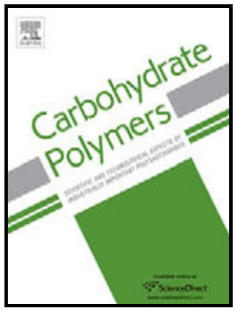

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Combined effect of fermentation, sun-drying and genotype on breadmaking ability of sour cassava starch

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Abstract

The influence of genotype and post-harvest treatments on expansion ability of sour cassava starch was investigated using 13 cassava genotypes from Colombia. Starches from cassava grown at 1000m and 1700m a.s.l. (3 lowland and 10 highland clones respectively) were modified by fermentation (0 or 30 days) and drying (oven or sun) treatments. RVA average peak viscosity decreased regularly from 952 cP in native starch to 699 cP in fermented and sun-dried starch. Granule size analysis revealed that fermentation hydrolysed lowland and highland granules by exocorrosion and endocorrosion respectively. This result was corroborated by significantly higher RVA breakdown and lower intrinsic viscosity in highland clones, reflecting different sensitivity to fermentation. For the first time, amylose contents ranging from 15.7 to 21.7% were correlated with expansion ability (3.0-8.6mL/g) of sour cassava starch. Therefore the combination of cassava genotypes (mainly amylose content) and post-harvest treatments is key for expansion ability. Supra-molecular granule structure influenced sensitivity to fermentation.

Key words: modified starch, breadmaking, loaf expansion, gluten-free, UV irradiation

1. Introduction

The Composite flour Program of FAO established in 1964 (Kent, 1985) financed research in tropical countries to substitute wheat flour with cassava flour. Consumer acceptability decreased in mixtures with > 30% cassava flour, due to noticeable color, texture, and flavor differences (FAO, 2004). In South America a traditional alternative to flour is the fermentation of cassava starch combined with sun-drying, which has excellent breadmaking capacity (Cereda, 1973 and Westby and Cereda, 1994, in Brazil and Cárdenas and de Buckle, 1980 and Zakhia, Dufour, Chuzel & Griffon, 1996, in Colombia). These authors described the process to obtain fermented or sour starch ("Polvilho azedo" in Brazil or "Almidón agrio" in Colombia). In this process, the main steps are starch extraction, natural fermentation for about 30 days and final sun-drying for 12 hours. The sour starch is used locally for bread and pastry production. There are also opportunities to use sour cassava starch as adjuvant for breadmaking or as the main ingredient for gluten-free breads. It is important, therefore, to understand better the mechanisms giving breadmaking ability of sour cassava starch.

Extensive works have been performed to develop a better understanding of breadmaking ability of sour starch (Dufour, Larsonneur, Alarcon Morante, Brabet & Chuzel, 1996; Mestres & Rouau, 1997; Mestres, Rouau, Zakhia & Brabet, 1996). Most studies focused on the effects of fermentation and sun-drying on the physicochemical properties and baking behaviour such as pH, solubility, specific volume, polymerization degree through intrinsic viscosity measurements (Marcon et al., 2009); degree of starch oxidation through carbonyl and carboxyl content measurements (Guerra Dias, Zavareze, Elias, Helbig, da Silva & Ciacco, 2011); and

starch paste thermomechanical properties (Bertolini, Mestres, Lourdin, Valle & Colonna, et al., 2001a). Evidence from these studies suggests that a degradation mechanism of starch occurs during fermentation and that sun-drying plays a key role in the breadmaking properties. However, the nature of the changes are not fully elucidated (Dufour, Brabet, Zakhia & Chuzel, 1995). Two hypotheses have been suggested to explain the link between starch degradation and breadmaking ability: firstly at molecular level, free-radical formation results in starch oxidation and depolymerisation (Bertolini, Mestres & Colonna, 2000; Bertolini, Mestres, Raffi, Buléon, Lerner & Colonna, 2001b; Demiate, Dupuy, Huvenne, Cereda & Wosiacki, 2000; Guerra Dias et al., 2011; Vatanasuchart, Naivikul, Charoenrein & Sriroth, 2005) or secondly at supramolecular level, the structure of the starch granules is altered and results in modified gelatinization behaviour (Camargo, Colonna, Buleon & Richard-Molard, 1988; Gomes, Mendes da Silva & Ricardo, 2005; Onitilo, Sanni, Oyewole & Maziya-Dixon, 2007).

In addition, few publications explored the influence of cassava genotype on breadmaking ability of sour starch (Escobar, Dufour, Sanchez, Giraldo & Dufour, 2009; Onitilo, Sanni, Oyewole & Maziya-Dixon, 2007) in contrast with most works which used only one or two genotypes and therefore did not take the into consideration genetic differences. Escobar et al. (2009) in particular, found that the altitude above sea level at which cassava is cultivated appears to be another determining factor for the breadmaking ability. Farmers traditional knowledge has often indicated that genetic and climatic factors influence breadmaking ability.

The originality of this work is to study in detail the genetic effect on breadmaking ability of sour cassava starch using 13 Colombian genotypes, cultivated in exactly the same conditions at two locations with low-intermediate (1000 meters above sea level, m.a.s.l.) or high (1700 m.a.s.l.) altitudes and referred to respectively as lowland and highland. The effect of post-harvest treatments (fermentation and sun-drying of the 13 native starches), was analyzed through characterization of both native and modified starches. Physicochemical parameters, pasting and thermal properties were quantified in order to provide information on the degradation mechanism(s) of sour starch. The aim of this work was to contribute to a better understanding of sour cassava starch properties, and to highlight the effects of genotype, altitude and process (fermented versus native starches) parameters on the breadmaking ability.

2. Materials and methods

2.1. Genotypes and growing conditions

Thirteen cassava genotypes from the germplasm collection at the International Center for Tropical Agriculture (CIAT, Cali, Colombia) were grown in two locations in Cauca Department in Colombia. Most of these genotypes are improved through conventional breeding approaches. Three lowland genotypes (HMC-1; CM6438-14; CM4574-7) were grown at Jamundi (altitude 1000 m.a.s.l.) and harvested at 18 months after planting (MAP). Ten highland genotypes (CM7436-7; CM7438-14; SM1498-4a; CM7138-7; SM7591-5; Cumbre 3; SM707-17; SM1495-5; SM1058-13; and Tambo 4) were grown in Morales (1700 m.a.s.l.) and harvested at 16 MAP. The difference in age of the plants at harvest time (2 months) was considered small enough to have no significant effect on starch properties, so that altitude and

genotype effects were predominant. It is not convenient to grow the 13 genotypes in the two locations as lack of adaptation to one of the environments would result in data that is not useful on one hand, and distortion of conclusions of the study on the other.

2.2. Treatments experimental conditions:

After harvest, all the cassava roots were transported to a traditional factory (*Rallanderia*) in Cauca Department (Colombia) for starch extraction, using the following operations: washing-peeling, rasping and extraction; whereby starch was drained off through sieving the mash under running water. After 24h of decantation, the top liquid phase was discarded. The sediment was divided into four equal subsamples and subjected to four different treatments. The first subsample was oven-dried at 40°C for 24 hours to obtain native, non-fermented, oven-dried cassava starch (NO). The second subsample was sun-dried for about 12 hours to obtain non-fermented, sun-dried starch (NS). The third and fourth subsamples were fermented together in an airtight PVC tank (100 L) at ambient temperature (35° C) for 30 days. The tank was filled with the wet starch and a layer of fermented starch was added. After fermentation, the third subsample was oven-dried for 24h at 40°C to obtain fermented, oven-dried starch (FO), and the fourth part was sun-dried for 12h to obtain fermented, sun-dried starch (FS).

2.3. Breadmaking test

Bread dough was prepared according to CIAT protocol (CIAT, unpublished protocol): 125 g of starch sample (12-13% moisture content), 75 g of ”*Costeño*“ cheese and 18.7 g of sunflower oil were hand mixed on a stainless steel plate. Secondly pre-

gelatinized starch was prepared by mixing 37.25 g starch (dm), with 31.25 g of cold water, then slowly adding 31.25 g of boiling water while stirring with a spoon. The native starch mix and pregelatinized starch were blended homogeneously with 100 mL of cold water. From the resulting dough, 12 rings of 33 g were shaped, each with a perimeter of 18 cm and 2 cm thick. The doughs were baked in an oven at 260 °C for 13 min, and then cooled for 20 min under ambient conditions. The weights and volumes of the breads were measured with a scale and a pycnometer using cauliflower seeds, and averages of the 12 replications (1 replication = 1 ring) were calculated. Breadmaking ability (or bread expansion) was characterized as the ratio: bread volume/bread weight (mL/g).

