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Cultivars and Sampling Regions Influence on Cassava Roots and Their Fermented Flours Characteristics

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Abstract: This study was carried out to check the influence of some factors (cultivars and sampling regions) on the physicochemical characteristics of two energizing products: cassava roots and derivative fermented flours. Cassava roots of three local cultivars were drawn from ten different regions of Cote d'Ivoire (Ivory Coast) and processed into fermented flours. These both products characteristics were significantly (P<0.05) affected by cultivars and sampling regions. The effects of the interaction cultivars-regions were also shown to be significant. These factors induced differences about physicochemical characteristics between the studied products.

Key words: Influence, cultivars, sampling regions, cassava roots, fermented flours, physicochemical characteristics

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is mainly cultivated for its roots which constitute an important calories source for many people in the world[1]. Despite of its importance as foodstuffs in many developing countries, the agricultural development policies often ignored it[2]. It could be, therefore, interesting to promote this important culture.

Once harvested, cassava roots are subject to rapid deterioration and must be consumed or treated quickly. Their processing is one of the solutions to this problem. Indeed, it leads to pleasant and healthy product that can be stored for a relative long time. Many derivative products of cassava roots exist. However, few of them are objects of important commercial transactions in Africa, particularly in Ivory Coast. This tendency could change, if competitive products that can be used in modern or traditional food industry are conceived.

For this purpose, we propose the processing of roots into fermented flour which can be used in bread making [3-5] or to reconstitute well known traditional meal like *placali*, a gelatinized and fermented cassava product. The use of fermented flour could decrease the cereal importations in developing countries and valorise the *placali* making. But, before choosing a cultivar for processing it into flour, it is important to know if differences about physicochemical characteristics exist between cultivars and what cultivar to use. Several authors have reported that several factors (e.g. soil composition, rainfall, cultivars, planting moment, cultural practices, regions…) have significant effects on roots characteristics [6-12].

This study aims to investigate, via chemometrics methods, the effects of different cultivars and sampling regions on chemical composition of cassava roots and its derivative fermented flours in order to determine their suitable food uses.

MATERIALS AND METHODS

Sampling: Fresh roots of three local cassava cultivars (Akaman, Yace and Zoklo) were used in this study. These roots were from Ivory Coast ten different regions, *i.e.* Lac (lac), Nzi comoe (N), Marahoue (mar), Sud-Bandaman (SB), Fromager (F), Lagune (lg), Agneby (A), Haut-Sassandra (HS), Moyen-Cavally (mcav) and Bas-Sassandra (BS).

In a region, three roots samples per cultivar were extracted. In total, 90 roots samples were collected. Each sample is separated in two sets:

- the first one, is used to determine physicochemical parameters of fresh roots and,
- the second set is processed into fermented flour.

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Process of Cassava Roots into Fermented Flour: The fermented flour process is summarised in Figure 1. Fresh roots were peeled with a knife and cut into large longitudinal pieces. These pieces were ground and a cassava inoculum was added at 8% dough [13]. The mass was packed into bags and let for three days at ambient temperature (25-28°C) without any compression. The fermented dough was then removed and squeezed. When the dough was sufficiently pressed, it was oven-dried for 48 hours at 55°C. The dried product was ground and sieved with a 200 µm mesh sieve to obtain the fermented flour.

Physicochemical Analyses: Moisture, ash, starch, proteins and lipids contents were determined using BIPEA method [14]. Total acidity and pH were obtained using method described by Dufour *et al*. [4]. Cyanide content was determined by FAO method [15]. Total carbohydrates and total sugars contents were quantified respectively by methods of Dubois *et al*. and Bertrand [16]. Atwater and Rosa coefficients were used to calculate caloric energy [17]. Minerals such as Ca, Mg, Zn and Fe were quantified by Atomic Absorption Spectrometer and by Spectrophotometer for P, after samples digestion using IITA method [18].

Chemometrics Methods: Statistical analysis was performed using cluster analysis and multidimensional analysis of variance (MANOVA). Whereas, the former consist in grouping identical individuals into sets, the later deals with comparing different means in order to identify if they are different or equal.

