International Journal of Food Properties, 13: 562–573, 2010

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942910802713172

GLUTEN-FREE BREAD MAKING TRIALS FROM CASSAVA (*MANIHOT ESCULENTA* CRANTZ) FLOUR AND SENSORY EVALUATION OF THE FINAL PRODUCT

Antonella Pasqualone[1], Francesco Caponio[1], Carmine Summo[1], Vito Michele Paradiso[1], Gabriella Bottega[2], and Maria Ambrogina Pagani[2]

[1]*University of Bari, Dipartimento di Progettazione e Gestione dei Sistemi Agro-Zootecnici e Forestali (PROGESA), Sezione di Industrie Agro-Alimentari, Bari, Italy*
[2]*University of Milano, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), Sezione di Industrie Alimentari, Milano, Italy*

The aim of this work was to evaluate the effectiveness of the use of cassava flour in bread making, and the sensory acceptability of the final product. Different baking trials were carried out by using egg white and extra-virgin olive oil, in consideration of their high nutritional value with respect to other food additives (i.e., hydrocolloids). Significant (p < 0.05) improvements of loaf specific volume (from 2.24 to 3.93 mL/g) and crumb firmness (from 9.14 to 4.67 N) were achieved by contemporarily including egg white and extra-virgin olive oil in the formulation. Cassava breads containing both these ingredients obtained the best scores from panelists for all the test breads examined and resulted attractive as the wheat bread prepared as reference.

Keywords: ***Cassava, Gluten-free bread, Celiac disease, Sensory properties.***

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, also known as manioc or yucca) is one of the leading food and feed plants in the world: it ranks fourth among staple crops, with a global production of about 160 million tons per year.[1] Most of this is grown in three regions, West Africa, and the Congo basin, tropical South America, South, and Southeast Asia,[2] while in Western countries it is not commonly used. Because of the presence of cyanoglucosides (linamarin and lotaustralin), a great number of recent studies have reported many biotechnological approaches to improve the safety and quality of cassava flour,[3-8] and the effect of the different processing modalities of the tuberous roots on the level of these toxic substances and functional properties has been assessed.[9-11]

Received 2 April 2008; accepted 25 December 2008.
Address correspondence to Antonella Pasqualone, University of Bari, Dipartimento di Progettazione e Gestione dei Sistemi Agro-Zootecnici e Forestali (PROGESA), Sezione di Industrie Agro-Alimentari, Via Amendola 165/A, 70126, Bari, Italy. E-mail: antonella.pasqualone@agr.uniba.it

Cassava is a mainly starchy raw material and contains no gluten, so it is suitable in case of celiac disease. Celiac patients suffer from a life-long intolerance to the prolamins of wheat and other cereals, and the only effective treatment is a strict adherence to a gluten-free diet throughout life. At present, many gluten-free foodstuffs are currently produced, but some problems still exist regarding bread making. In fact, many commercially available gluten-free breads are inferior in quality to their gluten-containing counterparts.[12]

In cassava growing areas, bread obtained from this flour is a flat, crunchy product similar to crackers, the "bread of the tropics." Examples of this are present in Nigeria, the Dominican Republic, Haiti, and some regions of the Amazon jungle.[13] The preparation of cassava loaf bread, more similar to conventional crumby bread than the flat type, could give a palatable product for celiac consumers in Western countries. The effect of partial substitution of wheat by cassava flour in making loaf bread has been extensively investigated by many authors at various replacement levels: 5–15%,[14] 10–40%,[15,16] 15–30%,[17] and 20–50%.[18] More recently, Miyazaki et al.[19] studied the effect of native or chemically modified cassava starches, used at the level of 18%, on wheat bread firming, while Ahlborn et al.[20] analyzed gluten-free composite breads obtained from rice flour with small amounts of cassava flour and potato starch.

Furthermore, the production of gluten-free loaf bread derived solely from cassava flour, without blending it with wheat flour, has been the object of various researches. All of them indicated the need of using various additives, due to the total lack of gluten. Some studies[21-23] pointed out the positive effect of emulsifiers, such as glyceryl monostearate, to improve air incorporation during mixing and gas retention during fermentation, and prehydrated extruded starch to increase batter viscosity. Other studies[24,25] evaluated the effect of combinations of xanthan gum, margarine, and egg white, indicating that also these ingredients increased the amount of air entrapped in the cassava batters at the mixing stages. Moreover, the fortification of cassava flour with 20% soy flour (natural or defatted) has been reported by all the studies[21-25] as fundamental, due to its high protein content, for increasing the nutritional value of the product, although this could pone a problem for consumers disliking the possible use of genetically modified soy.[26]

In this frame, the aim of this work was to evaluate the effectiveness of the use of cassava flour in bread making by adding only egg white and/or extra-virgin olive oil, in consideration of their high nutritional value, without the help of any hydrocolloid or industrial improver, and in absence of fortification with soy flour. For this purpose, different baking trials were carried out and the sensory acceptability of the final product was assessed.