2.4. Gelatinization temperature and amylose content

The methodology reported by Mestres and Rouau (1997) was used. DSC analyses were performed on a Perkin-Elmer DSC 7 device (Perkin-Elmer, Norwalk, VA) using sealed stainless-steel pans. The sample pan (10 - 11 mg of starch and 50 µL of lyso-phospholipid (SIGMA, 2% w/v in water) and the reference pan (empty) were heated from 25 to 160°C at 10°C.min^{-1}, held at 160°C for 2 min, and then cooled to 60°C at 10°C.min^{-1}. The temperatures of gelatinization (T_o, T_p and T_c) and enthalpies (ΔH) of each sample were determined on the thermograms. The gelatinization temperature range (ΔT) was calculated as ($T_c - T_o$) as described by Cavallini and Franco (2010). Amylose content was also measured from the energy of amylose-lysophospholipid complex formation. The analyses were performed in duplicate, and mean values were calculated.

178 **2.5. Granular size**

179 Starch granules average diameter was determined from the size distribution
180 evaluated by laser diffraction (Malvern Mastersizer 2000E, Malvern, Worcestershire,
181 UK). The sample was suspended in distilled water and pre-treated by ultrasound (30
182 seconds) in order to break down granules aggregates, according to Jinapong,
183 Suphantharika and Jamnong (2008). The obscuration was adjusted in the range 11-
184 14% during the measurements. The Fraunhofer model for non-transparent particles
185 was used for the size calculations, with the refractive index of the dispersant (water)
186 set at 1.33. The analyses were performed in triplicates, and mean values were
187 calculated.

188

189 **2.6. Pasting properties**

190 The methodology reported by Sanchez et al. (2009) was used. Hot starch dispersion
191 viscosity profiles were obtained with a Rapid Visco Analyser model RVA-4 Series
192 (Newport Scientific, Warriewood, Australia). Starch (1.25 g db) was dispersed in
193 distilled water (23 cm^3) to yield a 5% suspension. Viscosity was recorded using the
194 following temperature profile: holding at 50°C for 1 min, heating from 50°C to 90°C at
195 6°C min^{-1}, holding at 90°C for 5 min and then cooling down to 50°C at 6°C min^{-1} with
196 continuous stirring first at 960 rpm for 10 seconds and then at 160 rpm throughout
197 the rest of the experiment. Eight parameters were measured on the visco-
198 amylogram: pasting temperature and pasting time (PT and Pt), peak viscosity 1 (PV1:
199 first viscosity peak after beginning of pasting), peak viscosity 2 (PV2: second
200 viscosity peak after beginning of pasting), time of peak viscosity 1 and 2 (tPV1 and
201 tPV2, respectively), lowest hot paste viscosity or holding strength (HS), final viscosity
202 (FV). Five additional parameters were then calculated: cooking ability (CA) estimated

as tPV2-Pt, breakdown (BD) estimated as PV2-HS, relative breakdown (RBD) estimated as (BD/PV2) x 100, setback (SB) estimated as FV-HS and relative setback (RSB) estimated by (SB/FV) x 100. The analyses were performed in duplicate, and mean values were calculated.

2.7. Intrinsic Viscosity

A 3% starch solution was prepared by dissolving starch in potassium hydroxide solution (KOH 5 mol/L) and heated in a water bath at 95°C for 10 min. The samples were stirred for 20 hours (± 10 min) on a roller mixer in a temperature-controlled room at 22°C (± 0.5°C). The resulting solution was centrifuged at 10000 rpm for 5 min. 2 mL were removed and diluted in 28 mL of distilled water to reduce KOH concentration to 0.33 mol/L and starch concentration to 2 mg/mL. This solution was filtered through a syringe filter (pore size 0.45 µm). 10 mL of the filtered solution were collected to carry out a serial dilution with distilled water and to determine the following concentrations: 2.0, 1.7, 1.4, 1.1, 0.8 mg starch/mL.

The intrinsic viscosity was measured for each diluted solution using an Ubbelohde viscometer (U-tube, size 2 mL, Shott Gerate Gmbh, Hofheim, Germany) immersed in a water bath at 35°C.. The analyses were performed with a replication. For each dilution, the flow time was recorded three times, after an initial 10 min period for temperature equilibration. Preliminary replication tests showed that flow times did not vary significantly between replications. The intrinsic viscosity (mL/g) was determined by extrapolation to zero concentration of the reduced and inherent viscosities (Harding, 1997).

2.8. Statistical analysis

Statistically significant differences between samples means were determined using analysis of variance (ANOVA) followed by a Fisher's test at 95% confidence level. When relevant, a multivariate cluster analysis was performed to identify groups of samples with similar characteristics, using the "average linkage method" based on the calculation of the distances between the averages of samples (Sokal & Michener, 1958) and groups thereof using Statistica v7.1 software (StatSoft, Maison-Alfort, FRANCE). The Principal Component Analysis (PCA) was carried out to identify groups of samples with similar characteristics and performed with The Unscrambler X 10.2 software (Camo*).*

Results and discussion

3.1. Breadmaking ability

The breadmaking ability of the different samples was represented by the values of loaf expansion in Table 1. The general trend of this parameter was as follows: NO = FO < NS < FS. This sequence was in agreement with literature (Bertolini et al., 2000; Marcon et al., 2009; Mestres, Boungou, Akissoe & Zakhia, 2000) although some actual values were lower than in the literature. The dissimilarities could be due to the use of different breadmaking protocols and/or treatment of samples used in the different studies. For instance the bread recipe in this study has content lipids due to the use of cheese as ingredient, therefore amylose lipid complexes may have formed and inhibited swelling as reported by Tester and Morrison (1992), in contrast to other studies using recipes without added lipids.

Table 1
Loaf expansion of native, fermented and/or sun-dried cassava starches.

Genotype	Loaf expansion (mL/g) [y]			
	Treatments [z]			
	NO [z]	NS [z]	FO [z]	FS [z]
Lowland				
HMC-1	1.40(0.03)[a]	1.93(0.08)[b]	1.40(0.05)[a]	3.57(0.23)[c]
CM6438-14	1.71(0.04)[a]	1.76(0.05)[a]	1.55(0.05)[a]	4.02(0.35)[b]
CM4574-7	1.85(0.05)[a]	1.92(0.05)[a]	2.19(0.13)[a]	6.59(0.81)[b]
Highland				
CM7436-7	1.92(0.05)[a]	2.65(0.10)[b]	2.07(0.04)[a]	4.37(0.69)[c]
CM7438-14	1.94(0.06)[a]	2.11(0.04)[ab]	2.40(0.07)[b]	4.47(0.43)[c]
SM1498-4a	1.82(0.03)[a]	2.26(0.07)[a]	1.94(0.04)[a]	5.99(0.68)[b]
CM7138-7	1.96(0.06)[a]	3.72(0.07)[b]	2.15(0.05)[a]	6.17(0.39)[c]
SM7591-5	1.45(0.09)[a]	2.40(0.05)[b]	1.49(0.07)[a]	3.05(0.17)[c]
Cumbre 3	2.21(0.13)[a]	3.39(0.15)[b]	2.30(0.15)[a]	8.58(0.79)[c]
SM707-17	1.87(0.05)[a]	2.30(0.06)[b]	2.27(0.07)[b]	7.49(0.44)[c]
SM1495-5	2.12(0.07)[a]	2.79(0.08)[a]	2.51(0.17)[a]	7.57(1.38)[b]
SM1058-13	1.88(0.02)[a]	2.55(0.07)[b]	2.19(0.09)[ab]	5.29(0.48)[c]
Tambo 4	2.11(0.07)[a]	2.68(0.08)[b]	2.54(0.07)[ab]	7.92(0.68)[c]
Lowland	1.59(0.21)[a 1]	1.87(0.10)[a 1]	1.76(0.38)[a 1]	4.99(1.51)[b 1]
Highland	1.91(0.24)[a 2]	2.68(0.49)[b 2]	2.17(0.34)[a 2]	6.17(1.88)[c 2]
All genotypes	1.83(0.27)[a]	2.50(0.55)[b]	2.07(0.39)[a]	5.87(1.86)[c]

[y] Values obtained from breadmaking test.

[z] Treatments: NO: non-fermented, oven-dried ; NS: non-fermented, sun-dried; FO: fermented, oven-dried ; FS: fermented, sun-dried.