In this study, cluster analysis was based on Ward's method as agglomerate one. This method defines a class as a group in which the variance between members is relatively small and seems to create equal classes [19]. The square Euclidean distance was the chosen metric as recommended by Johnson and Wichern [19]. The influence of cultivar and region on the physicochemical parameters of the products was checked via a multidimensional analysis of variance (MANOVA). The MANOVA is a generalization of analysis of variance (ANOVA) method to one or several factors (qualitative variables) in which two or several dependent variables are measured simultaneously [20]. The MANOVA enables to examine the main effects and factors interaction. The statistics used is the Wilks lambda which indicates the part of variance not explained by factor considered. It varies from 0 to 1. Lower is its value, more significant is the effect of the factor considered.

In addition, Principal Components Analysis (PCA) was performed when needed. The PCA is a linear dimensionality reduction technique, which identifies orthogonal directions of maximum variance in the original data, and projects the data into a lower-dimensionality space formed of a sub-set of the highest-variance components [19, 21].

RESULTS AND DISCUSSION

Physicochemical Characteristics of Roots: Table 1 presents the results of cassava roots samples chemical analyses. Moisture content of cassava roots is 62.37 ± 3.62%; situated between 60 and 65% [22]. The high moisture content of roots is at the origin of their rapid deterioration once harvested. Total carbohydrates content on a dry weight basis is 93.01 ± 1.07%. Woolfe has reported carbohydrates contents ranged from 91% to 94.1% [6]. Starch content on dry weight basis is 75.77 ± 3.37%. Therefore, starch is the main carbohydrates of the roots. On the other hand, the roots are very poor in lipids and proteins with respective contents of 0.66 ± 0.28% and 2.32 ± 0.48%. This extreme poverty of the roots has been reported by Benesi [23].

In these roots, the pH value is 6.57 ± 0.23 and the total acidity reaches the value of 3.28 ± 1.89 meq/100 g on dry weight basis. This pH value is in agreement with earlier findings [23, 24]. Ash content (2.61 ± 0.5% on dry matter) is close to 2.49% when total sugars content (2.44 ± 0.35%) is lower than 5.65% [24]. The cyanide content (61.58 ± 36.95 mg/kg on fresh matter) is situated between 15 and 400 mg/kg [24].

The consumption of these roots brings 387.38 ± 2.65 kcal/100 g (dry matter) as caloric energy. Therefore, the roots constitute an energizing food. Indeed, the consumption of one kilogram of these roots can cover the daily energy needs of an adult man that is 3050 kcal [1].

Cassava roots also, contain minerals like phosphorus (121.84 ± 48.62 mg/100 g), magnesium (71.74 ± 33.81 mg/100 g), iron (7.85 ± 4.5 mg/100 g), zinc (3.99 ± 2.9 mg/100g) and calcium (112.45 ± 66.39 mg/100 g). The roots phosphorus content is relatively lower than 150 mg /100g when calcium and magnesium contents were in agreement with respective values of 130 mg/100 g and 140 mg/100 g on dry weight basis found by Oke [25]. Whereas iron content of studied roots is higher than 1.8 mg/100, zinc content is close to 2.4 mg/100 g [25].

The ratio of calcium on phosphorus, 0.88 ± 0.26, is situated between 0.5 and 2, indicating a good absorption of these two elements [26].

The analysis of the relative standard deviation (RSD) from table 1 shows that the moisture presents low variation from one root to the other (RSD=5.81%). It is the case of several other characteristics *i.e.* starch (RSD=4.45%), total carbohydrates (RSD=1.15%), energy (RSD=0.89%), pH (RSD=3.59%) and total sugars (RSD=14.37%). But considering ash (RSD=19.15%), proteins (RSD=20.64%) and the ratio Ca/P (RSD=30.94%), it appears that their RSD are relatively higher. Moreover, cyanide content varies considerably (RSD=59.99%) from a root to the other. Its mean value (61.58 ± 36.95 mg/kg) is not, therefore, really representative of a root to the other. Other parameters RSD vary in the same mood (*i.e.* total acidity (RSD=57.75%), lipids (RSD=41.97%) and the minerals like phosphorus (RSD=39.91%), magnesium (RSD=47.13%), iron (RSD=57.42%), zinc (RSD=52.54%) and calcium (RSD=59.04%)).