MATERIAL AND METHODS

Flours

Cassava flour was obtained from the experimental farm of Ali Mobarek Horticulture Research Station (Behera Governorate, Egypt), courtesy of Dr. Reda Abdalla Abdelaziz. Fresh tubers, harvested 12 months after planting, were peeled, washed, sliced to 4-mm thickness and pressed to squeeze out as much of the juice as possible, then dried for 8 hours at 60°C according to Nambisan and Sundaresan[10] to thermally inactivate the cyanogenic glucosides. The dry chips were ground with a disk crusher (Retsch, Haan, Germany), and the resulting flour was sifted through a coarse sieve to separate out the fibers. Wheat flour (dry gluten 8.9 g/100 g dry solids; gluten index 55; P/L 0.4; W 172 × 10^{-4} J) was purchased at a local retailer.

Chemical Analyses of Flours

Moisture content and proteins were determined according to AACC methods 44-15A and 46-11A[27], respectively. Damaged starch content was assessed according to the AACC method 76-31, using the "Starch Damage Assay kit" (Megazyme International Ltd., Wicklow, Ireland).

Pasting Characteristics of Flours

Starch properties were determined using a Brabender micro-viscoamylograph (Brabender Instruments, Duisburg, Germany), according to AACC approved methods 22–10 and 61–01[27], suspending 15 g flour (14.0% moisture basis) in 100 ml of distilled water. The instrument was programmed to perform a cycle of heating and cooling, beginning at 30°C and reaching 95°C, holding this temperature for 30 min, then cooling to 50°C and maintaining this temperature for 30 min, finally cooling to 30°C. The heating/cooling rate during the whole cycle was 1.5°C/min, with continuous stirring rate at 250 rpm. The viscosity values at the peak (peak viscosity, PV), at the end of the holding period at 95°C (minimum viscosity, MV), and at the end of cooling period to 30°C (cooling maximum viscosity, CMV) were detected. The differences between PV and MV, and between CMV and PV were evaluated as breakdown and setback, respectively.

Bread-Making Trials

Breads were prepared at a local bakery using cassava flour, either with or without adding extra-virgin olive oil and egg white. A reference bread was made from 100% wheat flour. Five types of bread were obtained: wheat bread (WB), cassava bread (CB), cassava bread with oil (CBO), cassava bread with egg white (CBE), and cassava bread with oil and egg white (CBOE). Detailed formulations of the breads are reported in Table 1, based on 100 g flour. The amount of water used in the different types of dough was

Table 1 Formulations of the bread loaves (g per 100 g flour)

	Type of bread				
Ingredients	WB	CB	CBO	CBE	CBOE
Bread wheat flour	100	—	—	—	—
Cassava flour	—	100	100	100	100
Fresh compressed yeast	2.5	2.5	2.5	2.5	2.5
Sucrose	9	9	9	9	9
NaCl	2	2	2	2	2
Extra-virgin olive oil	—	—	6	-	6
Egg white	—	—	—	40	40
Water	70	120	120	100	100

Note: WB = wheat bread; CB = cassava bread; CBO = cassava bread with oil; CBE = cassava bread with egg white; CBOE = cassava bread with oil and egg white.

assessed based on the experience of the baker to achieve similar consistency. The ingredients were mixed with a spiral kneader for 10 min. The batters were poured into paper baking pans, proofed for 30 min at 35°C, then baked at 200°C for 30 min. Eight loaves of 250 g were prepared for each type of bread. Two independent bread-making trials were performed.

Physical and Chemical Analyses of Breads

The bread loaves were analyzed two hours after baking. Specific volume was determined by rapeseed displacement, as in AACC method 10-10.[27] The protein and moisture contents of the crumb were determined according to AACC methods 46-11A and 44-15A, respectively.[27] Crumb firmness was measured on a Universal Testing Machine Galdabini (Varese, Italy), according to the AACC method 74-09.[27] Firmness was evaluated as the force, expressed in Newton (N), compressing the centre of a 25 mm height slice to 25% compression.

