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Effects of Cereal Malts Used as Improver on Physico-Chemical, Nutritional and Sensory Characteristics of Wheat and Millet Composites Breads

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Abstract

The aim of this study was to optimize composite breads of wheat and whole millet flour by the use of natural improvers. Three types of local malted cereals were used as natural improvers. The millet flour was fermented with EPSs producing LAB strain prior to use. The technological characteristics of the composite flours were determined using an alveograph. The physico-chemical and nutritional characteristics of the composite breads were determined using standard methods and their sensory profiles were evaluated by a panel of 35 consumers. The alveograph results showed an increase in dough resistance, deformation and a decrease in extensibility and elasticity with the level of incorporation of millet flour. From the results of physico-chemical analyses of composite breads, no significant difference ($p > 0.05$) was observed in the use of the three types of local cereal malts except for the incorporation of 50% of the millet flour. The control sample presented the lowest acidity and dry matter value, the highest water content and pH value. No significant difference ($p > 0.05$) was observed in the use of the three types of cereal malts for the macronutrient contents of the composite bread samples. However, differences were observed according to the levels of incorporation. Macronutrients results showed an increase in protein content (11.17% ± 0.28% - 14.01% ± 0.10%/DM); crude fat content (1.86% ± 0.05% - 2.48% ± 0.20%/DM) and a decrease in carbohydrates content (85.36% ± 0.54% - 81.06% ± 0.36%/DM). Regarding the content of mineral elements, significant differences ($p < 0.05$) were observed in the use of the three types of cereal malts for the incorporation of 15% (Mg and Fe content), 30% (Fe, Zn and K) and 50% (content of Zn, K, Na and Mg) of millet flour. The free amino acid profile revealed three essential amino acids such as valine, isoleucine and lysine. Breads incorporated with 30% of whole millet flour were the most appreciated by consumers.

Keywords

Composite Bread, Cereal Malts, Improver, Millet, Sensorial Characteristics

1. Introduction

Bread is a bakery product which main ingredients are water, flour, salt, yeast, sugar, and fat mixed and fermented to form a viscoelastic dough before baking [1] [2]. It is an important and mostly consumed staple cereal-based food globally and it contains useful nutrients such as starch, protein, fiber, vitamins, and minerals [3] [4] [5]. In addition, bread (wheat bread) is receiving a growing interest as a possible functional food due to its great diffusion and consumption [5] [6]. It is poor in protein while rich in carbohydrates, with a high glycemic index, which can lead to obesity and susceptibility to diabetes and biliary-tract cancer [7]. The consumption of bread in many countries, especially in sub-Saharan Africa is on the rise due to urbanization, but there is a challenge to meet the supply and demand of bread in order to match the eating habit of consumers [8]. Therefore, baking industries have a challenge of producing bread with improved nutritional, physicochemical and sensory characteristics due to increased consumer's demand for high quality and healthy bakery products [5] [9].

Wheat flour, the main ingredient in bread, is avoided by gluten intolerant patients as it contains high gluten content, low fiber and high glycemic level, and also contributes, to many disorders and diseases like diabetes, obesity, and atherosclerosis [10]. However, consumption of pearl millet has been linked to reduced risk of age related chronic diseases such as colorectal cancer, cardiovascular diseases (CVD), heart disease and obesity [11] [12]. Pearl millet is also known for its low glycemic index (GI) which is considered to be important for the management of type 2 diabetes [12] [13]. It is a rich source of carbohydrate, protein, dietary fiber, vitamins B complex and minerals such as calcium, phosphorus, magnesium, and manganese [14].

To ensure food security, Africa needs to take urgent steps to become self-sufficient in food production and also promote the use of indigenous grains such as millets in industrial food production [12]. Imported wheat flour, especially in its refined form, is used to make breads and other bakery products. A strategy to reduce wheat import is to utilize composite flour containing wheat and millet in bakery products, which will enhance the production and consumption of indigenous grains on an industrial scale [12]. The inclusion of millet flour in bread making is uncommon given the high demand for wheat-based bread. There are also technological challenges to replacing wheat flour with millet, which diminishes bread quality particularly with respect to loaf volume, texture, mouth-feel and staling rate [12] [15]. Gluten proteins are essential for structure building; they form a network in the solid matrix and allow gas retention and expansion, which improve the bread volume [12] [16]. Starch retrogradation and water molecule migration are the major causes of bread staling [17]. The gluten network can slow the movement of water, thereby maintaining softness and reducing bread staling [17]. The absence of gluten functionality in pearl millet restricts the high level of substitution in the wheat bread formula. One option is to employ hydrocolloids to mimic the properties of gluten. To do this, dextran produced by lactic acid bacteria (LAB) can act as a hydrocolloid in bread making [12] [18]. In the practice, exopolysaccharide (EPSs)-producing LAB are most often used to ferment gluten-free cereal flour dough into a slimy sourdough [12].

Some studies reported that the use of malt flour in controlled conditions improved loaf volume and crumb texture [19] [20]. These positive effects were attributed to the natural enzymes expressed during the germination process that might decrease or completely replace the quantity of commercial enzymes added to bread formulation. This technique makes it possible to enrich cereals with hydrolytic enzymes such as beta-amylase and alpha-amylase, in sugars, in free amino acids and in vitamins, thus improving the technological and nutritional quality of the derived products [21] [22] [23].

The aim of this study is to evaluate the effect of local cereals malts flours used as natural improvers on the physico-chemical and nutritional characteristics of composite bread produced based on millet and wheat flour with different substitution rate of wheat flour.

2. Material and Methods

2.1. Vegetal Material

The vegetal material were sorghum grains (*Sorghum bicolor* (L. Moench) and millet grains (*Pennisetum glaucum*), which were purchased from national institute of environmental and agriculture research (INERA/Saria at Koudougou, Burkina Faso). The grains of three types of variety (30 kg each other) were purchased, including "IKMP5" for millet grains, "Framida" for red sorghum grains and "Kapèlga" for white sorghum.

2.2. Origin of EPSs-Producing LAB Strain Used as Starter Culture

The EPSs producing LAB strain (A16) used as starter culture for millet dough fermentation was obtained from traditional fermentation process of *Massa* [24]. This strain was previously characterized as EPSs producer and identified as *W. confusa/cibaria* using 16S rRNA gene sequencing [24].