Physicochemical Characteristics of Fermented Flours: Fermented flours contain low moisture content (8.92 ± 1.33%) (Table 2). This content is in agreement with manufacturing standards of Codex Alimentarius [27] for the edible cassava flours. According to these standards, flours must have moisture content lower than 13%. The low moisture content of fermented flours inhibits the multiplication of alteration microorganism; what it is interesting for their conservation for a relative long period. Proteins content is 1.51 ± 0.21%. This value is higher than that of 0.82% found by Grace [28] for the same product. The flours obtained are poorer in lipids (0.38 ± 0.13%) like roots samples. The pH value of these flours is acid (4.19 ± 0.13). This low pH can prevent from pathogenic microorganisms multiplication [29, 30]. Low pH values associated to low moisture content contribute to increase the stability and therefore the shelf life of cassava fermented flours. The non toxicity is reinforced by the low cyanide content (0.44 mg/kg). This content is in agreement with the findings of Sant’ana *et al.* [31] who published values between 0.08 and 4.16 mg/kg. But, the herein cyanide value is lower than that of Ernesto *et al*. and Cumbana *et al*. whose values range from 26 to 186 mg/kg [32, 33]. In the other hand, cyanide and ash (1.27 ± 0.17%) contents are in agreement with the standards (< 10 mg/kg and 3%) [27].

Cassava fermented flours are rich in total carbohydrates (95.66 ± 0.73% on dry weight basis) with 81.53 ± 3.92% of starch. This confirms earlier report that starch is the cassava major constituent [34]. In these flours, the total sugars content is 1.74 ± 0.24%. They are energizing foods with 391.74 ± 2.61 kcal/100 g; which is superior to the one (361.17 kcal/100 g) found by Grace [28].

The calcium (74.82 ± 33.53 mg/100 g) and phosphorus contents (97.09 ± 33.56 mg/ 100 g) lead to a ratio of 0.75 ± 0.2. This value close to 0.7, which is the optimal absorption value of these two minerals, shows that they will be efficiently absorbed. Cassava fermented flours contain also magnesium (44.88 ± 16 mg /100 g), zinc (3.21 ± 1.67 mg/100 g) and iron (5.04 ± 2.63 mg/100 g on dry weight basis).

Concerning the RSD values (Table 2), it appears that some physicochemical parameters (*i.e.* lipids (34.85%), acidity (18.39%), zinc (52.65%), iron (52.32%), calcium (45.25%), phosphorus (34.85%) and magnesium (34.93%)) vary significantly from one flour to the other.

Roots and Flours Characteristics Comparison: Figure 2 presents the results of PCA analysis of roots and flours characteristics. According to this figure, two subsets can be easily distinguished. The first one is composed by roots and the second by flours. This regrouping is the sign of existence of physicochemical differences between roots and derivative flours. Indeed, it appears that some parameters such as moisture, pH, total acidity, cyanide, ash, starch and carbohydrates contents were significantly affected by processing. For instance, the moisture content changes from 62.37 ± 3.62% in fresh roots, to 8.92 ± 1.33% in fermented flours. The decrease of 85% is due to unit operations of manufacture process such as oven-drying, fermentation and squeezing. The role of fermentation in moisture content changes could be explained by the ability of some microorganisms to break down cassava tissue. Their activity led to, important liberation of water by tissue [35]. The pH value in the roots (6.57 ± 0.23) decreases significantly until 4.19 ± 0.13 in the fermented flours whereas total acidity raises from 3.28 ± 1.89 meq/100 g in roots to 12.33 ± 2.26 meq/100 g in fermented flours. These results are due to roots carbohydrates broken down, during fermentation, by some microorganisms to produce organic acids that decrease the pH value and increase therefore the acidity in flours.

Ash content is also affected by processing. It changes from 2.61 ± 0.5% in roots to 1.27 ± 0.17% in fermented flours, due certainly to unit operations like fermentation, squeezing and sieving [36]. Cyanide content decreases considerably during roots processing. It changes from 61.58 ± 36.95 mg/kg in roots to 0.44 mg/kg in flours. This important decrease (about 99%) is due to the fact that fermentation process reduces the cyanogenic potentials of roots to safe levels [31]. This confirms earlier reports that the fermentation is an important way of detoxifying roots [37-39]. Oven-drying operation is a technological treatment that can also explain partially this decrease [40]. Total carbohydrates and starch contents are also affected by processing. Total carbohydrates changes from 93.01 ± 1.07% to 95.54 ± 0.77% when, starch content varies from 75.71 ± 3.37% in roots to 81.53 ± 3.92% in flours. The increase of these contents during processing could be due mainly to sieving. This operation selects the carbohydrates molecules because of the sieve mesh. It could also be due to the fact of loss in constituents like ash, during processing.

