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Fortification with mushroom flour (*Pleurotus ostreatus* (Jacq.) P. Kumm) and substitution of wheat flour by cassava flour in bread-making: Nutritional and technical implications in eastern DR Congo

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Abstract

Background: The import of wheat flour is the major driver for the high prices and low use of bakery products in non-producing tropical countries such as the Democratic Republic of Congo (DRC). These products' high prices worsen the risk of malnutrition and food insecurity among rural and resource-poor populations. This study aimed at substituting the imported wheat flour with less expensive local cassava flour fortified with oyster mushroom flour in order to mitigate the nutritional crisis in the region.

Methodology: Series of experiments were conducted by substituting wheat flour with cassava flour at proportions of 10–25% to find the optimal combination. In addition, oyster mushroom flour (2.5–10%) was added to the composite flour to compensate for nutrient deficiencies of cereals and tuber crops. The overall aim was to identify the optimal wheat–cassava–mushroom combination, improving the nutritional value of breads while keeping their physico-chemical and organoleptic properties.

Results: Results showed that 0–10% mushroom flour increased bread protein from 19.63 to 22.66%. Besides, 7.5% mushroom flour allowed rising the bread calories from 311.8 to 354.5 kcal, and the dry matter from 77.33 to 87.86%. The wheat substitution for cassava fortified with mushroom flour negatively affected the bread volume, color and taste ($p < 0.001$). However, other organoleptic features remained unchanged. The different breads were microbiologically stable for bacteria, but susceptible to fungal attacks.

Conclusion: This study recommended 5–15–80% and 10–10–80% mushroom–cassava–wheat composite flour for better bakery results, good consistency and high protein and energy contents, for improving the nutritional status of populations in the tropical non-wheat producing regions such as DRC. Efforts are necessary to improve the taste and color of the mushroom-fortified bread to increase its uptake and competitiveness in the local markets.

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Keywords: Fortification, Oyster mushroom, Wheat, Cassava, Bread-making, DR Congo

Introduction

Bread has been a part of the human diet for 30,000 years; it provides energy (carbohydrates), essential minerals, dietary fiber and phytochemicals [1, 2]. Cereals, and more particularly wheat, are the most dominant in bakery. However, in most tropical countries like the Democratic Republic of Congo (DRC), the agro-ecological conditions are unsuitable for wheat cultivation [3], and thus, the wheat supply relies almost exclusively on the import [3, 4]. In DRC, the bread is still a luxurious food commodity and unaffordable to most rural and the predominantly resource-poor populations [5]. Indeed, the high cost of imported wheat is the key factor limiting its use in many villages, especially those far from the cities [4]. There is, therefore, a need to promote local resources in bakery and thus availing cheap, energy-balanced and proteinaceous food to these populations. Availing nutritious food products to rural and resource-poor farmers has been regarded as an efficient strategy for combating malnutrition, strengthening food security and boosting economic growth by optimizing human capital productivity [6].

Cassava (*Manihot esculenta* Crantz) is the main staple crop in DRC accounting for more than 70% of the national agricultural production [7, 8]. Cassava adapts in multiple agro-ecological conditions and cropping systems. Besides, it has flexible harvesting dates, tolerance to low soil fertility and high yields compared to cereals and legume crops [7]. Research centers, governmental and non-governmental organizations such as the Institut National d'Etude et Recherche Agronomiques (INERA), the International Institute of Tropical Agriculture (IITA) and the Technical Centre for Agricultural and Rural Cooperation (CTA) conducted research for substituting wheat flour by cassava flour in bakery to cut down the bread price in DRC. Although the cassava flour has become an integral part of the bakery production sector in the country, its final product is deficient in protein, iron and other micronutrients [2, 9]. There is need for its fortification to compensate this nutritional unbalance.

Research on the bakery product fortification mostly focused on legume grains such as beans, soybeans and sesame seeds [10, 11]. The multiple purposes of these crops increase their cost at local markets. Besides, their production is seasonal as they are dependent on the weather conditions such as temperature and rainfall. In this regard, mushroom flour is a valuable alternative

for bakery products' fortification. Mushroom is source of proteins, essential minerals, vitamins and therapeutic properties [12, 13]. It is cheaply produced on agricultural waste and independent from weather influences, and thus, produced throughout the year [14–16]. Oyster mushroom production techniques are already known in eastern DRC and its flour is accessible compared to other fortification products used in other regions [17]. Hence, it is practical and cost effective to incorporate mushroom flour into that of cassava to produce proteinaceous, energetic breads with a high concentration in micronutrients.

This study aimed at establishing optimum wheat–cassava–mushroom composite flour for highly nutritious, available and affordable bakery products in a non-wheat producing country like DRC. It will also help mitigate the high rate of wheat importation and to increase bread use by resource-poor and food insecure populations in rural areas of the South-Kivu Province, eastern DRC.

Materials and methods

Study area

The experimental trials were carried out in various laboratories including the microbiology laboratory of the Université Evangélique en Afrique (UEA/Bukavu), the Congolese Control Office (OCC) and the International Institute of Tropical Agriculture (IITA-Kalambo Station). All these institutions are located in South-Kivu, eastern DRC (Fig. 1).