2.3. Characteristics of Cereal Malts Used as Natural Improver

Three types of cereals malts were used as improver (**Table 1**) for composite bread production. It was red sorghum, white sorghum and millet malts. These malts were produced in controlled conditions at the IRSAT/DTA microbiology laboratory (Ouagadougou, Burkina Faso [23]). The choice of these malts was mainly based on their diastatic power, α and β-Amylases activities.

2.4. Preparation of EPSs Producing LAB Inoculum and Sourdough

The EPSs producing LAB strain A16 (previously stored in MRS-broth + glycerol at −20˚C) was subcultured onto MRS agar (composition) and incubated for 48 h at 37˚C. One colony of the LAB strain A16 was then subcultured in 10 mL of MRS-broth and incubated for 24 h at 37˚C. A volume of 0.1 mL of culture broth was subcultured in MRS-broth (10 mL) and then incubated for 16 to 18 h at 37˚C. The culture broth obtained was distributed in sterile cryotubes (1 mL/tube), then centrifuged (MIKRO 220R, Germany) at 8000 *g* for 10 min. The supernatant of each tube was removed and the pellet (cells) retained. To this pellet was added 1 mL of sterile diluent [0.1% (w/v) peptone (Difco), 0.85% (w/v) NaCl (Sigma), pH 7.2 ± 0.2]; after stirring, a further centrifugation was carried out at 8000 *g* for 10 min. The supernatant was again removed and the pellet was preserved. One millimeter (1 mL) of sterile distilled water was added to the pellet and, after stirring, the cells suspension which constitutes the inoculum was stored in the refrigerator at 4˚C. The concentration of viable cells of the inoculum was determined by enumeration on MRS agar. The inoculum (10^6 CFU/ml) was used for the fermentation of the millet dough which has been the main element for the composite bread production. The dough was inoculated with *W. confusa*/cibaria strain (A16) at 1% (v/w then incubated in an oven at 25˚C for 24 h to obtain a millet sourdough.

2.5. Composite Bread Production Process

For the preparation of composite bread (**Figure 1**), the total amount of water used for kneading was between 62% - 63% (v/w) of the total flour mass. The dough was produced by mixing all the ingredients (yeasts, salt, sugar, cereal malt flour, wheat flour and the millet dough fermented with the EPS-producing LAB strain A16) in a kneader for 4 min at low speed and 10 min at high speed quick. The dough obtained was put in to balls of 250 g and let to rest for 10 min. These balls were then mechanically molded into French baguette-type loaves of bread and let to rest in a proofing chamber for 45 min (30˚C, Relative Humidity 75%).

After the dough pieces had rested, the loaves were incised with a blade and baked in a rotary electric oven (RAMA hos, China), equipped with an automatic water injection system, with preheating at 240˚C and baking at 190˚C for 15 min. After baking, the loaves were cooled at room temperature. Composite breads without malt flour were used as control breads. Three formulations of composite bread (**Table 2**) were done using three types of cereals malt flour (red sorghum, white sorghum and millet malt flour).

2.6. Physico-Chemical and Nutritional Analyses

The strength and elasticity of the dough based on millet and wheat flours were measured using a chopin alveograph (Alveolab, French) according to the French standard NF ISO 5530-4: 1992.

Table 1. Characteristics of cereals malts used as improver.

Cereals malts samples	Diastatic power (UPD)	α-Amylases (CERALPHA units)	β-Amylases (CERALPHA units)	Water content (%)	Dry matter (%)
SRn72	251.22 ± 6.8	221.65 ± 7.1	29.57 ± 2.6	9.44 ± 1.5	90.56 ± 1.5
SBn72	266.17 ± 8.9	237.33 ± 11.5	28.84 ± 3.2	9.86 ± 2.8	90.14 ± 2.8
PMn48	239.85 ± 19.3	201.91 ± 19.7	37.94 ± 1.5	8.39 ± 1.5	91.61 ± 1.5

SRn72: Red sorghum malt 72 h of germination; **SBn72:** White sorghum malt 72 h of germination; **PMn48:** Pearl millet malt 48 h of germination [23].

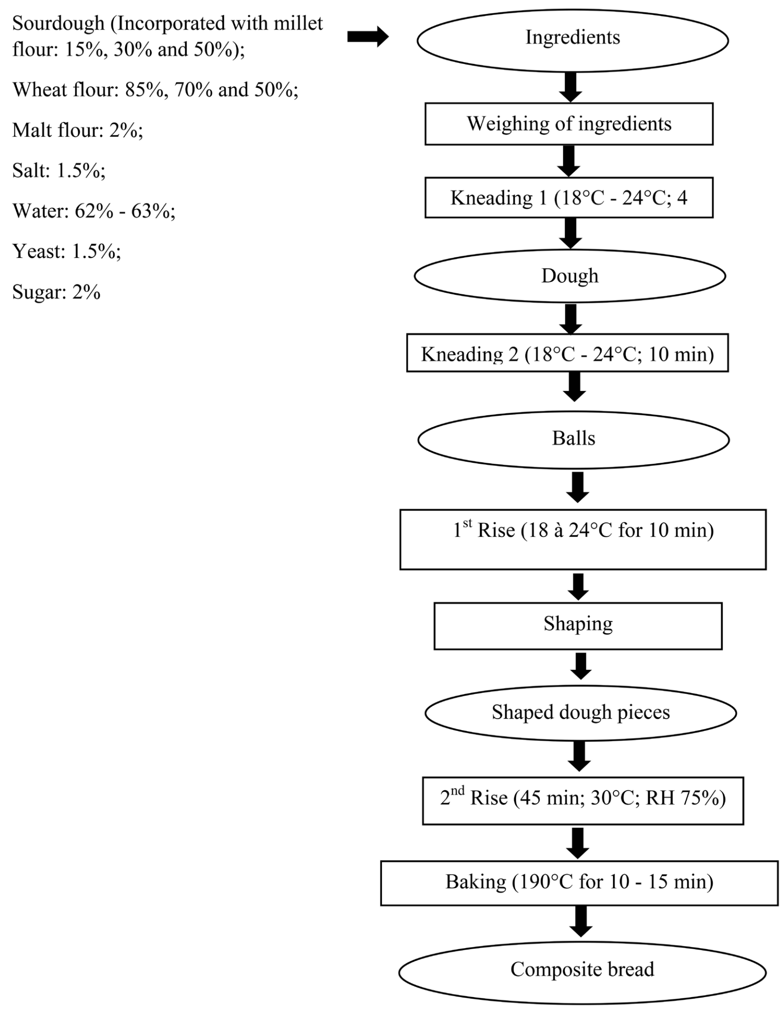


Figure 1. Composite bread production diagram.