Identification of Differences Between Roots, and Fermented Flours: Figure 3 presents the result of cluster analysis on cassava roots. The analysis of the hierarchical tree reveals the existence of physicochemical differences between roots. Indeed, when the tree is cut to a distance of 20 (arbitrary unit), more than 60% of the differentiations between roots are explained and three classes can be distinguished:

- a class formed by roots of the three cultivars coming from Lac, Marahoue, Nzi comoe, Sud-Bandaman, Agneby, and Lagune regions, and the roots of Zoklo cultivar and Akaman one from Moyen-Cavally and Fromager regions;
- a second class of only Yace cultivar from Moyen-Cavally, Fromager, Bas-Sassandra and Haut-Sassandra regions;
- a third class, composed by the roots of Zoklo cultivar from the regions of Bas-Sassandra and Haut-Sassandra, and the Akaman cultivar of Haut-Sassandra region.

This roots distribution in classes could be explained by the influence of some factors such as cultivars and sampling regions, on the physicochemical parameters.

The hierarchical tree (figure 4) obtained by cluster analysis reveals in the same mood, that fermented flours can be grouped in three classes. The first class is formed by flours from roots of the three cultivars coming from Agneby region. The second class is composed by flours from roots of the three cultivars coming from the region of Bas-Sassandra and Haut-Sassandra. The third class is formed by flours from roots of the three cultivars coming from the regions of Lac, Marahoue, Sud-Bandaman, lagunes, Moyen-Cavally, Nzi comoe and Fromager. This typology summarized at the rate of 70%, the differentiations between fermented flours and could also be due to the effects of some factors such as cultivars and sampling regions.

Influence of Cultivars and Sampling Regions: The influence of factors is checked out using MANOVA procedure. Table 3 presents the results on roots characteristics. The effects of cultivars and regions on the chemical composition of roots are significant ($P<0.05$). These factors are sources of changes noted on roots constituents. Cultivars effects could be explained by the existence of specific characteristics belonging to a cultivar [11]. This hypothesis is confirmed by the results of PCA (figure 5). Indeed, differences exist clearly between roots of these three cultivars studied. Statistical analysis [41], reveals that roots cyanide content is the only parameter which varied significantly ($P<0.05$) from a cultivar to the other (table 4). This cyanide content is therefore a specific characteristic that could be used to classify the cassava cultivars. This result confirms the roots classification proposed by Purseglove [42]. According to this classification:

- roots from Zoklo cultivar in which cyanide content (22.33 ± 7.73 mg/kg) is inferior to 50 mg/kg, are qualified as non toxics;
- roots from Yace cultivar in which cyanide content (108.67 mg/kg) is superior to 100 mg/kg are toxics;
- roots from Akaman cultivar with 53.75 ± 7.03 mg/kg are an intermediate variety.

In addition, the existence of specific characteristics lead Zoundjihekpon [43] to take into account biochemical characters in the description of the cassava cultivars.

The variations of roots characteristics due to factor regions could be explained by regional parameters such as soil type, soil composition, planting moment, relief and rainfall [6-10, 44].

The effects of cultivars and regions on changes in physicochemical characteristics of cassava roots have also been reported by Woolfe [6] and Asiedu [7]. Outside of the main effects of these factors, another significant ($P<0.05$) effect due to the interaction cultivars and regions exist (table 3).

The significant ($P<0.05$) effects of cultivars, regions and their interaction on fermented flours characteristics are shown in table 5. As expected, the flours are also influenced by region and cultivar.