Materials, formulation of composite flours and bread-making

Cassava flour used in bread-making was produced by IITA/Kalambo, DRC. The process of its production included: harvesting fresh cassava, peeling, washing, cutting, pressing, drying, and molding. AZAM trademark wheat flour imported from Tanzania and available in the local market was used in this study. Edible fungi (*Pleurotus ostreatus* var. P969) was produced by the Mushroom Research Unit of UEA-Bukavu, DRC, following the isolation and fruiting methods described by Mondo et al. [17] and Mushagalusa et al. [14]. Mature mushrooms were picked, dried in an oven and then crushed to a fine flour. The nutritional composition of these flours is presented in Table 1.

Six composite flour formulations were established by adjusting the amount of mushroom, cassava and wheat flours as presented in Table 2. The different formulations were compared with the controls: formulation

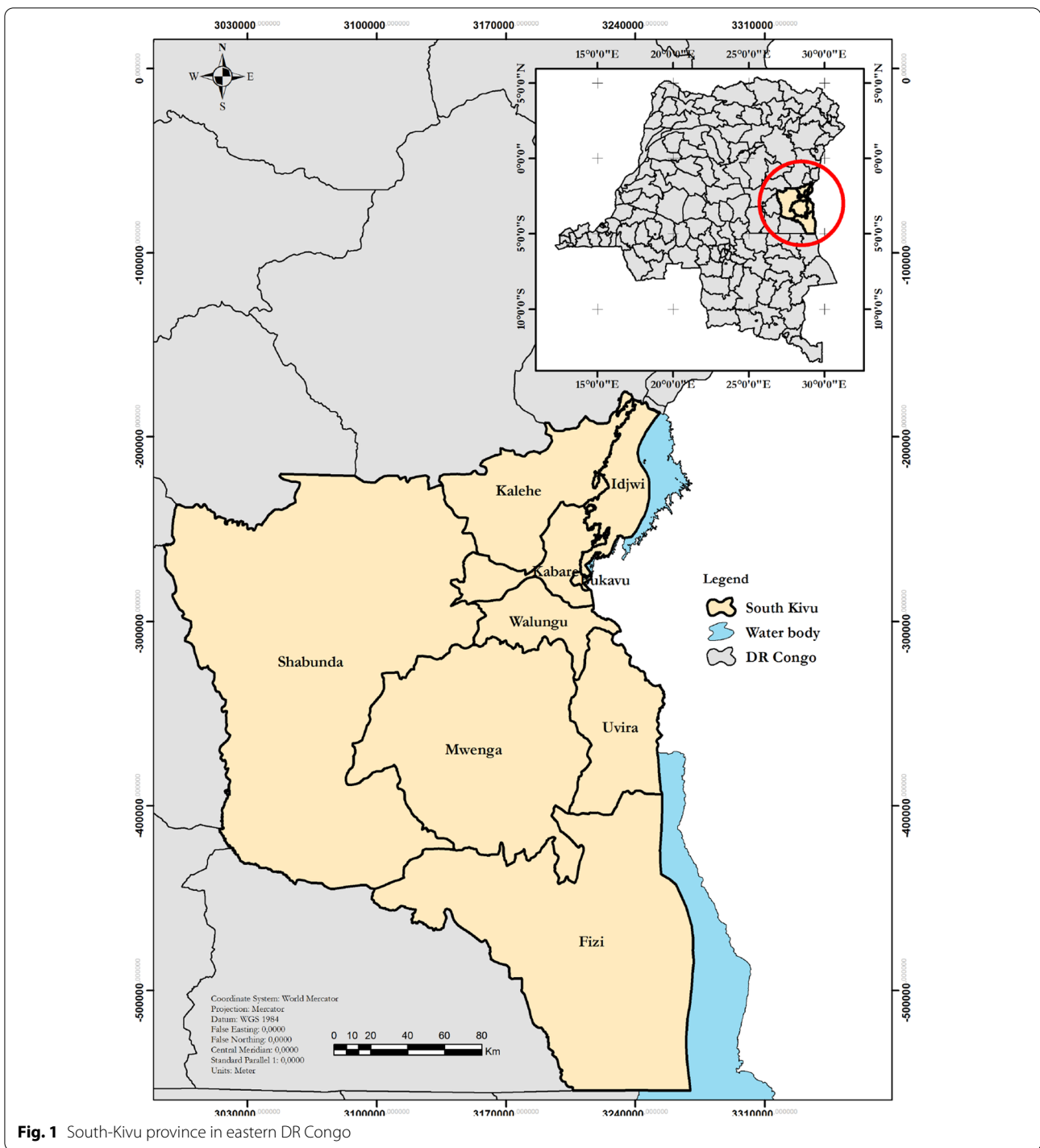


Fig. 1 South-Kivu province in eastern DR Congo

from local bakeries (100% wheat) and that from IITA (80% wheat flour and 20% cassava flour). The other ingredients such as sugar, yeast, salt, oil, etc. were added in equal proportions in all 8 formulations and according to the practices used by local bakeries (Table 3).