Sensory Analysis of Breads

A quantitative descriptive analysis of the sensory properties was carried out according to ISO standard no. 13299[28] by eight panelists (4 male, 4 female, age range 24 to 40 years), previously selected over a group of 16 people for their reliability, consistency, and discriminating ability. After a cycle of lectures on the basic notions of sensory analysis and some tests to monitor the performance in recognizing the basic tastes and odors, the selection involved to pass a test consisting in profiling also two equal bread samples in two different sessions, without anyone knowing. Prior to actual sample evaluation, the selected panelists were trained via two pre-test sessions held to familiarize with the vocabulary regarding the descriptors of cassava bread, and the intensity range of the attributes in testing cassava bread. The list of sensory terms included eight descriptors, plus overall acceptability, rated on an anchored line scale that provided a 0–9 score range. In Table 2,

Table 2 Descriptive terms used for sensory profiling the bread samples.

		Scale anchors	
Descriptor	Definition	min (0)	Max (9)
Crumb color	Color tone and intensity of crumb	Whitish	Grayish-brown
Crust thickness	Crust depth	Very thin	Very thick
Crumb moisture	Amount of moisture perceived by fingers at the surface of crumb and in the mouth during chewing	Very dry	Very humid, sticky
Crumb homogeneity	Homogeneity of internal structure	Heterogeneous	Homogeneous
Crumb cohesiveness	The way the crumb reacts when broken by fingers and held together when masticated	Poorly cohesive, it crumbles	Very cohesive, it sticks
Crumb consistency	Consistency of crumb evaluated by fingers and during chewing	Soft	Tough
Cassava odor	The typical odor of cassava flour	Weak, tends to sweet	Strong, tends to bitter and pungent
Fresh bread odor	The typical odor of fresh bread	Weak	Strong
Overall acceptability	The sum of the palatable characteristics of texture and taste	Low	High

the definitions of each descriptor and their scale anchors are reported. Descriptors of appearance (crumb color, crust thickness), visual-tactile and chewing characteristics (crumb moisture, homogeneity, cohesiveness, and consistency), and odor (cassava, fresh bread odor) were considered. Their choice was mainly based on current literature about cassava bread[24,25] as well as taking in account previous researches by the same authors about sensory properties of bread.[29,30] Samples were presented in coded dishes as slices 1-cm thick taken from the centre of the loaves. At each sensory session, the slices deriving from eight different loaves of each type of bread were randomly distributed to the eight panelists. The samples were distributed simultaneously and anonymously, coded using a three-digit number, to all panel members. Two sessions were carried out at a distance of 15 days—one after each bread-making trial. Sensory evaluations took place in a room where temporary partitions were used to set up isolated sensory tasting booths for separating panelists during analysis, and were performed at room temperature (20°C), according to ISO standard no. 8589[31]. Booths (width 70 cm, depth 60 cm) had white walls (height 60 cm). White lamps were used to enhance light quality.

Statistical Analysis

Analysis of variance (ANOVA), principal component analysis (PCA), and clusterization were performed using XLSTAT (Addinsoft, New York, NY, USA) to compare the experimental data.

RESULTS AND DISCUSSION

Chemical and Pasting Properties of Flour

The chemical characteristics of the flours (Table 3) evidenced that protein content of cassava flour was 1.4 g/100 g dry solids, so that it could be considered practically an entirely starchy raw material, while wheat flour showed a protein content of 10.7 g/100 g dry solids. The pasting characteristics of flours were evaluated because, particularly in absence of gluten as in cassava flour, it was essential to verify if the starchy fraction could assure a suitable consistency during the baking process. The gel from cassava flour showed the remarkably high

Table 3 Chemical and viscoamylographic characteristics of cassava and wheat flour samples.

Determination	Type of flour	
	Cassava	Wheat
Moisture content (%)	10.0	11.9
Protein (g/100 g dry solids)	1.4	10.7
Damaged starch (g/100 g dry solids)	3.0	6.2
Pasting temperature at peak (°C)	72.7	87.8
Peak viscosity (BU)	1184	394
Minimum viscosity (BU)	163	212
Cooling maximum viscosity (BU)	673	1126
Breakdown (BU)	1021	182
Setback (BU)	–511	732

BU = Brabender Units.

viscosity of 1184 Brabender Units (BU) (peak value) at the temperature of 72.7°C. This value encountered a sharp fall to the minimum of 163 BU (with breakdown of 1021 BU), when the gel was held at 95°C for 30 min. This trend indicated a rapid swelling of the native starch granules, but their inability to retain the swollen structure at cooking temperature and under stirring for a prolonged period. During subsequent cooling, the viscosity moderately increased, reaching the maximum value of 673 BU, thus indicating a low tendency to starch retrogradation, with a setback of –511 BU. The observed low gelatinization temperature and the high peak viscosity of cassava flour were in accordance with the findings of other authors.[23,32,33]

In the case of wheat flour, on the contrary, peak viscosity was much lower (394 BU) and was reached at a higher temperature (87.8°C) than in cassava, indicating a lesser extent of starch swelling, probably due to a more compact granule structure. Otherwise, the gel from wheat flour showed a minor decrease in viscosity, with a remarkably lower breakdown value (182 BU instead of 1021 BU). After cooling, the viscosity of the gel from wheat flour was higher (1126 BU) than that obtainable from cassava, indicating a firmer structure and a greater tendency to retrogradation of starch.