Conclusion: Cassava roots as well as their derivative fermented flours are energizing foods. During roots processing into flours, moisture, total acidity and pH, ash, starch, total carbohydrates and cyanide contents of roots are affected. Physicochemical differences between cassava roots exist and concern parameters such as proteins, ash, total acidity, lipids, minerals (P, Mg, Zn, Fe and Ca) and cyanide contents. Differences between manufactured flours also exist and concern parameters like lipids, acidity and minerals (P, Mg, Zn, Fe and Ca). These differences are due to effects of cultivars and regions, and their interaction. Before choosing cassava roots and fermented flours for valorisation, it is therefore important to take into account the cultivars and the sampling regions. The manufactured fermented flours characteristics are in agreement with Codex standards on edible cassava flour; so they can be valorised in modern and traditional food industries like bread making and *placali* reconstitution.

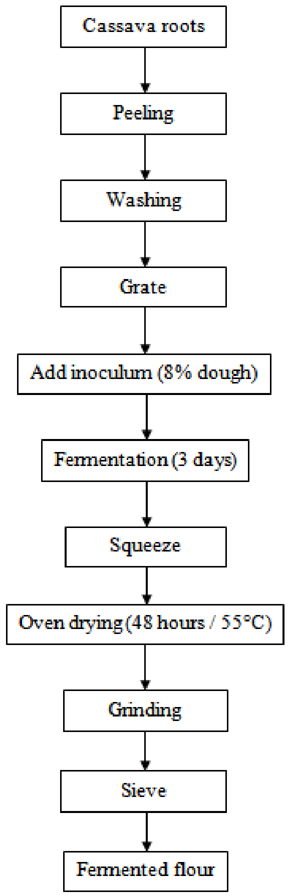

Fig. 1: Flow chart of cassava roots processing into fermented flours