This study's experiments took place from February to April 2017 and then repeated from June to July 2018, the period during which the different food crops are harvested, and the residues of which contributed to the local production of mushrooms. Practices for bread-making took into account the protocols from major bakeries (Pain Royal,

Table 1 Physico-chemical compositions of the different flours

Composition	Flour		
	Wheat	Cassava	Mushroom
Humidity (%)	13.9	15.0	89.2
Dry matter (DM) (g kg ⁻¹)	86.1	85.0	10.8
Proteins (g kg ⁻¹)	11.7	3.0	3.3
Fat (g kg ⁻¹)	8.0	0.6	0.4
Carbohydrates (%)	69.3	79.1	6.1
Fiber (%)	3.9	3.9	2.3
Ash (%)	5.6	3.0	–
Saturated fatty acid (g kg ⁻¹)	160.0	160.0	20.0
Total sugar (°Brux)	1.6	3.7	1.1
Energetic value (kcal)	339.6	357.5	33.0
Iron (Fe)(ppm)	89.0	59.0	133.0
Zinc (Zn) (ppm)	70.0	74.0	77.0
Phosphorous (P) (mg kg ⁻¹)	10.6	5.9	12.0
Potash (K) (mg kg ⁻¹)	15.8	59.1	42.0
Sodium (Na) (mg kg ⁻¹)	2.9	30.6	18.0
Magnesium (Mg) (mg kg ⁻¹)	24.8	45.8	18.0
Calcium (Ca) (mg kg ⁻¹)	24.0	34.9	3.0
Vitamin C (mg kg ⁻¹)	–	44.9	3.1

Table 2 Formulation of composite flours used in bread-making

Formulations	Mushroom flour (%)	Cassava flour (%)	Wheat flour (%)
F1	2.5	17.5	80.0
F2	5.0	15.0	80.0
F3	7.5	12.5	80.0
F4	10.0	10.0	80.0
F5	2.5	22.5	75.0
F6	5.0	25.0	70.0
Relative control (IITA) (F7)	0.0	20.0	80.0
Absolute control (local bread) (F8)	0.0	0.0	100.0

Table 3 Quantities of ingredients used for each formulation

Ingredients	Quantity for 25 kg of flour	Quantity for 1 kg of flour
Sugar	1–2 kg	100 g
Yeast	200–250 g	8–10 g
Vegetable oil	1 l	40 g
Water	6 l	240 g
Salt	100 g	4 g

Pain d’Or, Super Pain and Ikya-IITA) of the Bukavu City. After weighing different flours and ingredients, the kneading process of soaking flour with water, kneading it into dough was carried out for 10–12 min in an electronic mixer. The resulting dough was left to rest on a plate for 45 min before being shaped, molded and then placed in a fermentation chamber for 45 min to prepare the dough. The dough was baked in an electric stove at 250 °C for 30 min at moist heat. The bread was taken out of the stove and left to cool at room temperature between 17 and 21 °C. Each 1 kg of composite or simple flour gave 10 breads called “pistolet” and four breads with square shape (pain carré) or 20 breads of “oval” shape (Fig. 2). Thus, for each formulation of composite flour, we produced 20 breads which were allocated as follows: four breads for nutritional and chemical analyses, four for microbiological analyses (storage), and two for physical analysis. The remaining 10 breads were used for sensory/organoleptic evaluation by a randomly selected panel of 60 people. Figure 2 presents the “Pistolet” and “Pain carré” bread types made in this study.

Physico-chemical analyses and assessed bread parameters
Determination of the breads’ shape and volume

The shape of the manufactured breads was oval; the volume was determined by the following formula: $V = (d^2/4) * \pi * E$ with d the bread basis diameter and E the thickness. The specific volume of the bread (cm³/g) was the ratio of the bread volume by its mass (M). The average crust thickness was obtained by the average of the measurements of the upper, the lower and the side crusts.

The breads’ color assessment

Color is a perceptual, subjective attribute, elaborated in the visual system from the light reflected by objects and their environment [18]. We used the diagram of chromaticity and space $L * a * b * CIE$ for assessing bread color in the current study. The breads’ color was determined using a digital colorimeter operating according to the principle of the “CIEL” color space, corresponding approximately to the lightness (L^*), the “red–green” balance (a^*) and the “yellow–blue” balance (b^*) where the colors can be expressed semi-quantitatively thanks to the digital colorimeter.

Dry matter (DM) and moisture content (MC)

The DM content of the bread samples was determined by drying the samples in an oven for 2 h at 105 °C. After drying, the moisture content was determined by the following formula:

$$\text{Moisture content}(\%) = 1 + ((P2 - P3)/(P2 - P1)) \times 100.$$

In this formula, $P1$ is the weight of the empty metal jar; $P2$ the weight of the metal jar containing the sample;