[a-c] Different letter, within row, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.

[1-2] Different numbers, within column, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.

Highland genotypes displayed higher significant average values of loaf expansion than lowland's, regardless of the treatments. NO, FO, NS and FS treatments of highland genotypes were 16.8, 18.9, 30.2 and 19.1% significantly higher than those of lowland's, respectively. In lowland genotypes only FS improved breadmaking (214% significantly higher than NO). In highland genotypes both NS and FS improved breadmaking (40 and 223% significantly higher than NO, respectively). In

this work, the fermentation process (FO) alone did not improve the breadmaking ability, in agreement with a previous study by Demiate et al. (2000) and in contradiction with a study by Bertolini et al. (2000), who found a significant improvement in breadmaking after treatment by lactic acid mimicking the fermentation process. In conclusion, highland genotypes appeared more sensitive to sun drying (NS and FS) than lowland ones, and the latter were sensitive to the UV-irradiation only after fermentation.

Cluster analysis and a Fisher test (not shown) carried out on the FS samples confirmed the high variability between samples in terms of breadmaking ability. A clear genotype effect was found, and two main groups were identified as follows. The high breadmaking ability group includes 4 genotypes: Cumbre 3, SM707-17, SM1495-5 and Tambo 4 ranging from 7.49mL/g to 8.58 mL/g. The intermediate and lower breadmaking ability group was composed of 9 genotypes further separated into two subgroups: the intermediate breadmaking ability of 4 genotypes: CM4574-7, SM1498-4a, CM7138-7 and SM1058-13 ranging from 5.29 mL/g to 6.59 mL/g and the lower breadmaking ability consisting of 5 genotypes: HMC-1, CM6438-14, CM7436-7; CM7438-14 and CM7591-5 ranging from 3.05mL/g to 4.47mL/g. Altitude was not a definite factor for predicting breadmaking ability, with one lowland genotype having good breadmaking ability (CM4574-7) and one highland genotype having poor breadmaking ability (SM7591-5). Hence both genetic and environmental factors appeared important in the control of breadmaking ability, in addition to the drying and fermentation processes. Other factors, such as the bread recipe containing lipids, may also contribute explain the observed differences in breadmaking ability among samples.

3.2. Thermal properties

In this study, only non-fermented, oven-dried samples (NO) were investigated, because a preliminary study with a limited set of samples indicated that DSC parameters did not change with fermentation or/and drying, as also indicated in literature (Bertolini et al., 2001a; Bertolini et al., 2001b; Camargo et al., 1988). This suggests that starch degradation induced by fermentation and/or sun-drying targets mainly the amorphous regions of starch granules (Bertolini et al., 2001b).

The gelatinization temperatures (T_o, T_p and T_c), gelatinization temperature range (ΔT) and enthalpy (ΔH) were comparable to values reported in the literature (Table 2) (Bertolini et al. 2001a; Garcia, Colonna, Bouchet & Gallant, 1997; Marcon et al., 2009; Mestres & Rouau, 1997; Nwokocha, Aviara, Senan & Williams, 2009).

298

Table 2

Thermal properties of native cassava starches (NO).

Genotype	Thermal properties [y]				
	T_o [y] (°C)	T_p [y] (°C)	T_c [y] (°C)	ΔH (J g^{-1})	ΔT (°C)
Lowland					
HMC-1	61.64(0.66)^{a}	66.24(0.59)^{a}	72.08(0.25)^{e}	15.28(1.73)^{a}	10.45(0.40)^{a}
CM6438-14	61.07(0.39)^{ab}	65.51(0.32)^{ab}	71.78(1.51)^{e}	16.50(2.87)^{a}	10.72(1.12)^{a}
CM4574-7	61.39(0.42)^{a}	66.10(0.21)^{a}	72.03(0.80)^{e}	17.77(1.10)^{a}	10.64(0.89)^{a}
Highland					
CM7436-7	57.38(0.64)^{efg}	61.83(0.78)^{de}	67.73(1.47)^{ab}	17.67(0.82)^{a}	10.35(1.21)^{a}
CM7438-14	58.12(0.25)^{def}	62.75(0.09)^{d}	68.78(1.13)^{bcd}	15.43(0.09)^{a}	10.66(1.39)^{a}
SM1498-4a	56.64(0.21)^{g}	60.86(0.38)^{ef}	66.40(1.58)^{a}	16.47(1.24)^{a}	9.76(1.37)^{a}
CM7138-7	58.69(0.23)^{cd}	64.19(0.30)^{c}	71.95(0.23)^{e}	16.63(2.21)^{a}	13.26(0.00)^{c}
SM7591-5	57.21(0.92)^{fg}	62.14(0.77)^{d}	68.29(1.11)^{abc}	17.07(2.71)^{a}	11.08(0.38)^{ab}
Cumbre 3	59.90(0.23)^{bc}	64.70(0.78)^{bc}	71.03(1.00)^{de}	16.91(0.46)^{a}	11.13(0.78)^{ab}
SM707-17	58.44(0.08)^{de}	62.76(0.06)^{d}	70.11(1.51)^{cde}	16.82(2.29)^{a}	11.67(1.43)^{abc}
SM1495-5	57.54(0.85)^{defg}	62.07(0.98)^{d}	68.81(0.57)^{bcd}	16.93(0.19)^{a}	11.27(1.42)^{abc}
SM1058-13	54.77(1.01)^{h}	60.26(0.53)^{f}	67.59(0.41)^{ab}	18.54(0.24)^{a}	12.83(0.60)^{bc}
Tambo 4	57.22(0.57)^{efg}	62.06(0.48)^{d}	68.76(0.74)^{abcd}	17.09(0.53)^{a}	11.54(0.18)^{abc}
Lowland	61.37(0.45)^{b}	65.99(0.42)^{b}	71.98(0.79)^{b}	16.83(1.83)^{a}	10.61(0.74)^{a}
Highland	57.56(1.35)^{a}	62.33(1.34)^{a}	68.86(1.81)^{a}	16.99(1.38)^{a}	11.30(1.26)^{a}
All genotypes	58.58(2.08)	63.30(2.01)	69.69(2.12)	16.95(1.48)	11.11(1.17)

[y] Values obtained from DSC: T_o: onset gelatinization temperature ; T_p: peak gelatinization temperature; T_c: conclusion temperature.

[a-h] Different letter, within each column, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.

299

300 DSC parameters are linked to the cohesion in the structure of granule (Marcon et al.,
301 2009), the integrity and the crystalline structure of starch granule (Mestres & Rouau,
302 1997). T_o, T_p and T_c of lowland genotypes were 3.81, 3.66 and 3.12°C significantly
303 higher than those of highland. In contrast, no statistically significant difference was
304 found between lowland and highland genotypes in terms of gelatinization enthalpy
305 (ΔH). Thus, T_o, T_p and T_c could be used to differentiate starches from lowland and
306 highland genotypes. The narrow gelatinization temperature range (ΔT) suggested a
307 homogeneous size and stability of crystallites within our samples, in contrast to

previous studies which found a broader gelatinization temperature range of approximately 20°C (T_o = 61.35°C and T_c = 81.77°C) (Putri, Haryadi & Marseno and Cahyanto, 2011).

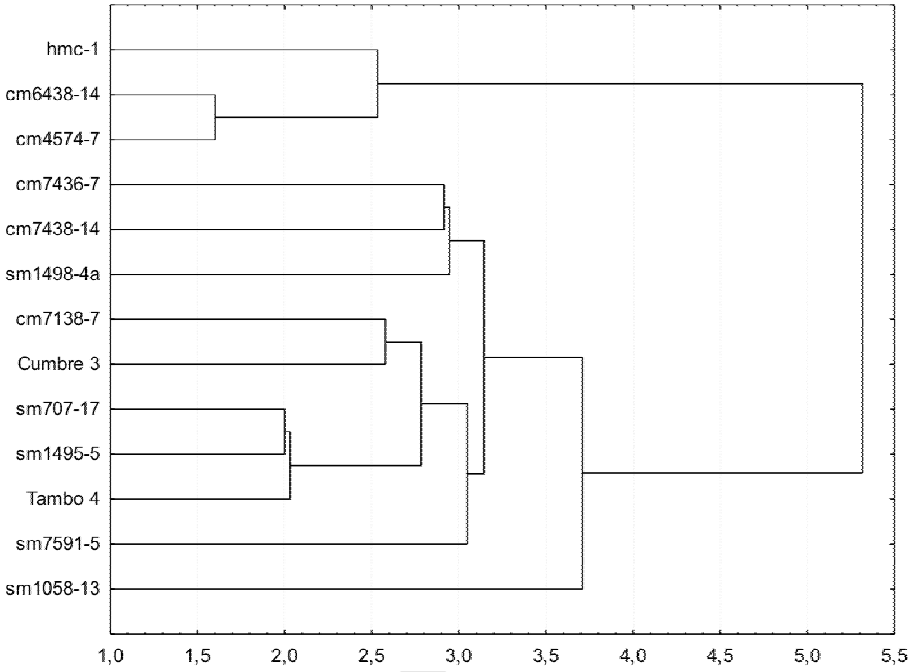


Fig. 1. Multivariate cluster analysis of the pooled DSC parameters (T_o, T_p, T_c and ΔH) identifying similarities and differences of between thirteen genotypes of cassava starch.