The pasting properties of the wheat flour were slightly different from those found by other authors at the same concentrations,[19] and more similar to those observed at lower concentrations,[34] probably due to the occurrence of starch granule damaging (damaged starch was found in an amount of 6.2 g/100 g dry solids), that showed a lowering effect on paste viscosity. It is well known that damaged starch shows a higher water absorbing capacity and a higher sensibility to α-amylase, with a consequent decrease in the maximum consistency detected by the Brabender amylograph.[35] The observed differences between cassava and wheat flour justify the main use of cassava starch in the food industry as a thickening agent to increase the viscosity of food products.[36]

Bread Characteristics

Table 4 reports the chemical and physical characteristics of the bread samples. As expected, the use of pure cassava flour resulted in a low bread-making potential, because the absence of gluten caused a marked reduction of the viscoelastic properties, and the pasting properties observed in this flour were not adequate to ensure sufficient standing attitude during baking. As a consequence, the processing of the cassava flour involved handling problems during both mixing and baking, particularly in the absence of egg white or oil. In these conditions, the batter did not show sufficient cohesive properties and tended to disaggregate and separate in different phases.

Table 4 Chemical and physical characteristics of bread samples and results of the statistical analysis at $p < 0.05$.

	Type of bread				
Determination	WB	CB	CBO	CBE	CBOE
Specific volume (mL/g)	4.04^{a}	2.24^{b}	2.75^{bc}	3.09^{c}	3.93^{a}
Protein content (g/100 g dry solids)	10.2^{a}	1.3^{b}	1.3^{b}	5.4^{c}	5.3^{c}
Crumb moisture (%)	31.7^{a}	53.3^{b}	52.4^{b}	37.0^{a}	35.8^{a}
Crumb firmness (N)	4.13^{a}	9.14^{b}	7.87^{c}	5.27^{d}	4.67^{ad}

[a-d]Different superscript letters among columns indicate significant differences. WB = 100% wheat bread; CB = 100% cassava bread; CBO = cassava bread with oil; CBE = cassava bread with egg white; and CBOE = cassava bread with oil and egg white.

Table 5 Sensory characteristics of bread samples and results of the statistical analysis at $p<0.05$.

	Type of bread				
Descriptor	WB	CB	CBO	CBE	CBOE
Crumb color	3.2^{a}	6.7^{b}	5.0^{c}	3.9^{ac}	3.3^{a}
Crust thickness	1.9^{a}	0.9^{b}	1.2^{bc}	1.4^{abc}	1.6^{ac}
Crumb moisture	6.7^{a}	8.9^{b}	8.6^{bc}	7.6^{abc}	7.3^{ac}
Crumb homogeneity	8.8^{a}	3.9^{b}	5.0^{b}	7.7^{a}	8.5^{a}
Crumb cohesiveness	7.1^{a}	8.8^{b}	7.8^{ab}	7.5^{ab}	7.3^{a}
Crumb consistency	4.5^{a}	7.7^{b}	6.9^{b}	6.8^{b}	4.7^{a}
Cassava odor	0.0^{a}	4.1^{b}	1.8^{c}	2.8^{d}	1.7^{c}
Fresh bread odor	7.8^{a}	0.1^{b}	2.0^{c}	6.2^{d}	7.8^{a}
Overall acceptability	8.7^{a}	0.7^{b}	4.2^{c}	5.1^{c}	8.4^{a}

[a-d]Different superscript letters among columns indicate significant differences. WB = wheat bread; CB = cassava bread; CBO = cassava bread with oil; CBE = cassava bread with egg white; and CBOE = cassava bread with oil and egg white.