Table 2. Composite bread formulations.

Composite bread recipe	Formulation 1 (%)	Formulation 2 (%)	Formulation 3 (%)
Millet flour	15	30.0	50
Wheat flour	85	70.0	50
Cereals malt flour	2.0	2.0	2.0
Yeast	1.5	1.5	1.5
Sugar	2.0	2.0	2.0
Salt	1.5	1.5	1.5

The pH of the breads samples was measured with an electronic pH-meter (Model HI 8520; Hanna Instrument, Singapore). For each sample, 10 g of product were mixed with 20 mL of distilled water prior to pH measurement. For titratable acidity determination, 5 g of sample suspended in 30 mL of ethanol (90˚) was mixed during 1 h and centrifuged for 5 min at 3500 g. From the supernatant, 20 mL was transferred to a 50 mL measuring flask and was titrated with NaOH 0.1 N using 1% phenolphthalein as indicator [25]. The titratable acidity was calculated according to [26]. Water content was determined by drying the sample at 105˚C ± 2˚C for 24 h according to NF V03-707: 2000; ash content was determined by incineration at 650˚C overnight according to international standard ISO 2171: 2007; crude protein content (N×6.25) was determined by the Kjeldahl method [27] after acid digestion according to NF V03 50: 1970; crude fat content was determined by soxhlet extraction using n-hexane according to ISO 659: 1998 and [28]. Total carbohydrates content was determined by spectrophotometric method at 510 nm using orcinol as reagent [29]. The energy value was calculated according to the Atwater method [30]. The determination of mineral elements was carried out by flame atomic absorption spectrometry (Perkin-Elmer model 303) according to [31]. The crude fiber content was determined by the formic insoluble method according to [32]. The free amino acid profile was carried out by high performance liquid chromatography (HPLC) using Waters PICO-TAG method [33], which consisted of three steps: hydrolysis of samples, sample derivatization pre-column and HPLC-reverse phase analysis. Amino acids identification and concentrations were determined with the Empower software by comparing retention times obtained with retention times of standards. The values were expressed in g/100g of dry matter.

2.7. Sensory Evaluation of Composite Bread Samples

The sensory evaluation consisted of evaluating the sensory profile of composite bread samples. A test of differentiation of the composite bread samples compared to the control sample (composite bread without malt flour) and a test of the classification of the composite bread samples were also performed according to the method describe by [34]. The tasting panel included 35 consumers composed of men and women with a minimum age of 15 whom were familiar with composite bread samples.

2.8. Statistical Analyses of the Data

All the data (except those for sensory analyses) were submitted to Analysis of Variance (ANOVA) with the statistical software XLSTAT-Pro 7.5.2: 2016 and the means were compared using the test of Student Newman-keuls to the probability level $p < 0.05$. The curves were obtained using Microsoft Excel 2013. Sensory evaluation data were analyzed using the Chi^2 test with the statistical software SPSS.

3. Results

3.1. Characteristics of Flours

It emerged from the results of **Table 3** that the characteristics of the dough from the composite flour (Wheat-Millet) in terms of tenacity (P), extensibility (L), swelling (G) and baking force or work (W) varied from 83 to 152 mm H_2O, from 14.7 to 66 mm, from 2.9 to 18.1 cm^3 and from 148 to 319×10^{-4} J, respectively, according to the incorporation rate of millet flour. These results showed that when the incorporation rate of millet flour increased, the quality of the dough in terms of strength, the elasticity decreased.

3.2. Physicochemical Characteristics of Composite Bread Samples

The results of the water content of the composite bread (**Table 4**) ranged between 28.87% ± 0.74% and 30.92% ± 3.68% for composite breads samples incorporated with 15% of millet flour. For the breads samples incorporated with 30% of millet flour the values ranged from 25.26% ± 0.14% to 30.34% ± 0.40% and for those incorporated with 50% of millet flour, the values ranged from 25.90% ± 0.13% to 30.91% ± 0.88%. The pH of composite breads varied from 5.29 ± 0.05 to 5.39 ± 0.00, from 5.19 ± 0.02 to 5.50 ± 0.00 and from 4.94 ± 0.02 to 5.19 ± 0.03, respectively for breads incorporated with 15%, 30% and 50% of millet flour (**Table 4**). Regarding the titratable acidity of the composite breads, its content increased with the incorporation rate of millet flour. Indeed, the bread samples incorporated with 50%of millet flour presented a higher acidity (0.49 ± 0.01 to 0.69 ± 0.07), followed by samples incorporated with 30% of millet flour (0.37% ± 0.01% to 0.41% ± 0.01%) and finally those incorporated with 15% of millet flour (0.21 ± 0.01 to 0.22 ± 0.00) (**Table 4**). Composite breads without malt (controls) showed the lowest values compared to breads containing malts. The ash content of the samples ranged from 1.58% ± 0.06%/DM to 1.77% ± 0.00%/DM for the composite breads incorporated with 15% of millet flour (**Table 4**). For those incorporated with 30% of millet flour, the ash content ranged between 1.85% ± 0.02%/DM and 2.00% ± 0.03%/DM. For the breads incorporated with 50% of millet flour, ash content ranged from 2.58% ± 0.02%/DM to 2.62% ± 0.16%/DM. The ash content increased with the incorporation rate of millet flour. For the crude fiber content, values ranged from 1.85% ± 0.28%/DM (bread samples without malt) to 2.84% ± 0.72%/DM (bread samples using malt flour). These crude fiber contents increased with the incorporation rate of millet flour (Table 4). No significant difference ($p > 0.05$) was observed in the use of the three types of malt for the physico-chemical parameters except for the incorporation of 50% of the millet flour. The control sample presented the lowest acidity and dry matter value, and the highest water content and pH value.

Table 3. Characteristics of flours (Wheat, Wheat-Millet) through the alveograph.