As seen in Table 2, a high variability of DSC parameters was observed, indicating a pronounced genotype effect. A Fisher test (Tab. 2) and a cluster analysis (Fig. 1) confirmed that lowland and highland genotypes can be segregated into two separate groups based on DSC data, in particular gelatinization temperature.

Figure 1 also shows that the best five breadmaking genotypes (CM7138-7, Cumbre 3, SM707-17, SM1495-5, Tambo 4) were clustered in two neighboring groups. Hence, information on breadmaking ability might be contained within DSC parameters, although more data would be needed to establish a more conclusive correlation.

3.3. Amylose Content

The amylose contents of NO samples are presented in Table 3. As for DSC gelatinization experiments, only unmodified samples were investigated as the amylose content is not influenced by the different treatments according to Franco et al. (2010). The amylose contents obtained in this study were in agreement with literature (Ceballos et al., 2008; Onitilo, Sanni, Oyewole & Maziya-Dixon, 2007; Sánchez et al., 2009).

Altitude appeared to be a determining factor for amylose content, with the lowland genotypes average (21.2%) being significantly higher than highland genotypes (18.0%). A possible explanation could be a difference in starch biosynthesis at different altitudes linked to different enzyme activities (probably related to the differences in temperature at the two locations), as well as genetic diversity.

A high variability in amylose content was found between genotypes. Hence, genotype effect was noticeable as confirmed by a Fisher test (Tab. 3) and a cluster analysis (not shown). These tests showed three groups: (i) a low amylose group including 4 genotypes (Cumbre 3, SM707-17, SM1495-5 and Tambo 4) ranging from 15.7 to 16.7%, (ii) an intermediate amylose group including 7 genotypes (CM6438-14, CM7436-7, CM7438-14, SM1498-4a, CM7138-7, SM7591-5 and SM1058-13) ranging from 17.6 to 20.1% and (iii) a high amylose group with 2 genotypes (HMC-1 and CM4574-7) at 21.6 and 21.7%. The four genotypes in the low amylose group matched those in the high breadmaking ability group. Hence, lower amylose contents appeared to improve breadmaking ability, which may be related to the formation of

less amylose-lipid complexes in low-amylose starches (Tester & Morrison, 1992). A weak negative correlation of R^2 = 0.46 was observed between the breadmaking ability of fermented, sun-dried starches (FS, both lowland and highland) and the amylose content (measured on non-fermented, oven-dried starches, NO) (Fig. 2). When one outlier (lowland CM4574-7) was removed, the correlation improved with R^2 = 0.71, which tends to confirm the link between amylose content and breadmaking ability. Mestres et al. (2000) and Shirai et al. (2007) also reported a link between amylose content and loaf expansion, with low expansion for oxidized normal corn starch, and high expansion for oxidized waxy corn starch, comparable to sour cassava starch or oxidized cassava starch.

Table 3

Amylose content of native cassava starches (NO).

Genotype	Amylose content (%) [y]
Lowland	
HMC-1	21.7(0.4)[g]
CM6438-14	19.6(0.3)[f]
CM4574-7	21.6(0.8)[g]
Highland	
CM7436-7	19.3(0.1)[ef]
CM7438-14	20.1(0.3)[f]
SM1498-4a	18.7(0.1)[de]
CM7138-7	17.6(0.1)[c]
SM7591-5	18.5(0.2)[d]
Cumbre 3	16.7(0.5)[bc]
SM707-17	15.7(0.0)[a]
SM1495-5	16.0(0.3)[ab]
SM1058-13	19.3(0.5)[def]
Tambo 4	16.7(0.1)[bc]
Lowland	21.2(1.1)[b]
Highland	18.0(1.5)[a]
All genotypes	18.8(2.0)

[y] Values obtained from DSC mesurements.

[a-g] Different letter indicate significant differences at

p < 0.05 (Fisher). Standard deviations are given within brackets.

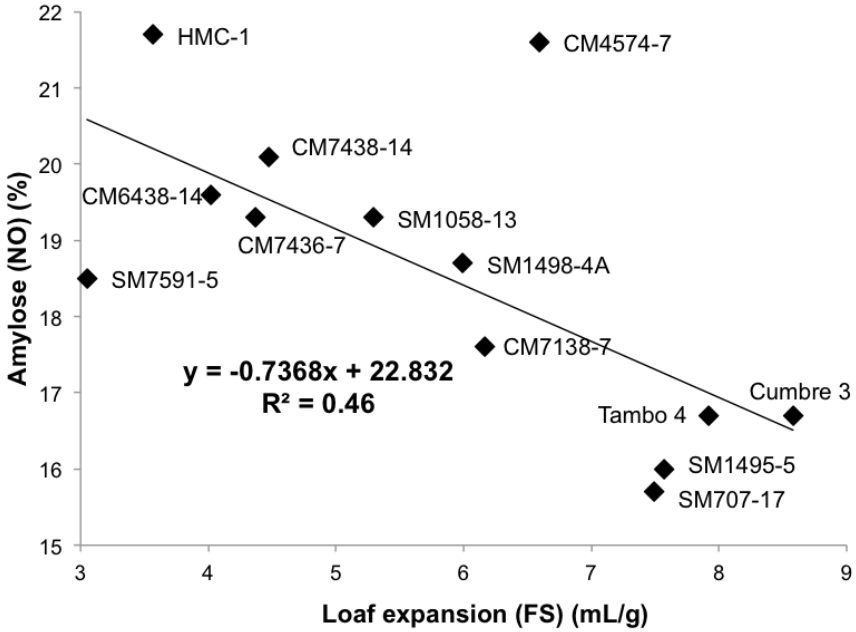


Fig. 2. Negative correlation (R^2 = 0.46) between loaf expansion of fermented, sun-dried starches (FS) and amylose content of non-fermented, oven-dried (NO) starches.

3.4. Starch granule size

The starch granular diameter (D[4,3]) of the 13 cassava genotypes ranged from 12.9 to 16.1 µm (Tab. 4), in agreement with literature data (Defloor, Dehing & Delcour, 1998; Huang, Lu, Li & Tong, 2007; Niba, Bokanga, Jackson, Schlimme & Li, 2002; Nuwamanya, Baguma, Emmambux & Taylor, 2010; Onitilo et al., 2007; Sriroth, Santisopasri, Petchalanuwat, Kurotjanawong, Piyachomkwan & Oates, 1999).

Table 4

Granular size of native, fermented and/or sun-dried cassava starches.

Genotype	Granular size (µm) [y]			
	Treatments [z]			
	NO [z]	NS [z]	FO [z]	FS [z]
Lowland	15.3(0.8)[c]	14.6(1.1)[b]	13.9(0.8)[a]	14.0(0.8)[a]
Highland	14.5(0.6)[a]	14.6(0.6)[a]	14.3(0.4)[a]	14.4(0.7)[a]

[y] Values obtained from laser diffractometry.

[z] Treatments: NO: non fermented, oven-dried ; NS: non fermented, sun-dried ; FO: fermented, oven-dried ; FS: fermented, sun-dried.

[a-c] Different letter, within row, indicate significant differences at p < 0.05 (Fisher). Standard deviations are given within brackets.

A small difference was observed in the average granular sizes of non-modified (NO) lowland and highland genotypes, which subsided after fermentation and drying treatments. A more significant difference between lowland and highland genotypes was observed in the decrease in granular size caused by the NS, FO and FS treatments (Tab. 4), as confirmed by Fisher tests. In lowland genotypes NS, FO and FS treatments decreased granule size by 4.6, 9.1 and 8.9 % respectively compared to NO, whereas, in highland genotypes, these treatments had no influence on the granular size. To explain this, it is hypothesized that different granular structures in lowland and highland genotypes resulted in varied levels of sensitivity to treatments. In lowland genotypes, NS, FO and FS treatments only shaved off the outer layers of the granules, leading to smaller granules with mostly intact cores. In contrast, in highland genotypes, granules were attacked more homogeneously throughout their structure during fermentation, resulting in weakened granules but with a non-significant reduction in granule average diameter. The RVA and intrinsic viscosities results tended to support this hypothesis, as discussed below.