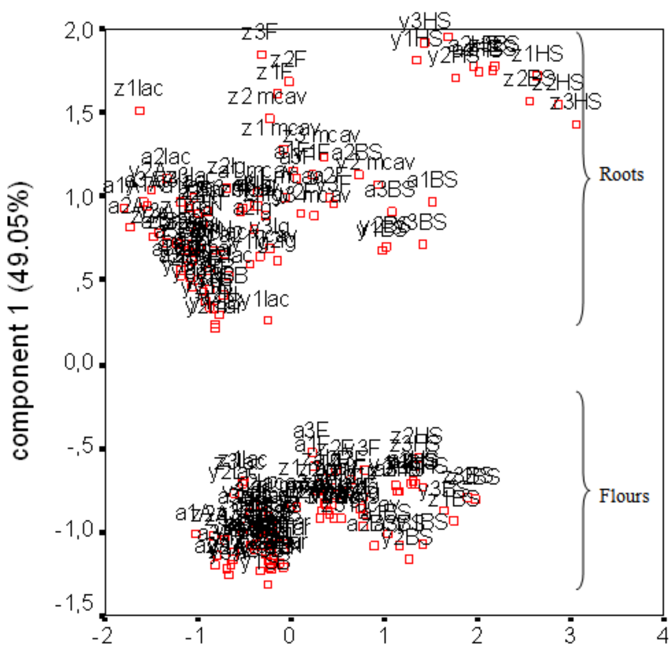

Fig. 2: Scatter plot of roots and flours

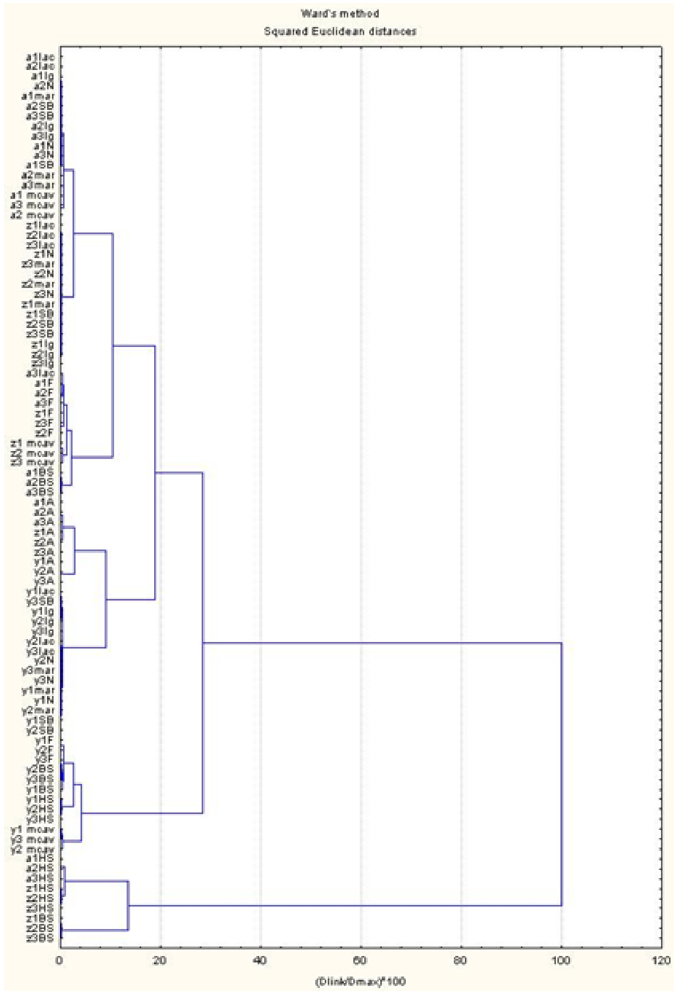

Fig. 3: Hierarchical tree of roots

Table 1: Physicochemical characteristics of cassava roots (dry weight basis)

	Number of observations	Minimum	Maximum	Mean	Standard deviation	Relative Standard deviation (%)
Moisture (%)*	90	53.41	73.94	62.37	3.62	5.81
Proteins (%)	90	1.45	3.76	2.32	0.48	20.64
pH	90	5.89	6.96	6.57	0.23	3.59
Acidity (meq/100g)	90	0.85	9.8	3.28	1.89	57.75
Lipids (%)	90	0.32	1.94	0.66	0.28	41.97
Ash (%)	90	1.4	3.81	2.61	0.50	19.15
Sugars (%)	90	1.51	3.01	2.44	0.35	14.37
Cyanide (mg/Kg)*	90	10.00	135.00	61.58	36.95	59.99
Starch (%)	90	65.78	83.58	75.71	3.37	4.45
Carbohydrates (%)	90	88.9	95.06	93.01	1.07	1.15
Energy (Kcal/100 g)	90	370.19	395.56	387.38	3.46	0.89

Table 1: Continue

Phosphorus (mg/100g)	90	41.41	278.05	121.84	48.62	39.91
Magnesium (mg/100g)	90	32.00	160.28	71.74	33.81	47.13
Iron (mg/100g)	90	4.31	25.05	7.85	4.50	57.42
Zinc (mg/100g)	90	1.3	7.95	3.99	2.09	52.54
Calcium (mg/100g)	90	29.82	342.37	112.45	66.39	59.04
Ca/P	90	0.44	2.13	0.88	0.27	30.94