Due to the dough's inability to retain CO_2, with consequent breakdown of the loaf structure, the specific volume was found to be extremely low in the CB samples, (accounting for 2.24 mL/g). Miyazaki et al.[19] observed a reduction of loaf specific volume due to the addition of 20% tapioca native starch to common bread formulation. The specific volume of the WB prepared under the same conditions as the reference was significantly higher (4.04 mL/g, $p < 0.05$). With reference to crumb moisture, the CB was notably more moist than the WB and showed a heterogeneous crumb structure (Table 5) with a gummy texture, as also observed by Eggleston et al.[24]

The lack of a regular alveolate structure in the CB crumb justifies its greater firmness in comparing with the WB crumb, in agreement with the results obtained by Defloor et al.[21] Similar problems were encountered by Ahlborn et al.[20] in evaluating gluten-free breads made from rice flour, which consistency was four times higher than in standard wheat bread. Firmness depends on the geometry of the crumb bubbles that, in time, is related to the components of the cell lamellae. In the case of common bread, the components of the crumb cells are composed of gelatinized starch dispersed in a continuous gluten matrix, while in gluten-free cassava bread only externally added proteins (soy flour, milk proteins, egg white, etc.) and hydrocolloids can form the matrix required for adequate gas retaining. All the pasting data on cassava flour (Table 3) indicated that starch granules could swell considerably and disperse during baking.

Egg white was added to the batter to obtain the formation of a continuous solid phase suitable for effectively retaining gases in a gluten-free product. This ingredient is capable of improving the coherence between starch granules, increasing the stability of the dough. In fact, albumins are reported to form a relatively stable aerated foam structure, potentially suitable for stabilizing other dough ingredients including lipids, without the addition of hydrocolloids. During the subsequent baking, the egg protein network coagulated, fixing the loaf shape.[24] Moreover, the addition of egg assured a higher protein content, rising from 1.3 g/100 g dry solids in CB to 5.4 g/100 g dry solids in CBE, and 5.3 g/100 g dry solids in CBOE ($p < 0.05$).

The breads containing egg white showed a significant ($p < 0.05$) improvement of loaf specific volume with respect to the corresponding samples prepared without this ingredient (from 2.24 mL/g of CB to 3.09 mL/g of CBE, and from 2.75 mL/g of CBO to

3.93 mL/g of CBOE). As expected, the positive effect on specific volumes corresponded to a softening of the crumb, with significantly lower ($p < 0.05$) firmness values of the CBE and CBOE samples compared to those of the CB and CBO, respectively. The use of egg white made it possible to use less water when forming the dough, so that moisture content significant decreased ($p < 0.05$) in the CBE and CBOE samples with respect to the CB sample. In particular, the CBE and CBOE appeared to be as moist as the reference WB.

The role of extra-virgin olive oil as improver was also investigated. This kind of oil, produced from olive fruits solely by mechanical processes, was chosen among various possible oils and fats for its high nutritional quality and its salutary features.[37,38] In addition, the balanced ratio SFA:MUFA:PUFA in extra-virgin olive oil, and the presence of various antioxidant substances (phenolic compounds, tocopherols, carotenoids), made it more suitable than refined seed oils to undergo the temperatures of bread baking.

The addition of extra-virgin olive oil alone did not lead to a significant increase of loaf volume, in agreement with Smith and Johansson.[39] On the contrary, in the presence of egg white, an enhanced stabilizing effect was observed, promoting a higher specific volume, as shown in Table 4. This parameter rose from 3.09 mL/g of CBE to 3.93 mL/g of CBOE ($p < 0.05$). The addition of olive oil did not show any influence on the moisture content, so that the moisture content of the CB was not significantly different to the CBO sample ($p < 0.05$).

Regarding the sensory attributes, eight descriptors were considered and overall acceptability was scored. The descriptors defined the appearance of the bread (crumb color, and crust thickness), the visual-tactile and chewing characteristics (crumb moisture, crumb homogeneity, crumb cohesiveness, and crumb consistency), and the aroma (cassava odor and fresh bread odor).

The results of the assessment of the sensory attributes (Table 5) were in agreement with those of the chemical and physical analyses, but also gave some additional information about color and aroma. The CB samples, as previously indicated by the values of specific volume and crumb firmness, showed an unappealingly stiff consistency with a gummy texture, more sticky in the central zone, and a significantly ($p < 0.05$) poorer internal homogeneity than WB. The whole loaf appeared heavy and very moist, with no formation of an acceptable crumb.

Preliminary trials carried out to set up the best working conditions indicated that these negative characteristics were even more pronounced (especially with a tougher consistency) when using lower amounts of water and/or higher baking temperatures. A tacky texture had already been observed by other authors when employing chemically modified tapioca starches.[19] The addition of egg white promoted an improvement of all the sensory parameters with respect to the CB, but the best results were observed in the CBOE samples, which also contained extra-virgin olive oil. They showed a thin crust and a homogeneous crumb structure.