3.5. Pasting behavior

The general shape of NO curves (Fig. 3) was similar to a standard RVA pasting profile (Franco et al., 2010; Mestres, Zakhia & Dufour, 1997), but with two viscosity peaks. Escobar et al. (2009) asserted that only highland genotypes had two viscosity peaks whereas in our work both lowland and highland genotypes had two peaks. In general, the first peak (PV1) was lower than the second (PV2) except for 3 genotypes for which PV1 was either higher than or similar to PV2 (CM7138-7, Cumbre 3 and Tambo 4). The difference between PV1 and PV2 decreased with treatments: on NS

curves, PV1 remained lower than PV2 except in two samples (CM7436-7 and SM1495-5), for which PV1 was similar to PV2. On FO curves, PV1 and PV2 were of similar magnitude in five genotypes (HMC-1, CM4574-7, CM7436-7, SM1495-5 and SM707-17), and PV1 disappeared altogether in the case of two other genotypes (CM7138-7 and Tambo 4). On FS curves only one viscosity peak remained in every genotype. This peak was arbitrarily referred to as PV2, and may be described as the result of the merger of the two peaks observed in other treatments, because the new peak time was in-between the peak times of PV1 and PV2 in other treatments (Fig. 3.). As in other study (Dufour et al. 1995), the peak viscosities PV1 and PV2 decreased after the NS, FO, FS treatments, with a stronger effect of fermentation compared to UV irradiation.

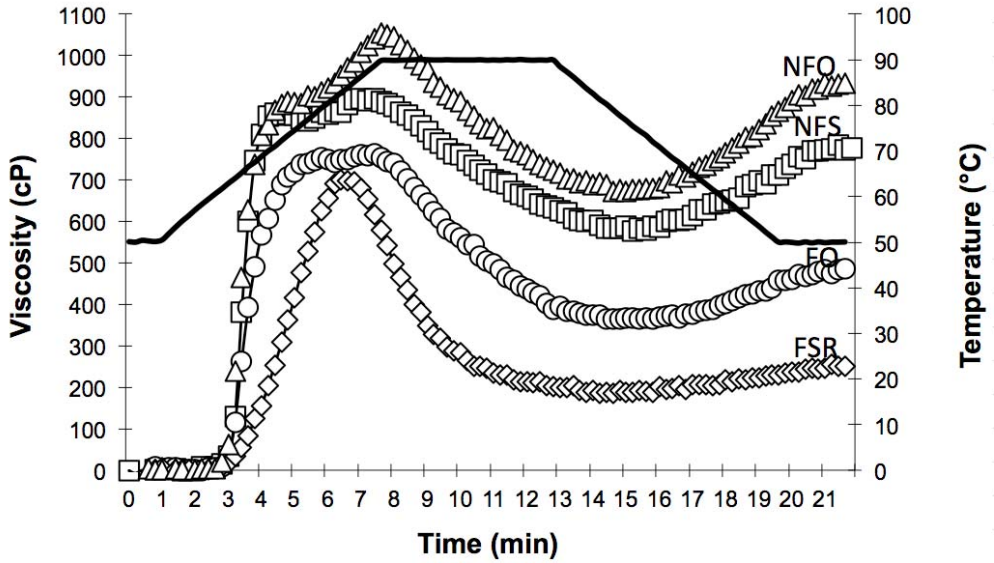


Fig. 3. Viscoamylograms of one cassava starch sample (sm 1495-5) after 4 treatments: NO (open black triangles), NS (open black squares), FO (open black circles) and FS (open black diamonds).

417

Table 5
Pasting properties of native, fermented and/or sun-dried cassava starches.

Genotype/ Treatment [z]	Pasting parameters [y]								
	Pasting temperature (°C) [y]	Peak viscosity 1 (cP) [y]	Peak viscosity 2 (cP) [y]	Breakdown (cP)	Relative Breakdown (%)	Final viscosity (cP) [y]	Setback (cP)	Relative Setback (%)	Cooking ability (s)
Lowland									
NO [z]	65.1(0.2)^{cd}	666(48)^{a}	914(65)^{de}	394(45)^{bc}	43.1(2.7)^{ab}	793(102)^{c}	273(79)^{c}	33.9(6.4)^{b}	263(14)^{cd}
NS [z]	65.0(0.7)^{c}	687(38)^{a}	913(37)^{de}	345(38)^{ab}	37.8(3.6)^{a}	847(47)^{c}	279(50)^{c}	32.9(4.9)^{b}	273(12)^{cd}
FO [z]	65.6(0.4)^{cd}	632(43)^{a}	663(40)^{ab}	310(39)^{a}	46.8(4.9)^{bc}	501(41)^{b}	149(12)^{b}	29.8(2.5)^{b}	266(24)^{cd}
FS [z]	66.2(0.7)^{d}	-	637(36)^{a}	403(21)^{bc}	63.4(3.9)^{d}	326(45)^{a}	92(15)^{ab}	28.3(2.4)^{b}	173(16)^{a}
Highland									
NO [z]	61.6(1.1)^{a}	835(62)^{c}	960(117)^{e}	378(42)^{b}	39.7(6.0)^{a}	844(166)^{c}	260(67)^{c}	30.8(4.0)^{b}	286(29)^{d}
NS [z]	61.6(1.2)^{a}	834(45)^{c}	889(87)^{d}	361(68)^{b}	40.9(9.0)^{ab}	771(189)^{c}	255(88)^{c}	32.6(4.3)^{b}	270(61)^{cd}
FO [z]	62.0(0.9)^{a}	777(46)^{b}	808(38)^{c}	425(49)^{c}	52.6(5.7)^{c}	522(64)^{b}	139(23)^{b}	26.7(3.2)^{b}	256(46)^{c}
FS [z]	62.8(1.1)^{b}	-	718(41)^{b}	504(50)^{d}	70.4(7.8)^{e}	286(98)^{a}	52(71)^{a}	13.9(20.7)^{a}	208(16)^{b}

[y] Values obtained from RVA.
[z] Treatments: NO: non fermented, oven-dried ; FO: fermented, oven-dried ; NS: non fermented, sun-dried ; FS: fermented, sun-dried.
[a-d] Different letter, within column, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.

418

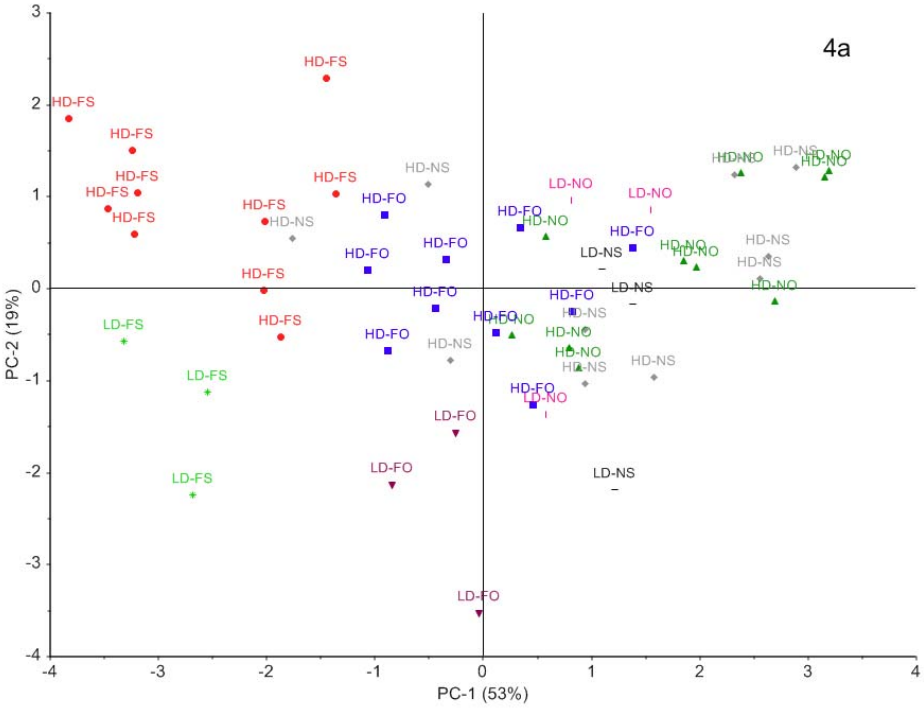
419 Considering the other pasting parameters of the 13 genotypes, the pasting
420 temperature (PT) of all treatments ranged between 59.1 and 66.9 °C, well within the
421 range of PT reported in the literature (Bertolini et al., 2000; Gomes et al., 2005). For
422 instance according to a study of over 4000 cassava genotypes by Sanchez et al.
423 (2009) PT ranged from 58.8°C to 71.2°C. For unmodified samples (NO), average
424 PV2, BD, FV and SB were 950, 382, 832 and 263 cP, respectively. Except for SB,
425 these results were close to the averages reported by Sanchez et al. (2009), and
426 therefore further confirmed that the 13 genotypes in this study represented typical
427 values for cassava.