(*) expressed on fresh matter basis

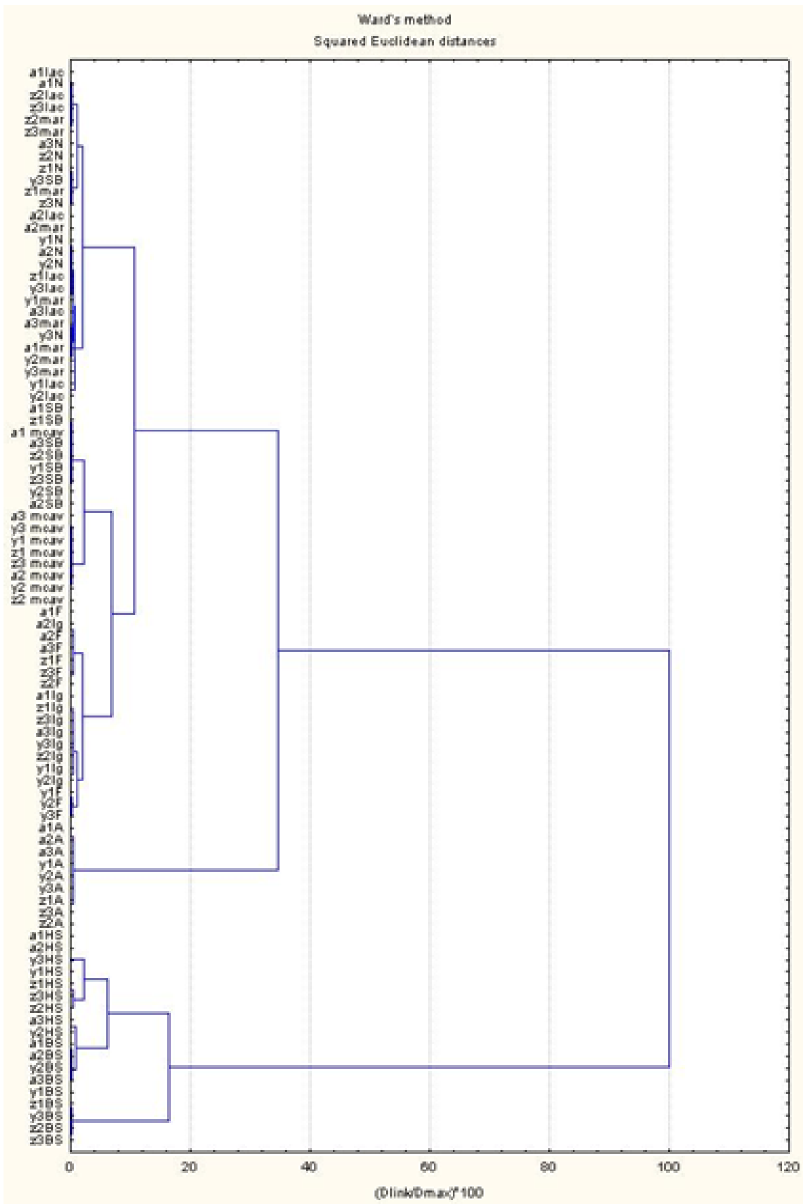

Fig. 4: Hierarchical tree of roots

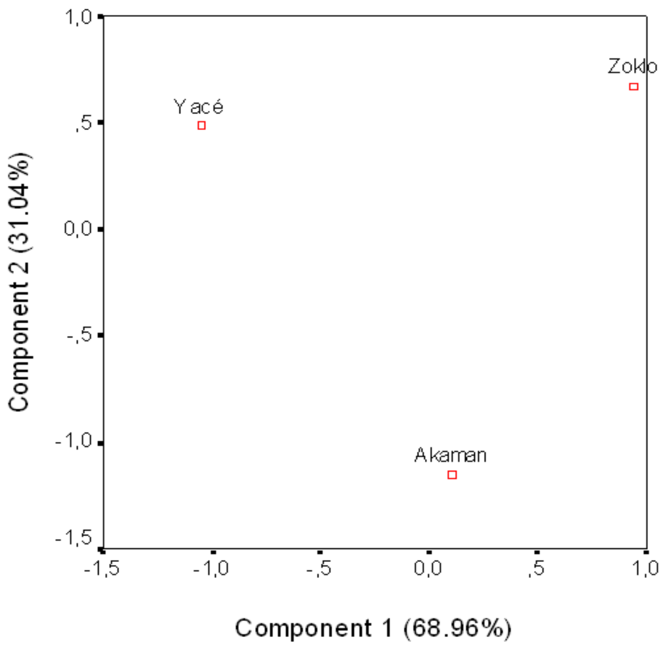

Fig. 5: PCA results of roots characteristics from three cultivars

Table 2: Physicochemical characteristics of fermented flours (dry weight basis)

	Number of observations	Minimum	Maximum	Mean	Standard deviation	Relative standard deviation (%)
Moisture (%)*	90	6.03	11.79	8.92	1.33	14.93
Proteins (%)	90	1.06	2.18	1.51	0.21	14.36
pH	90	3.92	4.53	4.19	0.13	3.17
Acidity (meq/100g)	90	7.89	16.62	12.33	2.26	18.39
Lipids (%)	90	0.13	0.88	0.38	0.13	34.85
Ash (%)	90	0.85	1.67	1.27	0.17	13.36
Sugars (%)	90	1.12	2.41	1.74	0.24	13.88
Cyanide (mg/Kg)*	90	0.00	15.00	0.44	2.44	550.21
Starch (%)	90	71.66	87.6	81.53	3.92	4.80
Carbohydrates (%)	90	92.91	97.03	95.54	0.73	0.77
Energy (Kcal/100 g)	90	380.57	396.4	391.74	2.61	0.66
Phosphorus (mg/100g)	90	30.33	197.32	97.09	33.83	34.85
Magnesium (mg/100g)	90	19.2	80.34	44.88	16.12	35.93
Iron (mg/100g)	90	2.1	13.41	5.04	2.64	52.35
Zinc (mg/100g)	90	1.16	7.1	3.21	1.69	52.65
Calcium (mg/100g)	90	17.57	180.55	74.83	33.88	45.27
Ca/P	90	0.47	1.33	0.76	0.21	28.44