Parameters	Wheat flour (From CANADA)	Composite flour (Wheat-Millet)		
Incorporation rate of millet flour	0%	15%	30%	50%
P (mmH_2O)	122	152	137	83
L (mm)	130	66	38	14.7
G (Cm^3)	25.4	18.1	13.7	2.9
W (10^{-4} J)	430	319	200	148
P/L	0.9	2.3	3.6	5.6

P: Tenacity; **L:** Extensibility; **G:** Swelling; **W:** Baking force or work.

Table 4. Physico-chemical characteristics of composite bread samples.

	Water content (%)	Dry matter (%)	pH	Titratable acidity (%)	Ash (%/DM)	Fiber (%/DM)
			15% incorporation of millet flour			
PMr1	29.07 ± 0.30^a	70.93 ± 0.30^a	5.29 ± 0.05^a	0.21 ± 0.01^a	1.71 ± 0.10^a	1.95 ± 0.16^a
PMb1	28.87 ± 0.18^a	71.14 ± 0.18^a	5.34 ± 0.00^a	0.22 ± 0.00^b	1.62 ± 0.00^a	1.95 ± 0.29^a
PMm1	29.72 ± 0.74^a	70.28 ± 0.74^a	5.34 ± 0.04^a	0.22 ± 0.01^b	1.77 ± 0.02^a	2.25 ± 0.04^a
PMt1	30.92 ± 3.68^a	69.08 ± 0.68^a	5.39 ± 0.00^a	0.22 ± 0.00^b	1.59 ± 0.06^a	1.85 ± 0.05^a
P-value	**0.864**	**0.864**	**0.298**	**0.043**	**0.235**	**0.541**
			30% incorporation of millet flour			
PMr3	25.26 ± 0.14^b	74.74 ± 0.14^a	5.19 ± 0.02^c	0.37 ± 0.01^a	2.00 ± 0.03^a	2.19 ± 0.15^a
PMb3	29.62 ± 0.74^a	70.38 ± 0.74^b	5.50 ± 0.00^a	0.39 ± 0.02^a	1.87 ± 0.04^a	2.84 ± 0.72^a
PMm3	29.58 ± 0.19^a	70.42 ± 0.19^b	5.26 ± 0.00^b	0.41 ± 0.01^a	1.91 ± 0.09^a	2.31 ± 0.29^a
PMt3	30.34 ± 0.40^a	69.66 ± 0.40^b	5.24 ± 0.00^{bc}	0.37 ± 0.00^a	1.85 ± 0.02^a	2.09 ± 0.13^a
P-value	**0.004**	**0.004**	**0.000**	**0.115**	**0.317**	**0.599**
			50% incorporation of millet flour			
PMr5	25.90 ± 0.13^b	74.10 ± 0.13^a	5.07 ± 0.03^{ab}	0.69 ± 0.07^b	2.58 ± 0.02^a	1.88 ± 0.28^a
PMb5	28.26 ± 0.03^b	71.74 ± 0.03^a	4.94 ± 0.02^b	0.53 ± 0.03^b	2.62 ± 0.16^a	2.17 ± 0.19^a
PMm5	27.58 ± 0.05^b	72.42 ± 0.05^a	5.07 ± 0.02^{ab}	0.56 ± 0.03^b	2.61 ± 0.05^a	2.11 ± 0.15^a
PMt5	30.91 ± 0.88^a	69.09 ± 0.88^b	5.19 ± 0.03^a	0.49 ± 0.01^a	2.62 ± 0.04^a	1.90 ± 0.07^a
P-value	**0.006**	**0.006**	**0.015**	**0.007**	**0.982**	**0.640**

PM: Pearl millet bread; **m:** millet malt; **r:** red sorghum malt; **b:** white sorghum malt; **t:** control (without malt) **1:** 15% incorporation of millet flour; **3:** 30% incorporation of millet flour; **5:** 50% incorporation of millet flour. Values with the same letters in the column are not significantly different at the 5% level according to Newman-keuls's test.

3.3. Macronutrient Composition of Composite Bread Samples

Results from Table 5 showed that the protein content of the bread samples varied from 11.17% ± 0.28%/DM (PMm1) to 14.01% ± 0.10%/DM (PMm5). The protein content increased according to the rate of substitution of wheat flour by millet flour, leading to an increase in this content in composite bread, in particular with pearl millet malt. The fat content also increased from 1.86% ± 0.05%/DM (PMb1) to 2.48% ± 0.20%/DM (PMm5). This increase followed the rate of incorporation of the millet flour. As for the carbohydrates content, it ranged from 81.63% ± 0.17%/DM to 85.36% ± 0.54%/DM and decreased with the increase in the incorporation rate of millet flour. The highest value was recorded for the 15% incorporation of millet flour, especially for the PMm1 sample. For the energy value, it varied from 401.10 ± 0.4 kcal/100g for the control bread to 404.86 ± 2.40 kcal/100g for the PMr1 sample. No significant difference ($p > 0.05$) was observed in the use of the three types of malt for the macronutrient contents of the bread samples. On the other hand, differences were observed according to the levels of substitutions.

Table 5. Macronutrient composition of composite bread samples.

	Protein (%/DM)	Crude fat (%/DM)	Carbohydrate (%/DM)	Energy value (kcal/100g)
		15% incorporation of millet flour		
PMr1	11.86 ± 0.02^{a}	2.34 ± 0.40^{a}	84.10 ± 0.32^{a}	404.86 ± 2.40^{a}
PMb1	11.39 ± 0.02^{a}	1.86 ± 0.05^{a}	84.78 ± 0.04^{a}	403.19 ± 0.24^{a}
PMm1	11.17 ± 0.28^{a}	2.05 ± 0.23^{a}	85.36 ± 0.54^{a}	402.84 ± 1.09^{a}
PMt1	11.50 ± 0.20^{a}	2.00 ± 0.11^{a}	84.91 ± 0.14^{a}	403.68 ± 0.81^{a}
P-value	**0.170**	**0.602**	**0.185**	**0.756**
		30% incorporation of millet flour		
PMr3	12.76 ± 0.05^{a}	2.93 ± 0.08^{a}	82.32 ± 0.00^{a}	406.62 ± 0.40^{a}
PMb3	12.60 ± 0.22^{a}	1.90 ± 0.20^{b}	83.64 ± 0.46^{a}	402.00 ± 0.85^{b}
PMm3	12.47 ± 0.23^{a}	2.04 ± 0.04^{b}	83.59 ± 0.10^{a}	402.55 ± 0.17^{b}
PMt3	12.58 ± 0.19^{a}	2.31 ± 0.20^{ab}	83.26 ± 0.38^{a}	404.17 ± 1.06^{ab}
P-value	**0.764**	**0.027**	**0.102**	**0.035**
		50% incorporation of millet flour		
PMr5	13.56 ± 0.07^{a}	2.34 ± 0.18^{a}	81.51 ± 0.08^{a}	401.41 ± 0.9^{a}
PMb5	13.50 ± 0.38^{a}	2.26 ± 0.37^{a}	81.63 ± 0.17^{a}	400.81 ± 2.4^{a}
PMm5	14.01 ± 0.10^{a}	2.48 ± 0.20^{a}	80.90 ± 0.05^{a}	401.99 ± 1.1^{a}
PMt5	14.00 ± 0.21^{a}	2.32 ± 0.12^{a}	81.06 ± 0.36^{a}	401.10 ± 0.4^{a}
P-value	**0.336**	**0.912**	**0.164**	**0.945**