428

429 Statistically significant differences between lowland and highland genotypes were
430 found, for all treatments, in pasting temperature and peak viscosity. PT was
431 consistently 3.5°C lower, and PV1 was consistently higher (81-168 cP higher),

including the case of the single viscosity peak in FS samples, in highland compared to lowland genotypes. Hence, the pasting temperature appeared to be a relevant parameter to differentiate lowland and highland genotypes, similar to the gelatinization temperature parameter measured by DSC.

Additionally fermentation treatments (FO and FS) resulted in significantly higher breakdowns in highland genotypes compared to lowland genotypes (BD 115 and 101 cP higher after FO and FS, respectively). This difference in breakdown may indicate that the granular structure was more severely damaged during fermentation in highland genotypes, resulting in more comprehensive granule disruption and a larger drop in viscosity after the viscosity peak is reached. This result would confirm the hypothesis introduced in section 3.4: highland genotypes granules were damaged throughout their structure, whereas lowland genotypes were attacked only on their surface, leading to more extensive granule breakdown in highland genotypes during cooking.



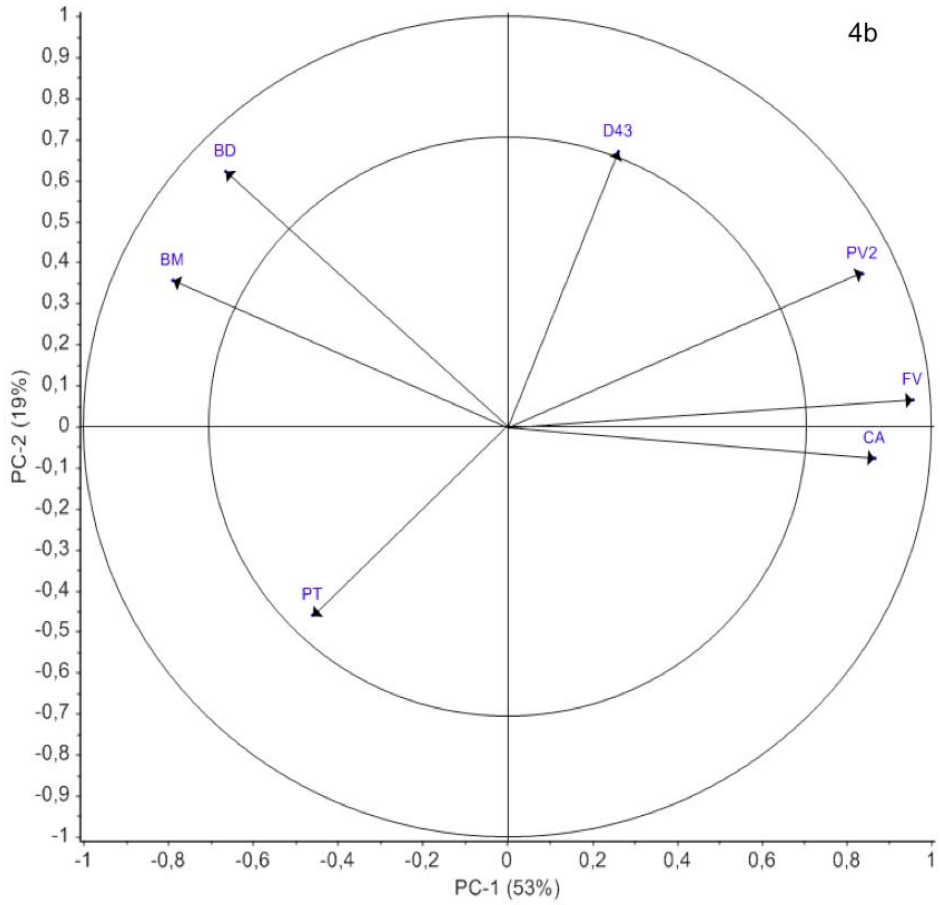


Fig. 4. Score plot (4a) and loading plot (4b) of principal components 1 and 2, describing the variation of the samples. HD = highland and LD = lowland. NO = non fermented, oven-dried; NS = Non-fermented, sun-dried; FO = Fermented, oven-dried; FR = Fermented, sun-dried. BM = Breadmaking ability, BD = RVA Breakdown, D43 = Average granule diameter, PV2 = Peak viscosity 2, FV = Final viscosity, CA = Cooking ability, PT = Pasting temperature. Colors correspond to samples grouped by altitude and post-harvest treatments.

With the aim of confirming our findings, a principal component analysis (PCA) was performed, using five selected RVA parameters (PT, PV2, CA, BD and FV), granular size and the bread loaf expansion values for the 13 samples (Fig. 4). The representation of the scores plot (Fig. 4a) allowed to confirm a stronger effect of fermentation over sun-drying on sour starch properties, with non-fermented samples (NO and NS) appearing mixed within one group, whereas fermented samples (FO and FS) samples were segregated along the first principal component (PC1). Nevertheless the synergy between fermentation and sun-drying was also confirmed, with FS samples markedly distributed along the second principal component (PC2),

while FO samples laid close to the NO+NS samples. Additionally, a differential effect of fermentation was observed depending on the altitude of cultivation, with fermented lowland and highland samples (FO and FS) segregated along PC2. The representation of the loading plot (Fig. 4b) confirmed the observations from other techniques, in particular the lower granule size of fermented lowland genotypes, the higher pasting temperature (PT) of lowland genotypes, the decrease in RVA viscosity (PV2, FV) with increasing strength of the treatments from NO to FS, the larger RVA breakdown (BD) and better breadmaking ability (BM) of highland FS samples. This latter observation confirmed the potential use of the RVA breakdown as predictive parameter for breadmaking ability of sour starch.

3.6. Intrinsic viscosity

Intrinsic viscosities data were determined for seven of the cassava genotypes (Table 6). The average values show the following sequence: NO > FO ≈ NS > FS. This result was in agreement with literature (Bertolini et al., 2000; Bertolini, Mestres, Colonna & Raffi, 2001c; Bertolini et al., 2001b; Marcon et al., 2009; Mestres & Rouau, 1997), although different protocols and/or sample treatments in previous studies resulted in different intrinsic viscosity values. The intrinsic viscosity values were sensitive to treatments: fermentation (FO) as well as sun-drying (NS) reduced the intrinsic viscosity (19.4 and 21.1 % respectively compared to NO) and this effect was even more pronounced for the combined fermentation and sun-drying FS treatment (34.6 % reduction). These results were in line with those determined by Fiedorowicz and Rębilas (2002) who found a molecular diminution of amylopectin in corn starch during a illumination.

The altitude may also be a determinant factor: for highland genotypes, NS and FO treatments decreased the average intrinsic viscosity by 23.8 % and 26.4 % respectively compared to NO, in contrast to respectively 5.9 % and 4.5 % for lowland genotypes. FS treatment reduced the intrinsic viscosity more effectively than other treatments and this change was similar in lowland and highland genotypes (36.4 and 33.9% respectively), compared to NO. These results further support the hypothesis that FO and NS treatments affected lowland genotypes only on the surface of the starch granules, whereas in highland genotypes modifications occurred throughout the granule structure. Thus, the core of starch granules in lowland genotypes could remain mainly unmodified while the outer layers could be removed (as indicated by the reduction in granule diameter discussed in section 3.4), resulting in high intrinsic viscosity. In contrast, starch molecular weight of highland genotypes could be reduced throughout the granules due to depolymerisation, resulting in lower intrinsic viscosity.

505

Table 6

Intrinsic viscosity of native fermented and/or sun-dried cassava starches.