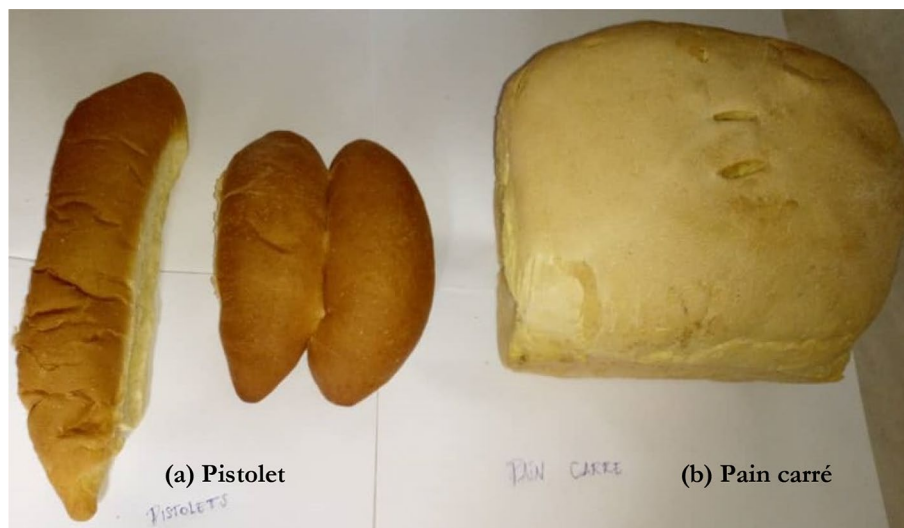


Fig. 2 Produced breads' shapes : **a** "Pistolet" and **b** "Pain carré" as they are referred to in South-Kivu, eastern DR Congo

and P_3 is the weight of the sample after drying and desiccation. The moisture content was estimated as the ratio between the dry weight and the wet weight, expressed in %. After calculating the moisture content, the dry matter was deducted as follows:

$$\text{Dry matter content (\%)} = 100 - \% \text{Water.}$$

Bread ash content and acidity and determination of soluble sugar

The determination of the ash content was by incinerating the samples in a muffle furnace at a temperature of 800 °C for 8 h according to the AOAC [19] recommendations. A 5-g sample of bread was ground, dissolved in 25 ml of distilled water, stirred for 5 min before reading the pH with a Mettler Toledo digital pH-meter. For estimating soluble sugar, a 5-g bread sample was added to 25 ml distilled water. After continuous stirring, the measurement of soluble sugar was determined by a refractometer and values were recorded in Dornic degree.

Proteins, carbohydrate content, fat content and energy value analyses

The protein content was determined by multiplying the bread nitrogen content obtained with the Kjeldahl method by 6.25 [19]. The method consisted of destroying the organic matter contained in the sample under the combined effect of sulphuric acid and a catalyst. In this case, the nitrogen contained in the sample was transformed into ammonium. The ammonia released from this salt was carried away by distillation in the presence of a mixed indicator. Then, the total nitrogen content

obtained was multiplied by a conversion factor of 6.25 to provide the sample protein content. The carbohydrate content was determined using the difference method as recommended by AOAC [19]. It consisted of subtracting moisture, fat (lipids), protein and ash from the sample. In this study, the carbohydrate content was calculated by the following formula:

$$\text{Carbohydrate content (\%)} = 100 - (\text{MC\%} + \text{Fat\%} + P + \text{As\%}).$$

In this formula, MC stands for moisture content, P is the protein and As% is the ash content. The determination of the fat content was done by extraction using the Soxhlet extractor as recommended by AOAC [19]. Total bread's energy value (TEV) was calculated according to the AOAC [19]'s protocol.

Sensory evaluation of breads and microbiological analysis

Prepared breads were evaluated on the basis of color, flavor, aroma and taste. The breads' samples were assessed by 60 randomly selected panelists. This assessment was made on a hedonic scale of four points: (1) very good, (2) good, (3) fairly good, and (4) bad. Three culture media were used, including PCA (plate count agar), MCCA (MacConkey agar) and SDA (Sabouraud dextrose agar) for identifying the different microorganisms that attacked 3-week conserved breads.

Statistical data analysis

Analysis of variance (ANOVA) was used to evaluate differences between breads from different formulations. The separation of means was done by the Tukey HSD test at 5% p -value threshold. A linear model was

used to assess the correlations between the different flour formulations and the carbohydrate, lipid, and protein contents. Sensory evaluation data were analyzed using the principal component analysis (PCA) followed by a hierarchical ascending classification (HAC). The sensory evaluation data were introduced as analyzed variables while the formulation types as supplementary variables. XLSTAT 2014 and R 3.4.3 statistical software were used for data analysis.

Results

Characteristics of different breads produced

The physical appearances of the various breads produced using wheat–cassava–mushroom composite flours are

presented in Fig. 3. There was a significant variation in protein content ($p < 0.001$) among breads from different flour formulations (Table 4). This variation in protein content ranged from 19.6% on the absolute control breads to 22.7% for breads fortified with 10% oyster mushroom flour. The lipid concentration varied with breads' flour compositions ($p < 0.001$). Wheat and cassava-only breads had 1.092 and 1.250% lipid, respectively, while those fortified with oyster mushroom (formulations F2 and F1) reached 1.687 and 1.623% lipid, respectively. There was a proportional increase in energy value with mushroom doses up to 7.5% and, beyond which, the energy value in breads decreased. The oyster mushroom flour positively influenced the protein content ($\gamma = 11.567$

