced using commercial baker's yeast only as leavening agent on composite flour and 100% wheat flour.

Dough was manually kneaded for 20 min, and then 500g dough was moulded into round shape and placed in oil greased baking pans. The dough was then proofed at 30°C for 60 min until dough doubled in volume. The developed dough was pre-heated in an oven and baked at 180°C for 25 min. The resultant baked bread was cooled to room condition, discharged from baking pans and stored in high density polythene bags at 4°C until analysed (Aboaba and Obakpolor, 2010).

Proximate Composition of Bread

Proximate values of the four and bread samples were analysed for moisture, ash, lipids (fat), protein and carbohydrate contents according to AOAC, (1995; 2000).

Sensory Evaluation

A total of 15 sensory panels of regular bread consumers consisting of staff and students of Ahmadu Bello University Zaria, Nigeria were employed. A randomized complete block design was used in which the bread samples were randomly assigned to each panelist at a time. The bread was sliced into uniform thickness coded and served in white coloured plates individually along with glass of drinking water. The panelists were asked to rate bread samples for appearance, taste, aroma, texture and overall acceptability on a 5-point Hedonic scale where score of 1 represented dislike very much and 5 = like very much as adopted previously (Eddy *et al.*, 2007; Aboaba and Obakpolor, 2010).

3. DATA ANALYSIS

The results of proximate composition and sensory acceptability obtained are expressed as means ± standard deviation. The value obtained were then subjected to one way analysis of variance using SPSS version 20.0 and significance was accepted at $p < 0.05$

4. RESULTS

Proximate composition of wheat and cassava flour is presented in Table 1. On overall, proximate contents of wheat flour are higher (p< 0.05) than in cassava flour except for its carbohydrate content. The result revealed that proximate composition of both flour followed a trend with values of carbohydrate >fats > protein> moisture > ash contents in decreasing order. The profile showed that wheat flour contained carbohydrate (83.16±0.80%), fats (7.66±0.53%), protein (3.05±0.28 %) and ash (1.64±0.24%) contents. However, proximate constituents of carbohydrate (88.76±0.80%), fats content (7.66±0.53%), protein (3.05±0.28%) and ash (1.64±0.24%) contents were observed in the high quality cassava flour (HQCF). Comparatively, finding of this study showed that proximate contents of high quality cassava flour (HQCF) are higher and significantly varied (p<0.05) from the locally processed cassava flour (LPCF) as shown in Table 1.

Proximate profile of wheat-cassava composite bread (Table 2) exhibited trends (p<0.05) with carbohydrate > moisture > protein > fats > ash contents in decreasing order. The result showed that wheat-cassava composite bread blend ratio maintained higher carbohydrate (51.49±0.64-58.78 ±0.64%) followed by moisture (30.16 ±0.41 to 33.15 ±0.39%) and least ash (0.41±0.08- 0.93 ±0.11%) contents. The carbohydrates (43.01±0.48 %), moisture (28.44±0.30%) ash (0.45 ±0.04%) protein (7.66%) and fat (3.05%) contents of wheat flour bread was however lower (p<0.05) compare to different blending ratio of wheat-cassava composite flour bread evaluated. In this study, the result showed that cassava flour inclusion ratio significantly (p<0.05) increased carbohydrate, moisture, fats and ash contents of the composite bread. Conversely, protein (7.07 ±0.12 to 3.35 ± 0.14%; p<0.05) and fat contents (8.84 ±0.46 to 8.50 ±0.27 %) of the composite bread decreased (p>0.05) with cassava flour inclusion.

Moreover, ash content (0.44 ±0.06%) fat contents (8.84 ±0.46 %) and protein contents (7.07 ±0.12%) of high quality cassava flour (HQCF) bread significantly varied (p<0.05) with values of 0. 37 ±0.11%, 8.22 ±0.20% and 5.17 ±0.38% respectively obtained for locally processed cassava flour (LPCF).

Development of bread dough using co-cultured amylolytic *L. plantarum* and *S. cerevisiae* influenced (p<0.05) the proximate content of the resultant bread (Table 2). In this study, the amylolytic *L. plantarum* and *S. cerevisiae* co- culture increased the proximate content of the wheat- cassava composite flour bread except its ash content. The result revealed that amylolytic *L. plantarum* and *S. cerevisiae* co-culture fermented bread increased (p<0.05) fat (9.87±0.40%) protein (7.09 ±0.12%) and carbohydrate (47.08 ±0.58 %). Correspondingly, a lower (p<0.05) fat (8.94 ±0.38 %) protein (6.47 ±0.17 %) and carbohydrate (43.01 ±0.48%) contents were observed in bread starter with the commercial baker's yeast.