Regarding crumb color, the CB showed a significantly grayer tone compared to all the other breads ($p < 0.05$). These findings confirmed the results of Ciacco and D'Appolonia,[14] who observed that the presence of increasing amounts of cassava flour in bread formulation led to a grayer crumb. In the presence of egg white (CBE and CBOE samples), a less lucid and wet appearance of the crumb was observed, significantly fading the grayish tone of CB bread. These results agreed with those of other authors in case of fortified cassava-soy breads, where egg white improved crumb color.[24] Also with regard to aroma, the bread showed to be more agreeable when both extra-virgin olive oil and egg white were included in the batter. Cassava flour conferred a distinctive flavor, unusual and different

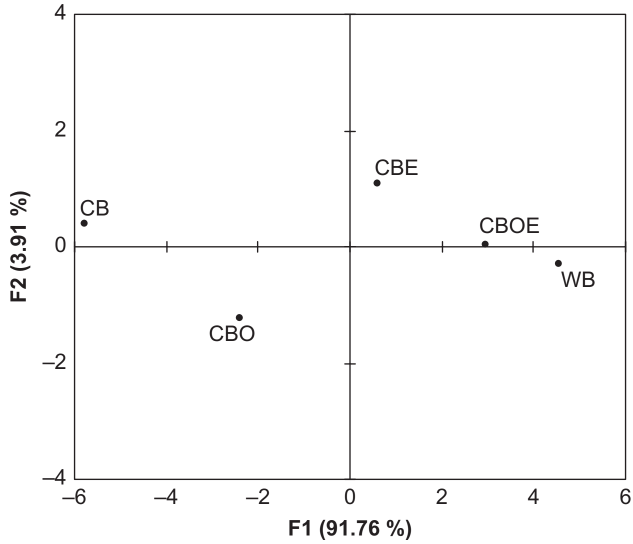

Figure 1 Score plot of the first two principal components. (WB = wheat bread; CB = cassava bread; CBO = cassava bread with oil; CBE = cassava bread with egg white; CBOE = cassava bread with oil and egg white).

from that of wheat bread, which was evident only in the CB sample. The addition of extra-virgin olive oil, as well as that of egg white, significantly reduced the distinctive cassava odor and flavor ($p < 0.05$). The CBOE bread gave an intense fresh bread aroma, statistically not dissimilar to that of the WB.

From the cassava breads, the CBOE was scored with a mean overall acceptability of 8.4. This value was quite similar to that attributed to the WB (8.7) and did not differ statistically from it, while the CB had the least preferable sensory qualities, with an overall acceptability of 0.7. The experimental results were subjected to PCA. The score plot of the first two principal components shows that the first component (PC1) accounted for more than 91% of the variability (Fig. 1). This indicates that all the variables were highly correlated and that egg white and olive oil had the effect of rendering the cassava bread more similar to WB, without conferring proper characteristics. The distribution of the samples was better evidenced by the dissimilarity dendrogram of Euclidean distances (Fig. 2) that clearly indicated the grouping of CBOE and WB samples together, quite distant from all the remnant breads.

CONCLUSIONS

A significant improvement of the sensory characteristics of gluten-free bread was achieved by contemporarily adding egg white and extra-virgin olive oil to the cassava bread formulation. The breads developed containing both these ingredients showed an improved loaf volume with a softer texture, a more regular crumb structure and reduced gumminess, compared with pure cassava bread. As a consequence, they obtained the best scores from panelists and resulted as attractive as wheat bread. These results demonstrated that nutritious and palatable gluten-free cassava breads, suitable for the diet of celiac patients, can be produced by using egg white and extra-virgin olive oil, even in the absence of hydrocolloids and industrial improvers.

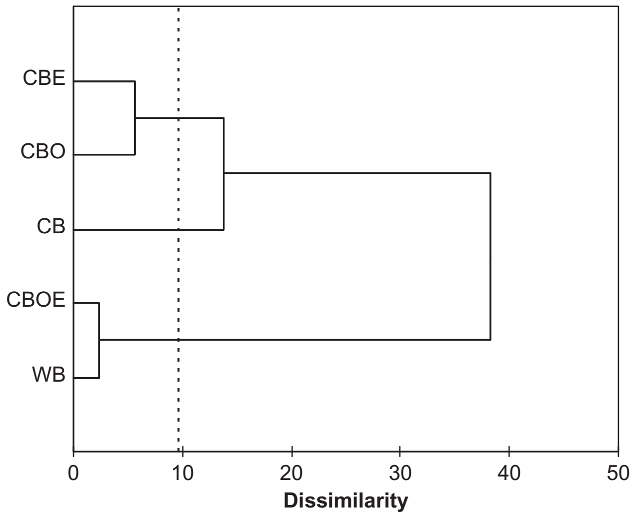

Figure 2 Dissimilarity dendrogram of the bread samples. (WB = wheat bread; CB = cassava bread; CBO = cassava bread with oil; CBE = cassava bread with egg white; CBOE = cassava bread with oil and egg white).