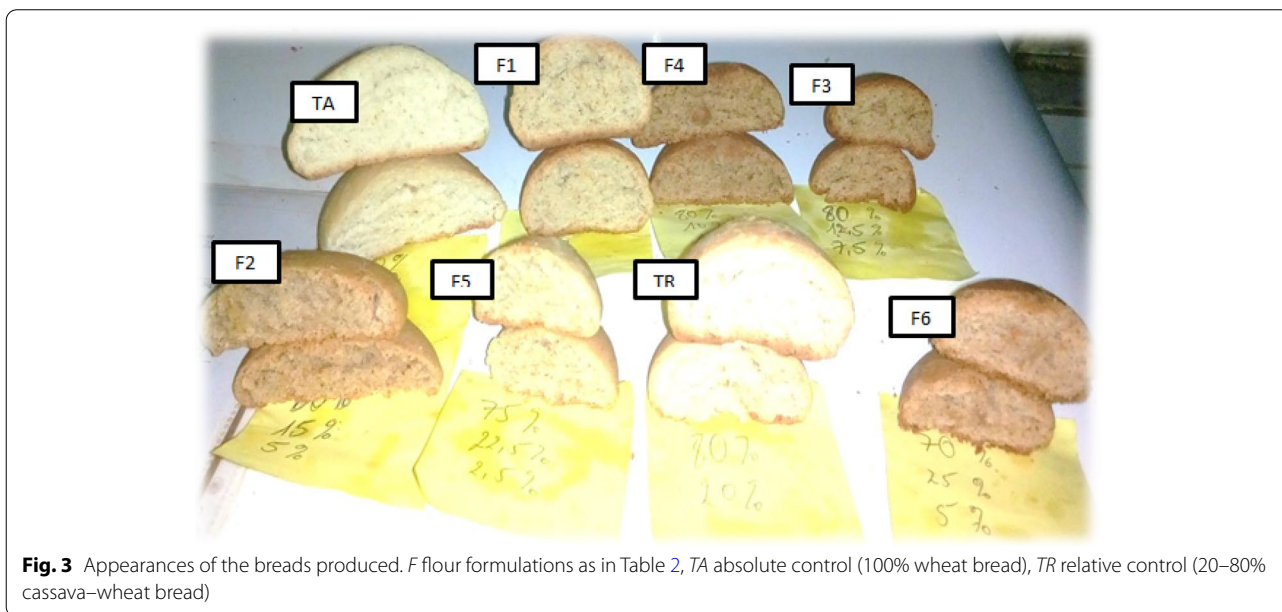


Fig. 3 Appearances of the breads produced. *F* flour formulations as in Table 2, *TA* absolute control (100% wheat bread), *TR* relative control (20–80% cassava–wheat bread)

Table 4 Physico-chemical compositions of the breads produced

Formulations	Lipid (%)	CH (%)	Protein (%)	TEV (Kcal)	Ash (%)	MC (%)	DM (%)	Acidity	TS °Brix
F1	1.623	59.781	20.512	335.778	0.801	17.283	82.717	6.05	3.8
F2	1.687	61.615	21.071	345.932	1.014	14.613	85.387	5.94	3.2
F3	1.492	63.323	21.937	354.471	1.112	12.136	87.864	6.12	3.8
F4	1.556	56.112	22.662	329.096	1.001	18.670	81.330	6.20	3.9
F5	1.302	56.396	22.508	327.335	1.805	17.989	82.011	5.89	4.8
F6	1.127	62.912	19.793	340.962	0.892	15.954	84.046	5.86	4.0
RC (F7)	1.250	54.874	20.793	313.917	0.445	22.638	77.362	6.45	3.6
AC (F8)	1.092	55.858	19.631	311.783	0.752	22.668	77.332	6.03	4.0
Mean	1.391	58.859	21.113	332.410	0.978	17.744	82.256	6.043	3.89
<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.023
CV (%)	16.432	5.861	5.497	4.489	40.128	20.628	4.450	3.072	10.85

MC moisture content, DM dry matter, TEV total energy value (kcal), TS total sugar in Brix degree, F formulation, AC absolute control, RC relative control, CH carbohydrate, CV coefficient of variation, italicized values represent *p*-values at 5% significance threshold

$\ln(x) + 5.4307$; $R^2 = 0.7396$). The dry matter decreased with wheat flour concentration ($y = -0.0065x^2 + 0.9036x + 52.181$; $R^2 = 0.2954$).

Variation in bread specific volume, crust thickness and bread color

Table 5 presents the different physical characteristics of the control and test breads. The specific volume of bread produced varied significantly with the different flour formulations ($p < 0.001$). It was found that the more cassava and oyster mushroom flours were added, the more the breads' specific volume decreased. However, F1 (2.375) and F2 (3.03) had a higher specific volume than the relative control (F7: 2.211) but lower than the absolute control bread (F8: 3.528). The crust thickness of the breads proportionally increased with the amount of non-bread/cassava flour. The colorimetric analysis of the breads showed that any incorporation of the composite flour had a significant effect on the color of the breads produced. The flour composition's influence was highly significant on the lightness and red–green balance of the breads produced. A high lightness was observed on F2 (40.6) compared to the two controls. Only the values of F3 (21) and F6 (23) were significantly different from the others and showed low lightness.