PM: Pearl millet bread; **m:** millet malt; **r:** red sorghum malt; **b:** white sorghum malt; **t:** control (without malt) **1:** 15% incorporation of millet flour; **3:** 30% incorporation of millet flour; **5:** 50% incorporation of millet flour. Values with the same letters in the column are not significantly different at the 5% level according to Newman-keuls's test.

3.4. Minerals Composition of Composite Bread Samples

The results in Table 6 showed a significant difference ($p < 0.05$) with regard to iron (Fe) content for breads samples incorporated with 15% and 30% of millet flour considering the different types of malt flour used. Values ranged from 3.58 ± 0.00 mg/100g to 3.88 ± 0.09 mg/100g; from 4.31 ± 0.07 mg/100g to 5.47 ± 0.20 mg/100g and from 7.73 ± 0.01 mg/100g to 8.65 ± 0.44 mg/100g respectively for the substitution of 15%, 30% and 50% of wheat flour by whole millet flour. Regarding zinc (Zn) content, a significant difference ($p < 0.05$) was found for the incorporation of millet flour at 30% and 50% depending on the type of malt used. These values ranged respectively from 2.54 ± 0.00 mg/100g to 2.75 ± 0.02 mg/100g and from 1.61 ± 0.00 mg/100g to 1.75 ± 0.00 mg/100g. However, for the 15% incorporation no significant difference was observed ($p > 0.05$). The potassium (K) content ranged from 206.13 ± 10.21 mg/100g to 222.21 ± 10.84

Table 6. Minerals elements composition of composite bread (Wheat-millet) samples.

Bread samples	Fe (mg/100g)	Zn (mg/100g)	K (mg/100g)	Na (mg/100g)	Mg (mg/100g)
15% incorporation of millet flour					
PMr1	3.88 ± 0.09^{a}	3.43 ± 0.01^{a}	222.21 ± 10.84^{a}	481.83 ± 22.36^{a}	33.15 ± 0.21^{b}
PMb1	3.58 ± 0.00^{b}	3.32 ± 0.05^{a}	206.13 ± 10.21^{a}	445.65 ± 0.81^{a}	30.31 ± 0.00^{c}
PMm1	3.88 ± 0.05^{a}	3.39 ± 0.05^{a}	214.58 ± 4.40^{a}	437.56 ± 1.30^{a}	29.35 ± 0.47^{c}
PMt1	3.81 ± 0.03^{a}	3.30 ± 0.01^{a}	213.25 ± 1.42^{a}	433.67 ± 1.77^{a}	35.87 ± 0.02^{a}
P-value	0.046	0.163	0.593	0.114	0.000
30% incorporation of millet flour					
PMr3	4.76 ± 0.18^{ab}	2.63 ± 0.00^{c}	245.88 ± 1.19^{a}	445.63 ± 1.45^{a}	41.73 ± 0.45^{a}
PMb3	4.31 ± 0.07^{b}	2.54 ± 0.00^{d}	243.71 ± 0.45^{a}	441.08 ± 0.29^{a}	42.11 ± 0.02^{a}
PMm3	5.47 ± 0.20^{a}	2.75 ± 0.02^{a}	249.82 ± 3.08^{a}	442.19 ± 0.73^{a}	41.00 ± 0.08^{a}
PMt3	5.16 ± 0.03^{a}	2.69 ± 0.00^{b}	231.74 ± 1.01^{b}	441.51 ± 7.27^{a}	41.68 ± 0.11^{a}
P-value	0.016	0.001	0.007	0.819	0.114
50% incorporation of millet flour					
PMm5	8.65 ± 0.44^{a}	1.75 ± 0.00^{a}	280.48 ± 0.22^{a}	445.02 ± 1.37^{a}	53.08 ± 0.57^{a}
PMb5	7.73 ± 0.61^{a}	1.61 ± 0.00^{b}	262.40 ± 1.19^{b}	448.48 ± 0.69^{a}	44.79 ± 0.10^{b}
PMr5	8.35 ± 0.04^{a}	1.74 ± 0.00^{a}	261.52 ± 2.52^{b}	442.10 ± 4.76^{a}	44.68 ± 0.02^{b}
PMt5	7.82 ± 0.00^{a}	1.73 ± 0.00^{b}	258.20 ± 4.13^{b}	436.41 ± 0.45^{b}	44.90 ± 0.01^{b}
P-value	0,376	0,000	0,010	0,101	0,000

PM: Pearl millet bread; **m:** millet malt; **r:** red sorghum malt; **b:** white sorghum malt; **t:** control (without malt) **1:** 15% incorporation of millet flour; **3:** 30% incorporation of millet flour; **5:** 50% incorporation of millet flour**.** Values with the same letters in the column are not significantly different at the 5% level according to Newman-keuls's test.