Genotype	Intrinsic viscosity (mL/g) [y]			
	Treatments [z]			
	NO [z]	NS [z]	FO [z]	FS [z]
Lowland				
HMC-1	140.8	147.1	134.6	115.9
CM4574-7	183.6	162.6	170.7	90.5
Highland				
CM7138-7	189.7	122.0	140.0	101.6
SM7591-5	217.2	176.4	162.5	163.3
Cumbre 3	176.6	128.6	152.1	135.9
SM707-17	188.2	149.7	142.7	146.2
SM1495-5	220.0	152.8	158.5	108.0
Lowland	162.2(30.2)[b]	152.6(25.6)[b]	154.9(11.0)[b]	103.2(17.9)[a]
Highland	198.3(19.2)[b]	151.2(9.7)[a]	145.9(21.6)[a]	131.0(25.9)[a]
All genotypes	188.0(26.6)[c]	148.4(18.7)[b]	151.6(13.1)[b]	123.0(26.2)[a]

[y] Values obtained from intrinsic viscosity measurements.

[z] Treatments: NO: non fermented, oven-dried ; NS: non fermented, sun-dried; FO: fermented, oven-dried ; FS: fermented, sun-dried.

[a-c] Different letter, within row, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.

506

507 In addition, an important genotype effect on intrinsic viscosity was observed
508 regardless of the treatments (Table 6). Cluster analyses (not shown) confirmed that
509 low, medium and high viscosity groups of cassava genotypes could be identified
510 within each treatment (NO, NS, FO, FS). However, no correlation was found between
511 breadmaking ability and intrinsic viscosity data.

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4. Conclusion

The influence of genotypes, altitude of cultivation and post-harvest treatments (fermentation and sun-drying) on the breadmaking ability of 13 cassava starches from Colombia was analyzed. Post-harvest treatments were prevailing factors in improving breadmaking ability, while the genotype factor had a smaller influence. Among post-harvest treatments, fermentation had a more pronounced effect than sun-drying, in particular on starch granule structure as evidenced by granule size analysis and RVA parameters such as PV1 and PV2. The combination of both treatments was nevertheless necessary to obtain the highest dough expansion. These results confirm several previous studies (Mestres et al. 1997, Bertolini et al. 2000, Bertolini et al. 2001a, Marcon et al. 2009, Guerra Dias et al. 2011), which showed that fermentation and sun-drying cause oxidative depolymerization and decrease RVA and intrinsic viscosities, and consequently increase loaf expansion. However no definitive linear correlation was found between depolymerization (measured by intrinsic viscosity) and expansion. One possible explanation, suggested by our results, is that other factors are at play.

In particular, the comparison of 13 different genotypes in this study showed that amylose content influenced negatively dough expansion, possibly because of amylose-lipid complex formation. Hence, selecting low-amylose cassava genotypes may help to obtain more consistent breadmaking quality. In particular, expansion tests with recently discovered amylose-free cassava genotypes (Rolland-Sabaté et al., 2013) could confirm this hypothesis.

Other physicochemical parameters did not show direct correlations with expansion properties. However, differences in sensitivity to the fermentation treatment for lowland and highland genotypes were apparent, in particular, through particle size, RVA and intrinsic viscosity analyses. These observations led to the idea that to understand the phenomena underpinning sour cassava starch breadmaking ability, it is important to make a distinction between molecular and supra-molecular levels.

Firstly at molecular level, fermentation and sun-drying caused depolymerization for all genotypes, which helped increase loaf volume due to the reduced viscosity of the dough during expansion, as predicted by the model of bubble expansion proposed by Fan, Mitchell and Blanshard (1999). Different models (Hailemariam, Okos and Campanella, 2007) predict that other phenomena can also have significant effects on loaf expansion at different stages of baking, including mass transfers, such as migration of CO2 or water from the surrounding matrix to the expanding bubbles, inertia and surface tension. Hence further work could focus on determining which of these phenomena are dominant for the expansion of sour cassava dough.

Secondly, at supra-molecular level, our results point to the hypothesis that depolymerization was different for lowland and highland genotypes: Lowland genotypes were attacked mainly on the surface, resulting in smaller but mainly intact granules containing high molecular weight starch; whereas highland genotypes underwent depolymerization throughout the granules, which was key to enhance breadmaking, due to the reduced molecular weight of starch, and possibly a more extensive granule collapse during gelatinization and better film formation around the bubbles of steam driving dough expansion.

Further work to characterize starch solubility and the proportions of A and B crystalline types could advance the understanding of molecular and supra-molecular level phenomena controlling the emergence of breadmaking properties during fermentation and sun-drying. The combination of cassava genotypes and fermentation and drying operations to tailor the properties of sour cassava starch is expected to open new avenues for developments of gluten-free ingredients for bakery products (bread, pizza dough, etc) and expanded snacks based on cassava starch.

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590 **Bibliography.**

591

592 Bertolini, A. C., Mestres, C., & Colonna, P. (2000). Rheological properties of acidified and
593 UV-Irradiated starches. *Starch-Starke, 52*(10), 340-344.

594 Bertolini, A. C., Mestres, C., Lourdin, D., Valle, G. D., & Colonna, P. (2001a). Relationship
595 between thermomechanical properties and baking expansion of sour cassava starch
596 (Polvilho azedo). *Journal of the Science of Food and Agriculture, 81*(4), 429-435.

597 Bertolini, A. C., Mestres, C., Raffi, J., Buléon, A., Lerner, D., & Colonna, P. (2001b).
598 Photodegradation of Cassava and Corn Starches. *Journal of Agricultural and Food*
599 *Chemistry, 49*(2), 675-682.

600 Bertolini, A. C., Mestres, C., Colonna, P., & Raffi, J. (2001c). Free radical formation in UV-
601 and gamma-irradiated cassava starch. *Carbohydrate Polymers, 44*(3), 269-271.

602 Camargo, C., Colonna, P., Buleon, A., & Richard-Molard, D. (1988). Functional properties of
603 sour cassava (Manihot utilissima) starch: Polvilho azedo. *Journal of the Science of Food*
604 *and Agriculture, 45*(3), 273-289.

605 Cárdenas, O. S., & de Buckle, T. S. (1980). Sour cassava starch production: a preliminary
606 study. *Journal of Food Science, 45*(6), 1509-1512.

607 Cavallini, C. M., & Franco, C. M. L. (2010). Effect of acid-ethanol treatment followed by ball
608 milling on structural and physicochemical characteristics of cassava starch. *Starch -*
609 *Stärke, 62*(5), 236-245.

610 Ceballos, H., Sánchez, T., Denyer, K., Tofiño, A. P., Rosero, E. A., Dufour, D., et al. (2008).
611 Induction and Identification of a Small-Granule, High-Amylose Mutant in Cassava
612 (Manihot esculenta Crantz). *Journal of Agricultural and Food Chemistry, 56*(16), 7215-
613 7222.

614 Cereda, M. P. (1973). Alguns aspectos sobre a fermentação da fécula de mandioca (PhD
615 dissertation). Faculdade de Ciências Médicas e Biológicas. Universidade do Estado de
616 São Paulo (UNESP). Botucatu, São Paulo, Brazil.
617 Defloor, I., Dehing, I., & Delcour, J. A. (1998). Physico-Chemical Properties of Cassava
618 Starch. *Starch - Stärke, 50*(2-3), 58-64.
619 Demiate, I. M., Dupuy, N., Huvenne, J. P., Cereda, M. P., & Wosiacki, G. (2000).
620 Relationship between baking behavior of modified cassava starches and starch chemical
621 structure determined by FTIR spectroscopy. *Carbohydrate Polymers, 42*(2), 149-158.
622 Dufour, D., Brabet, C., Zakhia, N., & Chuzel, G. (1995). Influence of fermentation and sun-
623 drying on the breadmaking capacity of cassava sour starch. In E. T. Agbor, A. Brauman,
624 D. Griffon & S. Trèche (Eds.). *Transformation alimentaire du manioc* (pp. 399-416).
625 Paris: ORSTOM.
626 Dufour, D., Larsonneur, S., Alarcon Morante, F., Brabet, C., & Chuzel, G. (1996). Improving
627 the breadmaking potential of cassava sour starch. In D. Dufour, G. M. O'Brien & R. Best
628 (Eds.). *Cassava flour and Starch: Progress in research and development*, 133-142. Cali,
629 Colombia: CIAT-CIRAD.
630 FAO. (2004). Domestic market opportunities. In *Global Cassava Market Study Business*
631 *Opportunities for the Use of Cassava* (6th ed., p. 184). Rome: FAO
632 Fan, J., Mitchell, J.R., Blanshard, J.M.V. (1999). A model for the oven rise of dough during
633 baking. *Journal of Food Engineering* 41, 69-77
634 Fiedorowicz, M., & Rębilas, K. (2002). Physicochemical properties of waxy corn starch and
635 corn amylopectin illuminated with linearly polarised visible light. *Carbohydrate*
636 *Polymers*, *50*(3), 315-319.
637 Escobar, A., Dufour, E., Sanchez, T., Giraldo, A., & Dufour, D. (2009). Evaluacion de yuca
638 (Manhiot esculenta Crantz) de altura para la produccion de almidon en Colombia.