Sensory appreciation of breads obtained from the different formulations tested

The bread color and taste varied with flour formulations used for bread-making (Table 6). The other descriptors showed no significant difference. Based on color (Fig. 4), all the breads produced were rated from “good to very

Table 6 Descriptive and sensory evaluation of the breads produced

Formulations	Color	Aroma	Taste	Crumb	Flavor
F1	1.45 _b	1.76 _a	1.52 _c	1.86 _a	1.62 _a
F2	1.62 _{ab}	2.24 _a	1.90 _{bc}	2.14 _a	1.83 _a
F3	1.62 _{ab}	2.55 _a	2.76 _a	2.07 _a	2.03 _a
F4	1.97 _{ab}	2.07 _a	2.10 _{abc}	2.17 _a	1.69 _a
F5	1.55 _{ab}	1.79 _a	1.79 _{bc}	1.86 _a	1.62 _a
F6	1.48 _{ab}	2.24 _a	2.07 _{abc}	1.93 _a	2.14 _a
RC (F7)	2.03 _{ab}	2.10 _a	2.41 _{ab}	2.07 _a	2.24 _a
AC (F8)	2.21 _a	2.14 _a	2.38 _{ab}	2.31 _a	2.24 _a
F-test	2.849	1.874	4.693	0.703	2.391
<i>p</i> -value	0.001***	0.075	0.001***	0.669	0.522

Scale: 1: very good, 2: good, 3: fairly good, 4: poor. *F* formulation, *RC* relative control, *AC* absolute control

***Highly significant. The values in each column with different letters are statistically different at the 5% *p*-value threshold according to the Tukey HSD test. Italicized values represent *p*-values at 5% significance threshold

good” except breads F1 and F6 which were “good”. For the taste, bread F3 was rated “fairly good” while breads F1, F2 and F5 were “good to very good”.

Produced breads were classified into three classes with 60% degree of similarity (Fig. 5). The first class comprised the breads from the formulations RC/F7 and AC/F8 (two controls); the second class had F2 and F4 while the third class contained F3, F5, F1 and F6. On the other hand, at 40% similarity, we had two classes: Group 1 had the two control breads while Group 2 had all the composite flours' breads. These different appreciations were linked to the oyster mushroom flour effects on the color and taste qualities.

Table 5 Physical parameters of the breads produced

Flour formulations	Specific volume (cm ³ /g)	Average crust thickness (cm ³)	Color		
			<i>L</i> *	<i>a</i> *	<i>b</i> *
F1	2.375 _{ab}	0.566 _{ab}	33.2 _{ab}	26.3 _{bcd}	19.8 _{bcd}
F2	3.037 _a	0.6 _{ab}	40.6 _a	24.6 _{cd}	15.4 _d
F3	1.620 _b	0.566 _{ab}	21.5 _b	17.1 _d	36.1 _{ab}
F4	2.381 _{ab}	0.7 _a	30.1 _{ab}	21.2 _{cd}	13.6 _d
F5	2.344 _{ab}	0.6 _{ab}	32.2 _{ab}	35.1 _{abc}	18.9 _{cd}
F6	1.514 _b	0.5 _{ab}	23.2 _b	22.8 _{cd}	40.3 _a
RC (F7)	2.211 _{ab}	0.433 _b	34.7 _{ab}	43.3 _b	32.2 _{abc}
AC (F8)	3.528 _a	0.333 _b	31.9 _{ab}	40.7 _{ab}	23.2 _{bcd}
F-value	6.99	50.11	6.45	5.43	4.95
<i>p</i> -value	< 0.001	< 0.001	0.0014	0.0159	0.0251
Tukey HSD	1.4	0.241	15.01	14.94	16.46

The values in each column followed by different letters are statistically different at the 5% *p*-value threshold according to the Tukey HSD test. Italicized values represent *p*-values at 5% significance threshold

*L** lightness, *a** balance “red–green”, *b** balance “yellow–blue”. *F* formulation, *RC* relative control, *AC* absolute control