mg/100g for the breads incorporated with 15% of millet flour, from 231.74 ± 1.01 mg /100g to 249.82 ± 3.08 mg/100g for breads incorporated with 30% of millet flour and from 258.20 ± 4.13 mg/100g to 280.48 ± 0.22 mg/100g for composite breads incorporated with 50% of millet flour. Sodium (Na) content ranged from 433.67 ± 1.77 mg/100g to 481.83 ± 22.36 mg/100g for bread samples incorporated with 15% of millet flour, from 441.08 ± 0.29 mg/100g to 445.63 ± 1.45 mg/100g for those with incorporation of 30% millet flour and ranged from 436.41 ± 0.45 mg/100g to 448.48 ± 0.69 mg/100g for samples with incorporation rate of 50% of millet flour. Magnesium (Mg) content of the composite bread samples (wheat-millet) ranged from 29.35 ± 0.47 mg/100g to 35.87 ± 0.02 mg/100g; from 41.00 ± 0.08 mg/100g to 41.73 ± 0.45 mg/100g and from 44.68 ± 0.02 mg/100g to 53.08 ± 0.57 mg/100g, respectively for breads samples incorporated with 15%, 30% and 50% of millet flour. The results of the minerals obtained from the samples of composite breads showed an increase in the values except for zinc where a decrease was observed with an increase in the incorporation rate of millet flour. The samples incorporated with 50% of millet flour presented a higher concentration of minerals compared to the others. Only zinc content remained low with values ranging from 1.61 ± 0.00 mg/100g to 1.75 ± 0.00 mg/100g. Regarding the content of mineral elements, significant differences ($p < 0.05$) were observed in the use of the three types of malt for the incorporation of 15% (Mg and Fe content), 30% (Fe, Zn and K) and 50% (content of Zn, K, Na and Mg) of millet flour.

3.5. Free Amino Acid Profile of Composite Bread Samples

Results of the analysis of free amino acid profile in composite breads (Table 7) showed the presence of six (06) free amino acids (Ser, Ala, Pro, Ile, Val and Lys) of which three are essential amino acids (Ile, Val and Lys). Proline was present in the composite bread samples with a lowest content of 0.3%/DM in the PMm5 bread and a highest content of 1.62%/DM in the bread produced with red sorghum malt at 30% incorporation of millet flour (PMr3). The alanine and isoleucine contents were observed in the control samples (PMt5 and PMt1) with respective contents of 0.23%/DM and 0.02%/DM. Serine content was also present in the control samples with a lowest content of 0.01%/DM for the 50% incorporation of millet flour and a highest content of 0.03%/DM at 30% of incorporation. From the result, it emerged that the serine content decreased with the level of substitution of wheat flour by millet flour. Results showed that valine content increased with the incorporation rate of millet flour. This content varied from 0.028%/DM to 0.115%/DM respectively in the control bread at 30% of incorporation of millet flour and in the breads samples incorporated with 50% of millet flour. Lysine content was observed in five (05) bread samples with a minimal value of 0.072%/DM obtained in the control sample at 15% of millet flour incorporation, and a maximal value of 0.174%/DM in bread produced with red sorghum malt at 50% of millet flour incorporation.

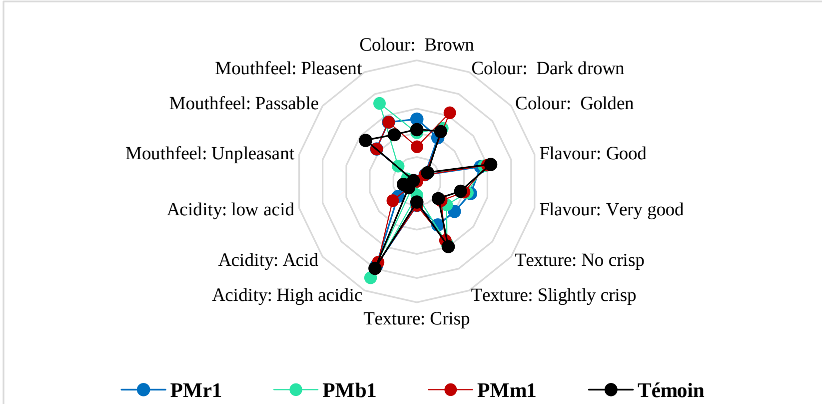
Table 7. Free amino acids profile of composite bread (wheat-millet) samples.

Samples	Ser (%/DM)	Ala (%/DM)	Pro (%/DM)	Val (%/DM)	Ile (%/DM)	Lys (%/DM)
15% incorporation of millet flour						
PMr1	0.000	0.000	1.365	0.000	0.000	0.000
PMm1	0.000	0.000	1.303	0.000	0.000	0.000
PMb1	0.000	0.000	1.409	0.000	0.000	0.079
PMt1	0.000	0.000	0.323	0.000	0.021	0.072
30% incorporation of millet flour						
PMr3	0.000	0.000	1.624	0.000	0.000	0.000
PMb3	0.000	0.000	1.271	0.000	0.000	0.000
PMm3	0.000	0.000	1.251	0.000	0.000	0.000
PMt3	0.027	0.000	0.597	0.028	0.000	0.136
50% incorporation of millet flour						
PMb5	0.000	0.000	0.885	0.115	0.000	0.092
PMm5	0.000	0.000	0.888	0.000	0.000	0.087
PMr5	0.000	0.000	0.934	0.000	0.000	0.174
PMt5	0.014	0.230	0.298	0.000	0.000	0.134

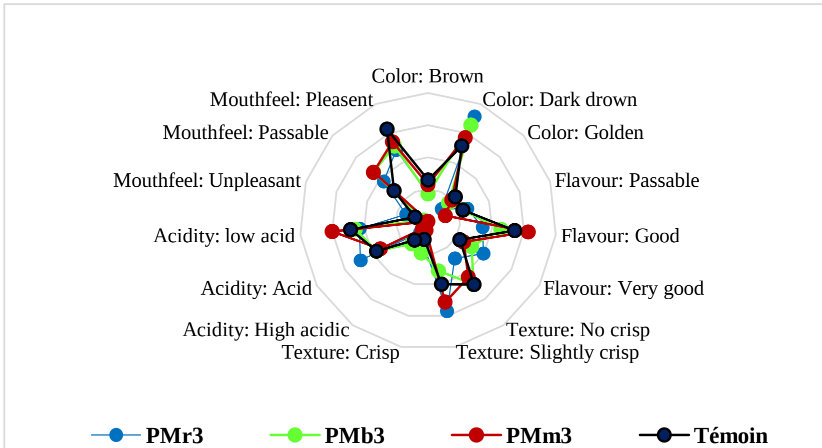
PM: Pearl millet bread; **m:** millet malt; **r:** red sorghum malt; **b:** white sorghum malt; **t:** control (without malt) **1:** 15% incorporation of millet flour; 3: 30% incorporation of millet flour; **5:** 50% incorporation of millet flour.