Sensory properties of wheat-cassava composite flour bread (Table 3) were acceptable within the range of 2.07±0.21 to 4.60±0.11. The acceptability however decreased (p<0.05) with increased ratio of cassava flour (HQCF) substitution. Significantly, bread with 40 and 50% flour inclusion had the least rating by the panellist for the attributes tested. On overall, the result showed that cassava flour inclusions at 10% and 20% were more acceptable and did not differ (*p*>0.05) with the control. Moreover, co-culture amylolytic *L. plantarum and S. cerevisiae* wheat bread increased acceptability and compared well with conventional bread in appearance, taste, texture, aroma and general acceptability.

5. DISCUSSION

The novelty to improve nutritional and sensory properties of the composite bread is therefore borne out of the reason that proximate composition of wheat-cassava flour bread varied with cassava inclusion (Eddy *et al*., 2007; Ogunbawo *et al*., 2008). An assessment of the proximate contents of wheat flour showed higher (p< 0.05) values than in cassava flour except for carbohydrate content. Therefore, composite flour played vital role in complementing the deficiency of essential nutrients (Igbabul *et al.*, 2014).

In this study, moisture content of the both wheat and cassava flour used falls within the recommended levels of less than 14% that advanced the keeping quality of cereal flour (Ajani *et al*., 2012). Cassava flour is a rich source of carbohydrate but low in protein and fat (Nwosu *et al.*, 2014) as evident in the result of this study. Commonly, carbohydrate content as high as 82.93% has been reported for cassava flour (Montagnac *et al*., 2009; Nwosu *et al*., 2014). Comparatively, higher ash content was observed in wheat flour (1.64±0.24%) than in cassava flour (0.60±0.08%) contrary to the report of Nwosu, *et al*. (2014) where cassava flour (2.15%) was reported to contain the higher minerals. However, the ash content of both wheat and cassava flour observed falls above recommended standard of 1.2%, indicating possible adulteration or reflection of poor processing (Ndife *et al*., 2011). The proximate content further affirmed that both wheat and cassava flours are poor in protein (%) and fat contents (Nwosu, *et al*., 2014).

Proximate contents of wheat flours bread are reported to be significantly reduced by composite flour inclusion (Nwosu, *et al*., 2014). In this study, the composite flour bread had higher moisture content (30.14±0.40% to 33.15±0.39%) compare to the control however, the average moisture content is within the range 37.07% reported in white bread, 37.22-41.23% for whole grain bread and 29.99% for commercial pan bread (Steller and Lannes, 2005; Ishida and Steel, 2014). The range of moisture content values obtained in this study conforms to 32-39% earlier reported in cassava wheat bread (Shittu *et al*., 2007). In conformity to the Nigerian regulatory standards, moisture contents of the bread samples were within regulatory specifications of 40% maximum moisture content (SON, 2004).

In this study, fats and protein contents of bread decreased consistently with increasing levels of cassava flour substitutions. This trend had similarly been reported (Ndife *et al*., 2011) and attributed to the poor content of both proteins and fat in cassava flour (Nweke, 2003; Montagnac *et al*., 2009; Eduardo *et al*., 2013). This finding is similar to breads produced from whole wheat and soya bean flour blends with protein content in the range of 8.13 to 12.50% (Ndife *et al*., 2011). In addition, fat content from 4.09% in conventional wheat bread to 9.87% in cassava composite bread. This is worrisome since high fat content promotes rancidity in foods with resultant development of unpleasant odour (Agiriga, 2014). The ash contents of the bread samples increased significantly ($P \leq 0.05$) as cassava flour ratio was increased. The progressive increase in ash content of the breads with cassava flour supplementation is in conformity with the findings of co workers (Ogunbanwo *et al*., 2008; Ndife *et al*., 2011). This has resulted in significantly higher minerals reported in the composite bread than that of wheat bread (Ogunbanwo *et al*., 2008).