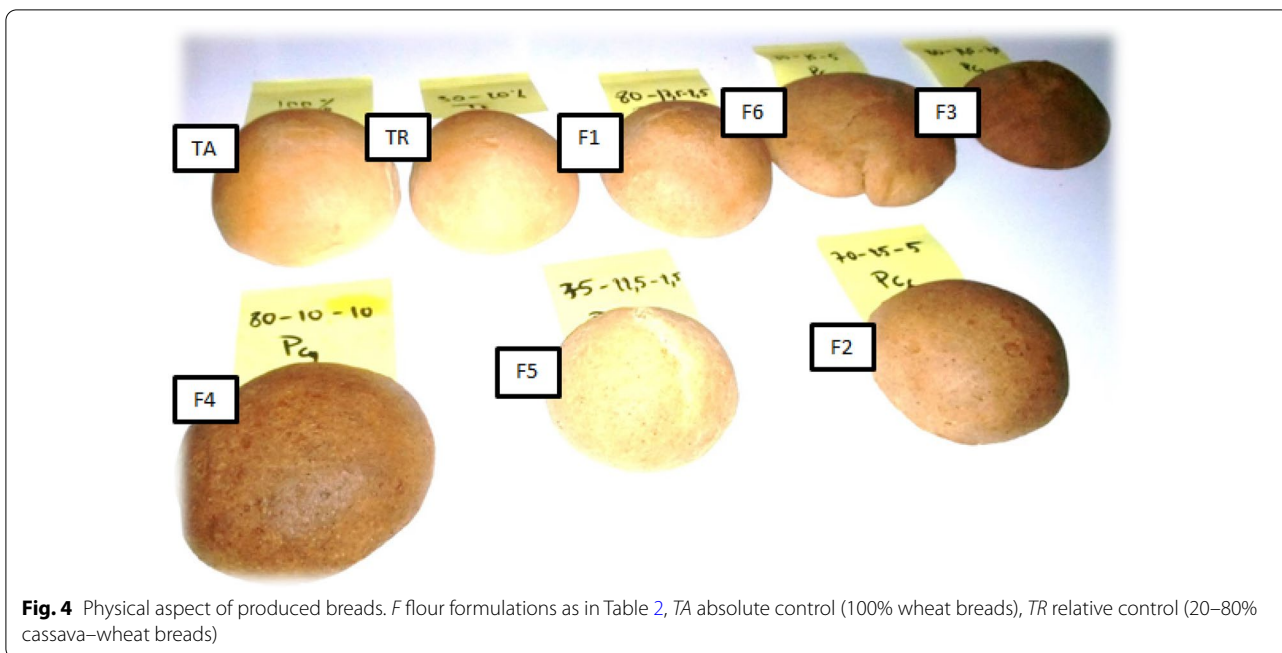


Fig. 4 Physical aspect of produced breads. *F* flour formulations as in Table 2, *TA* absolute control (100% wheat breads), *TR* relative control (20–80% cassava–wheat breads)

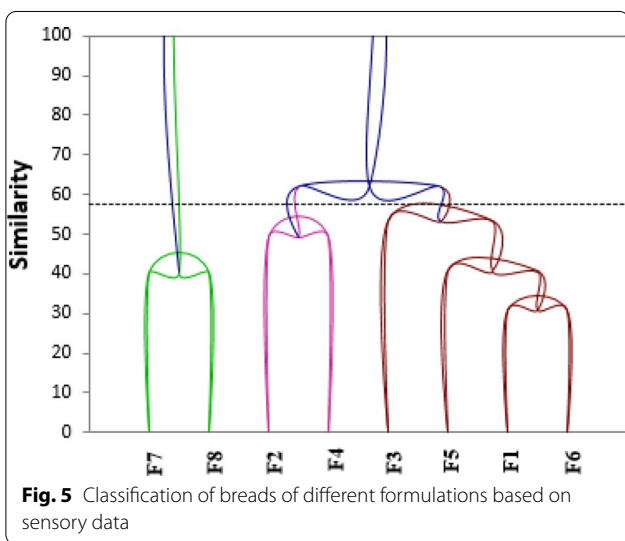


Fig. 5 Classification of breads of different formulations based on sensory data

Table 7 Microbiological stability analysis of breads produced

Formulations	SDA ^a	PCA (colonies)	MCC (<i>Bacillus</i>)
F1	Yeast	> 300	0
F2	Yeast	> 300	0
F3	Yeast	80	0
F4	Yeast	0	0
F5	Yeast	> 300	0
F6	Yeast	0	0
RC (F7)	Yeast	> 300	0
AC (F8)	Yeast	> 300	0

SDA Sabouraud dextrose agar, PCA plate count agar, MCC MacConkey agar. *F* formulation, *RC* relative control, *AC* absolute control

^a Yeast: fungi shape, > 300 colonies: high colony concentration

Microbiological analyses of the breads produced

After a 3-week storage, we noticed a deterioration of the breads by yeast fungus after a culture on SDA medium. The same result was confirmed by colony count on the PCA culture medium. No Gram-positive bacilli including Enterobacteriaceae were identified on the MacConkey agar (MCC) (Table 7). We noted that the MCC culture medium conventionally used in the diagnosis of *Enterobacterium bacilli* showed no presence of such microorganisms on stored breads. In conclusion, the breads produced were microbiologically stable for bacteria while susceptible to the effects of yeast fungi.

Discussion

Adding cassava flour as a substitute to wheat and oyster mushroom flour as a fortification supplement significantly improved the breads’ chemical composition. These results supported those found by Diallo et al. [20] showing improved chemical composition of breads when wheat flour was substituted with Faba bean flour. From our results, the protein content of the F4 (22.66%) at 10% mushroom fortification was higher than that of Risasi [21] while using sweet potato flour (9%) and that found by Meite et al. [22] who used *Citrullus lanatus* (20%). This difference could be explained by the high protein content in oyster mushroom flour, which

generally varies from 19 to 38% [23]. However, Akdowa [1] found that breads rich in protein could be made using wheat-based composite flours combined with taro and gum at proportions of 79.8, 17.6 and 2.6%, respectively, compared to 100% wheat breads. Diallo et al. [20] suggested a fortification based on Voandzou flour at a rate of 20%; this rate allowed an increase in protein in the end-products from 12.96 to 20.66%. The breads produced in this study were not only rich in protein, but also in total energy value (TEV), the latter increased from 311.78 to 354.47 kcal. Thus, the fortification of breads improved the product TEV as also reported by El-Soukary [24] using *Cucurbita moschata* seeds. Abdel-Kader [10] combining wheat flour with broad beans (*Vicia faba*) and sesame (*Sesamum indicum*) flours reached the same conclusions. However, this oyster mushroom-based fortification slightly increased TEV when compared to values reported by Diallo et al. [20]. As for the energy, breads' carbohydrate, ash and dry matter values varied with the substitution and fortification rates; optimum being set at 12.5% cassava flour and 7.5% oyster mushroom flour.