3.6. Sensory Evaluation Results of Composite Bread Samples

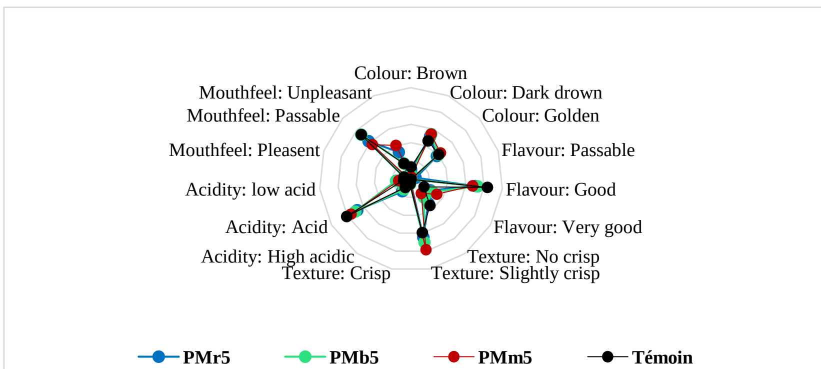
From the results of sensory evaluation (Figure 2), the consumers found that the breads made from wheat-millet presented a brown crust. Thus, 40.71% of the consumers reported that the PMr1 bread and the control bread resulting from the incorporation of 15% of the millet flour were brown. On the other hand, 49.29% of them brought their appreciation on the brown color in the PMm1 and PMb1 sample. As for the 30% and 50% incorporation of millet flour, the result indicated that all the bread samples with the malts were brown compared to the control breads. It also emerged that 41.43% of the consumers found that the breads had a very good flavor for the 15% incorporation of millet flour. Thus, 49.29% and 73.65% of consumers found that these breads presented good aroma, respectively, for the breads incorporated with 30% and 50% of millet flour. Regarding the texture of the bread samples, the incorporation of 15% and 50% millet flour was considered slightly crispy respectively by a proportion of 52.86% and 68.24% of the consumers. On the other hand, the breads samples incorporated with 30% of millet flour were appreciated as not crispy by 42.14% of the consumers, particularly for the PMb3 bread and the control. The tart taste sensation of the samples was pronounced with increasing rate of incorporation of millet which is fermented into millet sourdough. However, the incorporation of 15% (PMb3 and control) and 30% of millet flour (PMm3 and control) was



(a)



(b)



(c)

Figure 2. Sensory profile of composite bread made with wheat-millet flour. **Legend:** (a) Wheat-millet bread (15% incorporation of millet flour); (b) Wheat-millet bread (30% incorporation of millet flour); (c) Wheat-millet bread (50% incorporation of millet flour).

judged to be low acidic by 80% and 49.29% of the consumers, respectively. At 50% incorporation of millet flour, the consumers (73.65%) found that the bread became acidic particularly for the control bread, the PMb5 bread and the PMm5 bread. Mouthfeel was rated as pleasant by 57.71% and 54.29% of consumers for the 15% and 30% incorporation respectively. All breads produced with malts had a good mouthfeel for both of these incorporation rates (15% and 30%). For the 50% incorporation of millet flour, consumers found the mouthfeel to be fair and the bread very hard. For the hedonic test (**Figure 3**), the incorporation of 30% of millet flour was considered very pleasant by the consumers (48.6%) compared to those at 15% (31.4% of the consumers) and 50% (25% of the consumers) taking into account of different criteria of sensory attributes.

4. Discussion

The incorporation of whole millet flour in composite breads resulted in an increase in the resistance of the dough to deformation, a decrease in extensibility and elasticity. The extensibility of a dough is dependent on both the extension rate due to viscous flow, as well as the elastic properties of the dough influence the amount of stress required to stretch the dough [35]. High P/L indicates a resistant and inextensible dough, while low P/L indicates a weak and extensible dough. P/L is often used industrially together with W to assess flour quality, as P/L indicates the shape of the alveogram and thereby the balance between tenacity and extensibility [35]. W is one of the industrially most applied alveograph parameters, as it is used for prediction of processing behaviour of flour cultivars. The incorporation of 30% of millet flour leads to good baking strength comparable to that obtained with 100% of certain wheat flours. On the other hand, beyond 30% incorporation of millet flour, the volume of loaves during resting step decreases, which leads to breads of high density with a low volume. This could be justified by the absence of gluten in millet flour. The absence of gluten functionality in pearl millet restricts the high level of incorporation of millet flour in composite bread formula. In fact, gluten, starch and water are major components of bread crumb [12]. Gluten proteins are essential for structure building, as they form a network in the solid matrix and allow gas retention and expansion, which improve the bread volume [12]. To overcome this, EPS-producing LAB

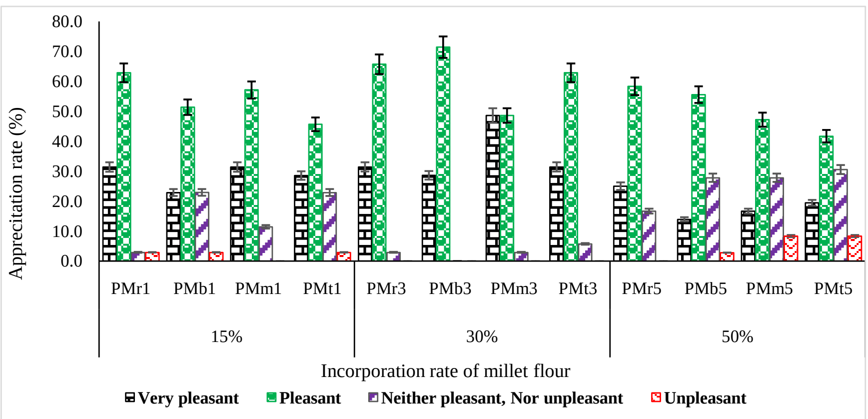


Figure 3. Hedonic characteristics of composite bread made with wheat-millet flour (**PM:** Pearl millet bread; **m:** millet malt; **r:** red sorghum malt; **b:** white sorghum malt; **t:** control (without malt) **1:** 15% incorporation of millet flour; **3:** 30% incorporation of millet flour; **5:** 50% incorporation of millet flour).