(*) expressed on fresh matter basis

Table 3: Roots characteristics MANOVA table

Error source	Test	Value	F	Degree of freedom	p
Intercept	Wilks	0.000014	188385.0	17	0.00
Cultivars	Wilks	0.002945	45.1	34	0.00
Regions	Wilks	0.000000	44.4	153	0.00
Cultivars*regions	Wilks	0.000000	7.0	306	0.00

Table 4: Physicochemical composition of roots from three cultivars (dry weight basis)

	Akaman	Yace	Zoklo
Moisture (%)*	62.31 ± 2.54^a	62.20 ± 3.95^a	62.6 ± 4.26^a
Proteins (%)	2.39 ± 0.32^a	2.22 ± 0.49^a	2.36 ± 0.58^a
pH	6.65 ± 0.18^a	6.41 ± 0.19^b	6.64 ± 0.24^a
Acidity (méq g/100g)	3.05 ± 1.51^a	4.18 ± 2.33^a	2.62 ± 1.4^a
Lipids (%)	0.79 ± 0.4^a	0.52 ± 0.11^a	0.67 ± 0.15^a
Ash (%)	$2.67 \pm 0.34^{a,b}$	2.34 ± 0.48^a	2.83 ± 0.53^b
Sugars (%)	2.48 ± 0.24^a	2.12 ± 0.31^b	2.72 ± 0.17^a
Cyanide (mg/Kg)*	53.75 ± 7.03^a	108.67 ± 11.38^b	22.33 ± 7.73^c
Starch (%)	76.37 ± 3.04^a	$76.21 \pm 3.33^{a,b}$	74.56 ± 3.52^b
Carbohydrates (%)	92.79 ± 0.78^a	93.67 ± 0.89^b	92.57 ± 1.19^a
Energy (Kcal/100 g)	387.93 ± 2.77^a	388.33 ± 2.66^a	385.89 ± 4.29^a
Phosphorus (mg/100g)	129.62 ± 58.85^a	111.65 ± 35.21^a	124.24 ± 48.75^a
Magnesium (mg/100g)	65.03 ± 33.49^a	77.73 ± 34.80^a	72.47 ± 33.05^a
Iron (mg/100g)	7.17 ± 2.85^a	7.41 ± 4.43^a	8.96 ± 5.71^a
Zinc (mg/100g)	3.84 ± 2.05^a	3.94 ± 2.21^a	4.19 ± 2.07^a
Calcium (mg/100g)	107.85 ± 58.53^a	103.20 ± 46.44^a	126.30 ± 87.39^a
Ca/P	0.80 ± 0.12^a	0.89 ± 0.21^a	0.95 ± 0.39^a

(*) expressed on the fresh matter
The chemical composition values with different letters in each row are significantly different ($P<0.05$)

Table 5: Fermented flours characteristics MANOVA table

Error source	Test	Value	F	Degree of freedom	p
Intercept	Wilks	0.000007	363757.2	17	0.00
Cultivars	Wilks	0.037077	10.9	34	0.00
Regions	Wilks	0.000000	39.9	153	0.00
Cultivars*regions	Wilks	0.000002	3.7	306	0.00

NOMENCLATURE

a : cultivar Akaman
y : cultivar Yace
z : cultivar Zoklo
a1lac : 1st sample of Akaman root or flour from Lac region
a1N: 1st sample of Akaman root or flour from Nzi comoe region
a1mar: 1st sample of Akaman root or flour from Marahoue region
a1SB: 1st sample of Akaman root or flour from Lac

region
a1F: 1st sample of Akaman root or flour from Fromager region
a1lg: 1st sample of Akaman root or flour from Lagunes region
a1A: 1st sample of Akaman root or flour from Agneby region
a1HS: 1st sample of Akaman root or flour from Haut-Sassandra region
a1mcav: 1st sample of Akaman root or flour from Moyen-Cavally region
a1BS: 1st sample of Akaman root or flour from Bas-Sassandra region

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