Ghorai et al. [25] and Akdowa [1] showed that rich crops in terms of nutrients could qualitatively improve bakery products. In our case, for example, the bread specific volumes were comparable to those reported by Dhingra and Jood [11] and Svec and Hruskova [26] after substituting wheat flour by soybean flour. We also observed that the higher cassava and/or mushroom flours were added to the bakery flour, the lower the bread specific volumes. This could be explained by the reduced structure of the wheat and a low capacity of the dough to trap air. Thus, the increase in the quantity of protein-rich flours in bread-making directly influenced the baking quality of different composite flours [2, 27]. However, Vittadini and Vodovotz [28] reported that adding soybean flour to bread lowered the bread volume compared to breads made of 100% wheat flour. Hence, fortification of bread with oyster mushroom flour resulted in consistent breads with medium sizes.

As for the sensory aspects, a highly significant difference between the breads was observed in terms of color and taste as also reported by Eissa et al. [29], Okafor et al. [27] and Majeed et al. [30]. Bread rating decreased for taste and color when adding the mushroom flour. Okafor et al. [27] showed that the higher the mushroom content, the lower the taste and color appreciation scores would be. As for the crumb, aroma and flavor, no significant differences were observed among produced breads regardless of the flour composition. This result agreed with that of Majeed et al. [30] as their study showed that incorporating mushroom flour in bakery production had not influenced these traits' assessment

scores. Efforts are, therefore, necessary to improve the taste and color of mushroom-fortified breads to increase its uptake and competitiveness in the local markets.

This study recommended the following oyster mushroom–cassava–wheat formulations: 5–15–80 and 10–10–80% as they provided breads with good consistency, high protein and energy values. In fact, 5–15–80% formulation breads had highest energy values, highest specific volume and an overall high organoleptic acceptability. On the other hand, 10–10–80% breads had the highest protein content, high specific volume (second to 5–15–80%), and they were the most stable microbiologically with an overall high organoleptic score. We can, therefore, conclude that better formulations are those with 80% wheat flour and 5–10% mushroom flour. The choice of mushroom concentrations will, however, depend on baking objectives: 5–7.5% formulations are highly energetic while 7.5–10% are highly proteinaceous.

In South-Kivu, eastern DRC, food insecurity and malnutrition among children under 5 years and women, especially pregnant women, is one of the major public health problems. It is often associated with high infant mortality rates. Some reports have even indicated that in South-Kivu rural areas, severe malnutrition (translated by low weight-for-height, also referred to as wasting) affected 7.8% of children, and while the province had an infant mortality rate of 125/1000 [31]. Despite actions undertaken by the governmental, international and local non-governmental organizations (NGOs) to improve the nutritional status among vulnerable groups, much effort is yet to be deployed. On the other hand, most of the South-Kivu food is imported from neighboring countries (Rwanda, Burundi and Uganda) and this even for products for which the region holds some comparative advantages. In 2014, ~13% of rural households were food insecure, and most of the nutritional insecurity status is linked to nutrient deficiencies among consumed products and food shortages [31]. As wheat is not locally produced in the province, this study results suggested that substituting wheat for cassava enriched with mushroom flour holds potential in alleviating both food quality and quantity deficiencies among vulnerable groups as supported by Azeez et al. [32]. There was significant improvement in the bread protein content and nutritional quality when adding mushroom powder in bread-making. Okafor et al. [27] reported 21.60–83.66% crude protein content increase in fortified bread samples. Also, more than 50% increase in the ash and crude fiber contents were achieved through fortification. The protein increase will allow reducing nutritional issues among resource-poor and rural populations of eastern DRC and other tropical areas of the world sharing similar

struggles. In fact, Santeramo and Shabnam [6] recommended that availing highly nutritious food products to rural and resource-poor farmers is an efficient strategy for combating malnutrition, strengthening food security and boosting economic growth by optimizing human capital productivity.

Conclusions

Bread-making trials showed that incorporating cassava flour fortified with *Pleurotus ostreatus* mushroom flour in the dough formulation had significantly affected its nutritional composition. The nutritional composition and sensory aspects confirmed that incorporating up to 10% oyster mushroom flour in the bread-making is optimum for producing breads that are not only rich in protein, but also have a total energy value (TEV) close to that of locally made and acceptable breads. Mixing local crop flours such as cassava and oyster mushroom flour in wheat for bread-making could, therefore, help improve the nutritional status of the South-Kivu populations, in eastern DRC. This study, therefore, suggests incorporating oyster mushroom flour up to a rate of 5–10% for a good balance of nutritional composition because beyond this, the TEV and dry matter (DM) decrease proportionally. It turns out that the following oyster mushroom–cassava–wheat formulations: 5–15–80 and 10–10–80% yield breads with good consistency, high protein and energy value, and thus, should be promoted.

Abbreviations

TEV: Total energy value; DRC: Democratic Republic of Congo; DM: Dry matter; SDA: Sabouraud dextrose agar; PCA: Plate count agar; MCC: MacConkey agar; IITA: International Institute of Tropical Agriculture; INERA: Institut National pour l'Etude et la Recherche Agronomique; CTA: The Technical Centre for Agricultural and Rural Cooperation ACP-EU; HSD: Honestly significant differences of means; PCA: Principal component analysis; UEA: Université Evangélique en Afrique.

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Authors' contributions

PNI, AMM and TZL conducted laboratory analysis and sensory survey. GBC and JMM conceived and coordinated the study, did the statistical analysis and drafted the manuscript. PKZ, SM, AZB and GNM supervised the research and helped revising the draft manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors want to declare that they can submit the data at whatever time based on your request. The data used for the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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