REFERENCES

1. Lawrence, J.H.; Moore, L.M. United States Department of Agriculture Plant Guide. Cassava, Manihot esculenta Crantz. USDA: Washington, DC, 2005, http://plants.usda.gov/plantguide/doc/pg_maes.doc (accessed March 24, 2008).
2. Kawano, K. Thirty years of cassava breeding for productivity – Biological and social factors for success. Crop Science. **2003**, *43*, 1325–1335.
3. Salehuzzaman, S.N.I.M.; Jacobsen, E.; Visser, R.G.F. Isolation and characterisation of a cDNA encoding granule-bound starch synthase in cassava (*Manihot esculenta* Crantz) and its antisense expression in potato. Plant Molecular Biology **1993**, *23*, 947–962.
4. Koch, B.M.; Sibbesen, O.; Swain, E.; Kahn, R.A.; Liangcheng, D.; Bak, S.; Halkier, B.A.; Moller, B.L. Possible use of a biotechnological approach to optimise and regulate the content and distribution of cyanogenic glucosides in cassava to increase food safety. Acta Horticulturae **1994**, *375*, 45–60.
5. Luong, H.T.; Shewry, P.R.; Lazzeri, P.A. Transient gene expression in cassava somatic embryos by tissue electroporation. Plant Science **1995**, *107*, 105–115.
6. Santana, M.A.; Vásquez, V.; Matehus, J.; Aldao, R.A. Linamarase expression in cassava cultivars with roots of low- and high-cyanide content. Plant Physiology **2002**, *129*, 1686–1694.
7. Shittu, T.A.; Sanni, L.O.; Awonorin, S.O.; Maziya-Dixon, B.; Dixon, A. Use of multivariate techniques in studying the flour making properties of some CMD resistant cassava clones. Food Chemistry **2007**, *101*, 1606–1615.
8. Onitilo, M.O.; Sanni, L.O.; Oyewole, O.B.; Maziya-Dixon, B. Physicochemical and functional properties of sour starches from different cassava varieties. International Journal of Food Properties **2007**, *10*, 607–620.
9. Cooke, R.D., Maduagwu, E.N. The effects of simple processing on the cyanide content of cassava chips. Journal of Food Technology **1978**, *13*, 299–306.
10. Nambisan, B.; Sundaresan, S. Effect of processing on the cyanoglucoside content of cassava. Journal of Science of Food and Agriculture **1985**, *36*, 1197–1203.