Studies have shown the contribution of *L. plantarum* to amino acid turnover during dough fermentation (Gobetti *et al*., 2005; Loponen *et al*., 2007; Rizzello *et al*., 2007). It is believed that the growth of lactic acid bacteria is promoted in co-culture with yeast mainly due to the excretion of specific amino acids and small peptides (Cheirship *et al*., 2003; Paramithiotis *et al*., 2006). The released amino acids are accumulated in dough, responsible for increased proteins content of amylolytic *L. plantarum* and *S. cerevisiae* co-culture fermented bread comparatively.

Bread made from composite flours has to be subjectively assessed to determine its quality and acceptance. The finding of this study revealed that bread made with 10%, 20% composite flours were widely accepted by consumers in agreement with previous reports (Sobowale *et al*.,2007; Ogunbanwo *et al*., 2008; Abdelghafor *et al*., 2011 ; Eduardo *et al*., 2013). Many researchers have indicated that acceptability of bread formulations does not depend on fermenting organism, but on the ingredients added (Gomez *et al*., 2003). In contrary, the texture scores decrease with cassava flour content however, amylolytic *L. plantarum* and *S. cerevisiae* co-culture fermented bread had higher acceptability compare to the *S. cerevisiae* fermented bread. Studies had revealed that lactic acid fermentation influences the structure and starch granules porosity by acidic and enzymatic hydrolysis, promoting starch granule damage (Bertolini *et al*. 2001; Putri *et al*., 2011). Previously also, interaction between ALAB and yeast in dough had been found to primarily influence texture and flavour of bread (Rehman *et al*., 2007; Di Cogno *et al*., 2014).Our result shows that the use of *L.plantarum and S. cerevisiae* as starter culture greatly improved the quality of bread produced. Evident, high tastes and aroma rating of *L. plantarum and S. cerevisiae* starter bread was observed. This could be attributed to the production of compounds such as organic acid, alcohols, aldehydes, and carbonyls which impacted appealing flavour during fermentation of the dough (Thiele *et al*., 2002; Rehman *et al*., 2006).

6. CONCLUSION AND RECOMMENDATION

Co culture of amylolytic *L plantarum* (AMz5) and *S. cerevisiae* (YSg2) during fermentation of bread dough apart from functional properties as leavening agent also enhanced nutritional component of the baked product. In this study, wheat-cassava composite bread produced by co-cultured amylolytic *L. plantarum* and *S. cerevisiae* improved the nutritional components and compared ($P < 0.05$) with the conventional wheat flour bread. Wheat-cassava composite dough up to 20% level of substitution co-culture with amylolytic *L. plantarum* and *S. cerevisiae* exhibited good quality bread and was more acceptable than wheat bread by consumers. The result of study is suggesting promising applications of co cultured amylolytic *L. plantarum* and *S. cerevisiae* in the production of bread and other fermented starch-containing foods.

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Table 1: Proximate composition of wheat and cassava flours

Flour Source	**Moisture** (%)	**Ash** (%)	**Fats** (%)	**Protein** (%)	**Carbohydrate** (%)
HQCF	2.54 ±0.35[a]	0.60±0.08[a]	6.51±0.12[a]	2.59±0.38[a]	88.76±0.80[a]
LPCF	2.28 ±0.22[c]	1.76 ±0.26[a]	6.08±0.28[c]	2.05 ±0.37[c]	87.84±0.80[b]
WF	5.16 ±0.29[b]	1.64±0.24[b]	7.66±0.53[b]	3.05±0.28[b]	83.16±0.8[b]

Values are means of triplicate determinations and means values with different superscripts along columns differ significantly ($p < 0.05$).

Key: high quality cassava flour (HQCF), locally processed cassava flour (LPCF) Wheat Flour (WF)