Alimentos Hoy : Revista de la Asociacion colombiana de ciencia y tecnologia de alimentos, 2009. (18). Congreso Iberoamericano de Ingenieria en Alimentos. Bogota, Colombia 06-09 September.

Franco, C. M. L., Ogawa, C., Rabachini, T., Rocha, T. d. S., Cereda, M. P., & Jane, J.-l. (2010). Effect of lactic acid and UV irradiation on the cassava and corn starches. *Brazilian Archives of Biology and Technology, 53*, 443-454.

Garcia, V., Colonna, P., Bouchet, B., & Gallant, D. J. (1997). Structural Changes of Cassava Starch Granules after Heating at Intermediate Water Contents. *Starch - Stärke, 49*(5), 171-179.

Gomes, A. M. M., Mendes da Silva, C. E., & Ricardo, N. M. P. S. (2005). Effects of annealing on the physicochemical properties of fermented cassava starch (polvilho azedo). *Carbohydrate Polymers, 60*(1), 1-6.

Guerra Dias, A. R., Zavareze, E. d. R., Elias, M. C., Helbig, E., da Silva, D. O., & Ciacco, C. F. (2011). Pasting, expansion and textural properties of fermented cassava starch oxidised with sodium hypochlorite. *Carbohydrate Polymers, 84*(1), 268-275.

Hailemariam, L., Okos, M., Campanella, O., (2007). A mathematical model for the isothermal growth of bubbles in wheat dough. *Journal of Food Engineering*, 82, 466-477.

Harding, S. E. (1997). The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. *Progress in biophysics and molecular biology, 68*(2-3), 56.

Huang, Z.-Q., Lu, J.-P., Li, X.-H., & Tong, Z.-F. (2007). Effect of mechanical activation on physico-chemical properties and structure of cassava starch. (Vol. 68, p. 8). Kidlington, England: Elsevier.

Jinapong, N., Suphantharika, M., & Jamnong, P. (2008). Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. *Journal of Food Engineering, 84*(2), 194-205.

Kent, N. L. (1985). *Technical compendium on composite flours: Technologies available for application*. Etiophia: FAO, United Nation Economic Commission for Africa.

Marcon, M. J. A., Kurtz, D. J., Raguzzoni, J. C., Delgadillo, I., Maraschin, M., Soldi, V., et al., (2009). Expansion Properties of Sour Cassava Starch (Polvilho Azedo): Variables Related to its Practical Application in Bakery. *Starch-Starke, 61*(12), 716-726.

Mestres, C., Boungou, O., Akissoe, N., & Zakhia, N. (2000). Comparison of the expansion ability of fermented maize flour and cassava starch during baking. *Journal of the Science of Food and Agriculture, 80*(6), 665-672.

Mestres, C., & Rouau, X. (1997). Influence of Natural Fermentation and Drying Conditions on the Physicochemical Characteristics of Cassava Starch. *Journal of the Science of Food and Agriculture, 74*(2), 147-155.

Mestres, C., Rouau, X., Zakhia, N., & Brabet, C. (1996). Physicochemical properties of cassava sour starch. In D. Dufour, G. M. O'Brien & R. Best (Eds.). *Cassava Flour and Starch : Progress in Research and Development* (pp. 143-149). Cali, Colombia: CIAT-CIRAD.

Mestres, C., Zakhia, N., & Dufour, D. (1997). Functional and physico-chemical properties of sour starch. In P. J. Frazier & P. Rich- mond & A.M. Donald (Eds.). *Starch: Structure and Functionality* (pp. 42-50). Cambridge, UK : The Royal Society of Chemistry. Special publication no. 205.

Niba, L. L., Bokanga, M. M., Jackson, F. L., Schlimme, D. S., & Li, B. W. (2002). Physicochemical Properties and Starch Granular Characteristics of Flour from Various Manihot Esculenta (Cassava) Genotypes. *Journal of Food Science, 67*(5), 1701-1705.

687 Nuwamanya, E., Baguma, Y., Emmambux, N., & Taylor, J. (2010). Physicochemical and
688 functional characteristics of cassava starch in Ugandan varieties and their progenies.
689 *Journal of Plant Breeding and Crop Science, 2*(1), 1-11.
690 Nwokocha, L. M., Aviara, N. A., Senan, C., & Williams, P. A. (2009). A comparative study
691 of some properties of cassava (Manihot esculenta, Crantz) and cocoyam (Colocasia
692 esculenta, Linn) starches. *Carbohydrate Polymers, 76*(3), 362-367.
693 Onitilo, M. O., Sanni, L. O., Oyewole, O. B., & Maziya-Dixon, B. (2007). Physicochemical
694 and Functional Properties of Sour Starches from Different Cassava Varieties.
695 *International Journal of Food Properties, 10*(3), 607-620.
696 Putri, W. D. R., Haryadi, D. W., & Marseno and Cahyanto, M. N. (2011). Effect of
697 biodegradation by Lactic Acid Bacteria on physical properties of cassava starch.
698 *International Food Research Journal, 18*(3), 1149-1154.
699 Rolland-Sabaté, A., Sanchez, T., Buléon, A., Colonna, P., Ceballos, H., Zhao, et al.. (2013).
700 Molecular and supra-molecular structure of waxy starches developed from cassava
701 (Manihot esculenta Crantz). *Carbohydrate Polymers, 92*(2), 1451-1462.
702 Sánchez, T., Mafla, G., Morante, N., Ceballos, H., Dufour, D., Calle, F., et al. (2009).
703 Screening of Starch Quality Traits in Cassava (Manihot esculenta Crantz). *Starch -*
704 *Stärke, 61*(5), 310-310. (Erratum: Starch/Stärke (61) 310 (2009))
705 Shirai, M.A., Haas, A., Ferreira, G.F., Matsuguma, L.S., Franco, C.M.L, Demiatte I.M.
706 (2007). Características físico-químicas e utilização em alimentos de amidos modificados
707 por tratamento oxidativo. *Ciência e Tecnologia de Alimentos, 27*(2), 239-247.
708 Sokal, R. R., & Michener, C. D. (1958). A Statistical Method for Evaluating Systematic
709 Relationships. *The University of Kansas Scientific Bulletin*, *38*, 1409–1438.
710 Sriroth, K., Santisopasri, V., Petchalanuwat, C., Kurotjanawong, K., Piyachomkwan, K., &
711 Oates, C. G. (1999). Cassava starch granule structure–function properties: influence of

712 time and conditions at harvest on four cultivars of cassava starch. *Carbohydrate*
713 *Polymers, 38*(2), 161-170.
714 Tester, R. F., & Morrison, W. R. (1992). Swelling and gelatinization of cereal starches. III.
715 Some properties of waxy and normal nonwaxy barley starches. *Cereal Chemistry, 69*(6),
716 654–658.
717 Vatanasuchart, N., Naivikul, O., Charoenrein, S., & Sriroth, K. (2005). Molecular properties
718 of cassava starch modified with different UV irradiations to enhance baking expansion.
719 *Carbohydrate Polymers, 61*(1), 80-87.
720 Westby, A., & Cereda, M. P. (1994). Production of fermented cassava starch (polvilho azedo)
721 in Brazil. *Tropical science, 34*(2), 203-210.
722 Zakhia, N., Dufour, D., Chuzel, G., & Griffon, D. (1996). Review of sour cassava starch
723 production in rural Colombian areas. *Tropical science, 36*(4), 247-255.
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Highlights

- Cassava starch breadmaking ability is enhanced by fermentation and sun-drying.
- Amylose content decreased breadmaking ability of sour cassava starch.
- Fermentation/sun-drying decrease starch molecular size, as shown by RVA and intrinsic viscosity.
- Edaphoclimatic conditions influence susceptibility of raw cassava starch to hydrolysis.
- Sour cassava starch is promising for development of gluten-free ingredients.