(A16) was used to produce EPS during the fermentation of millet dough. In fact, EPS can act as a hydrocolloid in bread making to mimic gluten properties [12] [18]. The flours characteristics obtained from alveograph in this study were superior to those found (P = 54, mmH_2O, L = 36 mm and W = 76 × 10^{-4} J) by [36] who produced bread with the incorporation of 30% sorghum flour.

It also emerged from this study that the pH value decreased significantly ($p < 0.05$) with the increase in the incorporation rate of millet sourdough with an increase in the acidity content. This could be explained by the fact that during the fermentation of millet dough by the LAB strain A16 for the production of EPS, there was acidification leading to a decrease in pH. These results corroborate those of [12] and [25]. Indeed, LAB are well known to produce antimicrobial substances such as organic acids (lactic, acetic, formic and caproic phenolic), carbon dioxide, hydrogen peroxide, ethanol and bacteriocins during fermentation [37]. As the amount of millet sourdough used for substitution of wheat flour increased with the incorporation rate, the titratable acidity increased and the pH decreased. Moisture content decreased with the increasing of incorporation rate, while dry matter content increased. This would probably be due to millet flour which has low water absorption compared to wheat flour [2] [5]. These results corroborate those of [12] who found that replacing wheat flour with 50% unfermented millet flour significantly reduced the Farinograph water absorption. Also, the decrease in moisture content of composite bread could be attributed to denaturation of protein which resulted into more interactions between proteins and polysaccharides through electrostatic forces [38]. This led to intermolecular network, water entrapment of water and lower free water content which is associated with decreased moisture content in foods [38]. The fiber content increased with the incorporation rate of millet flour, in particular at 30%. An explanation of this result is due to the fact that the millet flour was obtained from whole millet grains which are therefore rich in dietary fiber [2]. These results are in line with those of [39], who found a crude fiber content between 1.76% to 2.91% in bread consisting of wheat bread enriched with rice bran. These results are also in line with those of [5] in the production of composite bread based on wheat and millet flour. The millet fibers are also helpful in reducing the risk of gall stones. Food rich in insoluble fibers can speed up the transit of undigested food through the colon and also reduce the secretion of bile acids which prevents the formation of gall stones [40]. Also, as a low glycemic index, insoluble fibers help in the slow releases of carbohydrates into human organism thus proving energy over a longer period and keeping blood glucose in check [41].

The results highlighted also an increase in protein and fat contents. This increase in protein could be explained by the fact that whole grains of millet were used for the production of composite bread. These whole grains of millet are rich in fiber, protein and lipids [2] [41]. While refined wheat flour which is in shelled form is poor in these elements. An increase in the incorporation rate of millet flour in the composite bread increased the protein and fat content. Some authors found that high amount of protein, fiber, fat, amino acids and energy value in millet flour compared to wheat flour which contain less nutritional elements due to removal of bran and germ of the outer seed coat [2] [7] [14], and [41]. However, a decrease in the carbohydrate content related to an increase in the incorporation rate of millet flour was observed. These results could be explained by the fact that whole millet flour is less rich in carbohydrates compared to refined wheat flour, and in addition millet flour was added as a fermented dough. During fermentation, lactic acid bacteria use a part of the carbohydrates to produce lactic acid resulting in a decrease of carbohydrate content and an increase of the acidity of the composite bread. These relative low carbohydrate contents could be an advantage for the consumption of this type of bread by diabetics. Moreover pearl millet is also known for its low glycemic index (GI) which is considered to be important for the management of type 2 diabetes [12] [13]. The results of the energy value are higher than those found by [41] during the production of composite bread of wheat-millet with different incorporation rates (379.55 Kcal/100g) and wheat bread without added millet flour (Normal Bread: 312.7 Kcal/100g). An increase in the content of mineral elements (Fe, K, Na and Mg) was observed in relation to the increase in the rate of incorporation of millet flour except for Zn. Wheat is richer in Zn than millet [42], which explain the decrease in Zn content as a function of the increase in the rate of incorporation of millet flour in composite bread. As for the other mineral elements, bread being produced with whole millet flour, this would explain the fact that the content of these mineral elements increases with the incorporation rate of millet flour. Indeed, pearl millet is rich in dietary fiber, vitamins B complex and minerals [2] [14] [43].

Six types of free amino acids have been found, including three essential amino acids (valine, isoleucine and lysine). The proportions of amino acids in the composite bread were relatively low. This could be due to the fact that pearl millet, like other cereals, has some limitations due to its low content in protein and some essential amino acids, such as lysine [44]. Amino acids are important biological components needed in the human body for biosynthesis, neuro-transmission and other metabolic activities [45]. The sensory evaluation results showed that the color of most of the composite breads was maroon, brown and golden, gradually becoming darker with the increase of millet flour. This darkening could be explained by the effect of heat leading to the billion reaction in the presence of amino acids, reducing sugars and humidity levels during cooking [46]. The main compounds that influenced the aroma of breads are volatile compounds derived from the fermentation and baking stages [36]. The production of EPS in the fermented millet dough improved the texture of the mixed bread, making it slightly crispy and less brittle. Several studies on composite and whole grain breads have confirmed the usefulness of dextran in improving the technological and sensory quality of composite breads [47] [48]. The composite bread obtained with the 30% incorporation was the most appreciated by the panelists because of its texture, its slightly acidic taste and a good feeling in the mouth. Some authors found also that bread with 20% pearl millet flour would be appropriate formulations to use in bakery goods [2].

5. Conclusion

The results found in this study showed that the baking strength of the composite flours decreases considerably with increasing levels of millet flour incorporation. The physico-chemical and sensory characteristics of the composite bread revealed that the use of sorghum and millet malt as natural improvers in bread-making contributed to improve the availability of macronutrients and micronutrients. Overall, the results of the sensory evaluation showed that the composite bread samples presented a good aroma, a good texture, a slightly acidic taste and a pleasant mouthfeel. The 30% incorporation was the most appreciated by the panelists. The production of composite bread based on wheat-millet, using local cereal malts as a natural improver, could be an alternative to replace imported improvers in the production of bread. This could contribute to reducing the importation of wheat and improvers in developing countries and an increase in the income of producers through the increase in the production of local cereals such as millet which will be used in the production of bread to replace wheat.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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