Table 2: Proximate constituents of wheat-cassava composite flour bread

Flour Sample	**Moisture (%)**	**Ash (%)**	**Fat (%)**	**Protein (%)**	**Carbohydrate (%)**
HQCF **1**	30.16 ±0.41[e]	0.44 ±0.06[f]	8.84 ±0.46[c]	7.07 ±0.12[a]	51.49 ±0.64[h]
LPCF **1**	30.18 ±0.58[e]	0.37 ±0.11[g]	8.22 ±0.20[e]	5.17 ±0.38[d]	52.46 ±0.68[g]
HQCF **2**	30.14 ±0.40[e]	0.41 ±0.08[fg]	9.05 ±0.16[b]	7.05±0.13[b]	53.96 ±0.74[f]
LPCF **2**	31.31 ±0.55[d]	0.58 ±0.24[e]	8.60 ±0.37[cd]	5.11 ±0.81[e]	53.79 ±0.84[f]
HQCF **3**	31.67 ±0.44[d]	0.67 ±0.03[d]	8.74 ±0.31[c]	5.06 ±0.31[e]	55.62 ±0.35[e]
LPCF **3**	30.45 ±0.20[e]	0.65 ±0.18[d]	8.94 ± 0.27[b]	5.09 ±0.13[e]	54.27 ±0.63[f]
HQCF **4**	32.49 ±0.33[c]	0.93 ±0.05[b]	8.44 ±0.40[d]	3.30 ±0.12[g]	58.78 ±0.64[c]
LPCF **4**	31.15 ±0.51[d]	0.90 ±0.10[b]	8.00 ±0.38[f]	4.02 ±0.85[f]	57.90 ±0.88[d]
HQCF **5**	33.15 ±0.39[b]	0.89 ±0.03[bc]	8.50 ±0.27[d]	3.35 ±0.14[g]	58.71 ±0.50[c]
LPCF **5**	34.45 ±0.35[a]	0.85 ±0.13[c]	8.25 ±0.22[e]	4.03 ±0.50[f]	64.22 ±0.97[a]
WBLS	28.44 ±0.30[f]	0.45 ±0.04[f]	9.87 ±0.40[a]	7.09 ±0.12[a]	43.01 ±0.48[j]
WBCY	28.24 ±0.18[f]	0.93 ±0.11[b]	8.94 ±0.38[b]	6.47 ±0.17[c]	47.08 ±0.58[i]
CB	24.44±0.19[g]	1.48±0.25[a]	4.09±0.34[g]	1.46±0.18[h]	60.09±0.52[b]

Values are means of triplicate determinations and values with different superscripts along columns differ significantly ($p < 0.05$).

Key: HQCF and LPCF I = 10%, 2=20%, 3 = 30%, 4=40%, 5 =50% (Bread produced with 10-50% high quality cassava flour (HQCF) and locally processed cassava flour (LPCF) inclusion), WBLS = Wheat bread with *Lactobacillus plantarum* (AMz5) - *Saccharomyces cerevisiae* (YSg2) co culture, WBCY= Wheat bread with commercial baker's yeast, CB= Commercial wheat bread,

Table 3: Sensory properties of wheat-cassava composite flour bread

Bread Sample	% Cassava	Appearance	Taste	Aroma	Texture	Overall Acceptability
HQCF 1	10	4.27 ±0.18[ab]	4.33±0.16[ab]	4.47±0.13[b]	4.27±0.18[ab]	4.60±0.11[b]
HQCF 2	20	3.53±0.13[bc]	3.60±0.19[bc]	4.07±0.15[de]	4.00±0.17[de]	4.33±0.13[ab]
HQCF 3	30	3.33±0.13[bc]	2.67±0.15[abc]	3.20±0.15[abc]	2.87±0.13[ab]	3.67±0.13[c]
HQCF 4	40	2.67±0.13[abc]	2.13±0.13[a]	2.13±0.19[a]	2.67±0.19[abc]	3.00±0.17[abc]
HQCF 5	50	2.00±0.17[abc]	1.87±0.09[a]	1.87±0.13[a]	2.53±0.19[cde]	2.07±0.21[abcd]
WBLS	-	4.67±0.13[de]	4.67±0.13[de]	4.53±0.13[cde]	4.80±0.11[d]	4.47±0.13[cde]
WBCY	-	4.20±0.11[ab]	4.07±0.15[abc]	4.00±0.17[abc]	3.93±0.15[abc]	4.40±0.13[bc]

Values are means ± standard deviation of triplicate determinations and values with different superscripts along columns differ significantly ($p < 0.05$).

Key: HQCF I = 10%, HQCF 2=20%, HQCF 3 = 30%, HQCF 4=40%, HQCF 5 =50% (Bread produced with 10-50% cassava flour inclusion leavened with *Lactobacillus plantarum* (AMz5)-*Saccharomyces cerevisiae* (YSg2) co culture), WBLS = Wheat bread leavened with *Lactobacillus plantarum* (AMz5) - *Saccharomyces cerevisiae* (YSg2) co culture, WBCY= Wheat bread leavened with commercial baker's yeast only.

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