11. Udensi, E. A.; Ukozor, A. U. C.; Ekwu, F. C. Effect of fermentation, blanching, and drying temperature on the functional and chemical properties of cassava flour. International Journal of Food Properties **2005**, *8*, 171–177.
12. Gallagher, E.; Kunkel, A.; Gormley, T.R.; Arendt, E.K. The effect of diary and rice powder addition on loaf and crumb characteristics, and on shelf life (intermediate and long-term) of gluten-free breads stored in a modified atmosphere. European Food Research and Technology **2003**, *218*, 44–48.
13. Food and Agriculture Organization (FAO) of the United Nations. Cassava bread. In Roots and Tubers Processing Toolkit; FAO: Rome, Italy, 2002, http://www.fao.org/inpho/content/food_proc_tlkt/Roots/gbread.htm
14. Ciacco, C.F.; D'Appolonia, B.L. Baking studies with cassava and yam. II. Rheological and baking studies of tuber-wheat flour blends. Cereal Chemistry **1978**, *55*, 423–435.
15. Almazan, A.M. Effect of cassava flour variety and concentration on bread loaf quality. Cereal Chemistry **1990**, *67*, 97–99.
16. Eggleston, G.; Omoaka, P.E.; Arowshegbe, A.U. Flour, starch and composite breadmaking quality of various cassava clones. Journal of the Science of Food and Agriculture **1993**, *62*, 49–59.
17. Defloor, I.; Nys, M.; Delcour, J.A. Wheat starch, cassava starch, and cassava flour impairment of the bread-making potential of wheat flour. Cereal Chemistry **1993**, *70*, 526–530.
18. Khalil, A.H.; Mansour, E.H.; Dawoud, F.M. Influence of malt on rheological and baking properties of wheat-cassava composite flours. Lebensmittel-Wissenschaft und Technologie **2000**, *33*, 159–164.
19. Miyazaki, M.; Maeda, T.; Morita, N. Starch retrogradation and firming of bread containing hydroxypropylated, acetylated, and phosphorylated cross-linked tapioca starches for wheat flour. Cereal Chemistry **2005**, *82*, 639–644.
20. Ahlborn, G.J.; Pike, O.A.; Hendrix, S.B.; Hess, W.H.; Huber, C.S. Sensory, mechanical and microscopic evaluation of staling in low-protein and gluten-free breads. Cereal Chemistry **2005**, *82*, 328–335.
21. Defloor, I.; De Geest, C.; Schellekens, M.; Martens, A.; Delcour, J.A. Emulsifiers and/or extruded starch in the production of breads from cassava. Cereal Chemistry **1991**, *68*, 323–327.
22. Defloor, I.; Leijskens, R.; Bokanga, M.; Delcour, J.A. Impact of genotype and crop age on the breadmaking and physico-chemical properties of flour produced from cassava (*Manihot esculenta* Crantz) planted in the dry season. Journal of Science of Food and Agriculture **1994**, *66*, 193–202.
23. Defloor, I.; Leijskens, R.; Bokanga, M.; Delcour, J.A. Impact of genotype, crop age and planting season on the breadmaking and gelatinisation properties of cassava (*Manihot esculenta* Crantz) flour. Journal of Science of Food and Agriculture **1995**, *68*, 167–174.
24. Eggleston, G.; Omoaka, P.E.; Ihedioha, D.O. Development and evaluation of products from cassava flour as new alternatives to wheaten breads. Journal of Science of Food and Agriculture **1992**, *59*, 377–385.
25. Eggleston, G.; Omoaka, P.E.; Arowshegbe, A.U. Flour, starch and alternative (wheatless) breadmaking quality of various cassava clones. Journal of Science of Food and Agriculture **1993**, *62*, 61–66.
26. Costa-Font, M.; Gil, J.M.; Traill, W. Consumer acceptance, valuation of and attitudes towards genetically modified food: review and implications for food policy. Food Policy **2008**, *33*, 99–111.
27. American Association of Cereal Chemists. AACC International Approved Methods of Analysis. AACC Press: St Paul, MN, 2000.
28. International Organization for Standardization (ISO). *Sensory Analysis. Methodology. General Guidance for Establishing a Sensory Profile*, Standard ISO no. 13299; ISO: Geneva, Switzerland, **2003**, 1–24.
29. Raffo, A.; Pasqualone, A.; Sinesio, F.; Paoletti, F.; Quaglia, G.; Simeone, R. Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura bread. European Food Research and Technology **2003**, *218*, 49–55.

30. Pasqualone, A.; Summo, C.; Bilancia, M.T.; Caponio, F. Variation of the sensory profile of durum wheat Altamura PDO (Protected Designation of Origin) bread during staling. Journal of Food Science **2007**, *72*, 191–196.
31. International Organization for Standardization (ISO). *Sensory Analysis. General Guidance for the Design of Test Rooms*, Standard ISO no. 8589; ISO: Geneva, Switzerland, **2007**,1–16.
32. Raja, K.C.M.; Ramakrishna, S.V. Compositional and pasting characteristics of plain-dried and parboiled cassava (*Manihot esculenta* Crantz). Food Chemistry **1990**, *38*, 79–88.
33. Moorthy, S.N. Physicochemical and functional properties of tropical tuber starches: a review. Starch-Stärke **2002**, *54*, 559–592.
34. Vansteelandt, J.; Delcour, J.A. Characterisation of starch from durum wheat (*Triticum durum*). Starch-Stärke **1999**, *51*, 73–80.
35. Farrand, E.A. Flour properties in relation to the modern bread processes in the United Kingdom, with special reference to alpha-amylase and starch damage. Cereal Chemistry **1964**, *41*, 98–111.
36. Balagopalan, C.; Padmaja, G.; Nanda, S.K.; Moorthy, S.N. *Cassava in food, feed, and industry*; CRC Press: Boca Raton, FL, **1988**, 1–205.
37. Manna, C.; Galletti, P.; Cucciolla, V.; Moltedo, O.; Leone, A.; Zappia, V. The protective effect of the olive oil phenol (3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. Journal of Nutrition **1997**, *127*, 286–292.
38. Visoli, F.; Galli, C. Olive oil phenols and their potential effects on human health. Journal of Agricultural and Food Chemistry **1998**, *46*, 4292–4296.
39. Smith, P.R.; Johansson, J. Influences of the proportion of solid fat in a shortening on loaf volume and staling of bread. Journal of Food Processing and Preservation **2004**, *28*, 359–367.