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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 1000 m and 1700 m.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 (3.0–8.6 mL/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.



## 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; Westby & Cereda, 1994, in Brazil and Cárdenas & 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 h. 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 behavior 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 et al., 2011); and starch paste thermomechanical properties (Bertolini, Mestres, Lourdin, Valle, & Colonna, 2001). 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

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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, et al., 2001; 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 behavior (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 et al., 2007) in contrast with most works which used only one or two genotypes and therefore did not take the into consideration the 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 have been 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 12 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 (4 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 casava roots were transported to a traditional factory (*Rallandería*) 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 24 h 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 h to obtain native, non-fermented, oven-dried cassava starch (NO). The second subsample was sun-dried for about 12 h 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 24 h at 40 °C to obtain fermented, oven-dried starch (FO), and the fourth part was sun-dried for 12 h 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 (dry matter), 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 ($\Delta H$) of each sample were determined on the thermograms. The gelatinization temperature range ($\Delta T$) 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.

### 2.5. Granular size

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

### 2.6. Pasting properties

The methodology reported by Sánchez et al. (2009) was used. Hot starch dispersion viscosity profiles were obtained with a Rapid Visco Analyser model RVA-4 Series (Newport Scientific, Warriewood, Australia). Starch (1.25 g db) was dispersed in distilled water (23 $cm^3$) to yield a 5% suspension. Viscosity was recorded using the following temperature profile: holding at 50 °C for 1 min, heating from 50 °C to 90 °C at 6 °C $min^{-1}$, holding at 90 °C for 5 min and then cooling down to 50 °C at 6 °C $min^{-1}$ with continuous stirring first at 960 rpm for 10 s and then at 160 rpm throughout the rest of the experiment. Eight parameters were measured on the visco-amylogram: pasting temperature and pasting time (PT and Pt), peak viscosity 1 (PV1: first viscosity peak after beginning of pasting), peak viscosity 2 (PV2: second viscosity peak after beginning of pasting), time of peak viscosity 1 and 2 (tPV1 and tPV2, respectively), lowest hot paste viscosity or holding strength (HS), final viscosity (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) × 100, setback (SB) estimated as FV-HS and relative setback (RSB) estimated by (SB/FV) × 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 h (±10 min) on a roller mixer in a temperature-controlled room at 22 °C (±0.5 °C). The resulting solution was centrifuged at 10,000 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 just a single 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

Statistical signifcance of differences between sample means was 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).

**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] |

[a–c] Different letters, within row, indicate significant differences at $p < 0.05$ (Fisher).
[1,2] Different numbers, within column, indicate significant differences at $p < 0.05$ (Fisher).
[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.

## 3. 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.

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,

**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) | $\Delta H$ (J g$^{-1}$) | $\Delta T$ (°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) |

[a–h] Different letters, within each column, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.
[y] Values obtained from DSC: $T_o$: onset gelatinization temperature; $T_p$: peak gelatinization temperature; $T_c$: conclusion temperature.

SM707-17, SM1495-5 and Tambo 4 ranging from 7.49 mL/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 S-5 ranging from 3.05 mL/g to 4.47 mL/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 explaining the observed differences in breadmaking ability among samples.

### 3.2. Thermal properties

Thermal properties were investigated only in non-fermented, oven-dried (NO) samples, 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, Mestres, Lourdin, et al., 2001; Bertolini, Mestres, Raffi, et al., 2001; 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, Mestres, Raffi, et al., 2001).

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

DSC parameters are linked to the cohesion in the structure of granule (Marcon et al., 2009), the integrity and the crystalline structure of starch granule (Mestres & Rouau, 1997). $T_o$, $T_p$ and $T_c$ of lowland genotypes were 3.81, 3.66 and 3.12 °C significantly higher than those of highland. In contrast, no statistically significant difference was found between lowland and highland genotypes in terms of gelatinization enthalpy ($\Delta H$). Thus, $T_o$, $T_p$ and $T_c$ could be used to differentiate starches from lowland and highland genotypes. The narrow gelatinization temperature range ($\Delta T$) suggested a 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, & Cahyanto, 2011).

As seen in Table 2, a high variability of DSC parameters was observed, indicating a pronounced genotype effect. A Fisher test (Table 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.

Fig. 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.

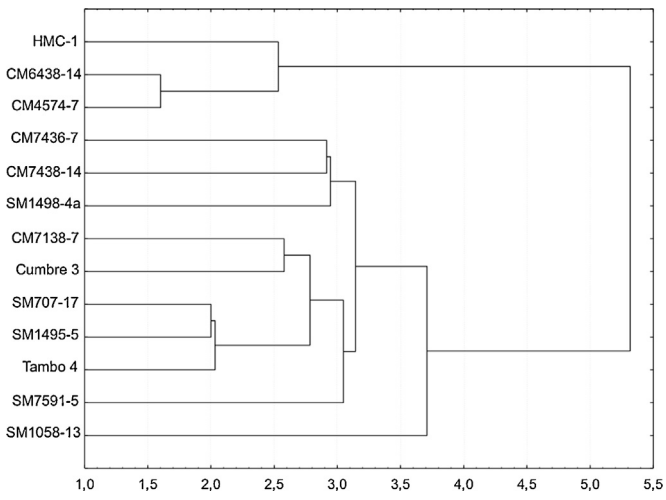


**Fig. 1.** Multivariate cluster analysis of the pooled DSC parameters ($T_o$, $T_p$, $T_c$ and $\Delta H$) identifying similarities and differences of between thirteen genotypes of cassava starch.

**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) |

[a–g] Different letters indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.
[y] Values obtained from DSC measurements.

### 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 et al., 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 (Table 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 association 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 relationship 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.

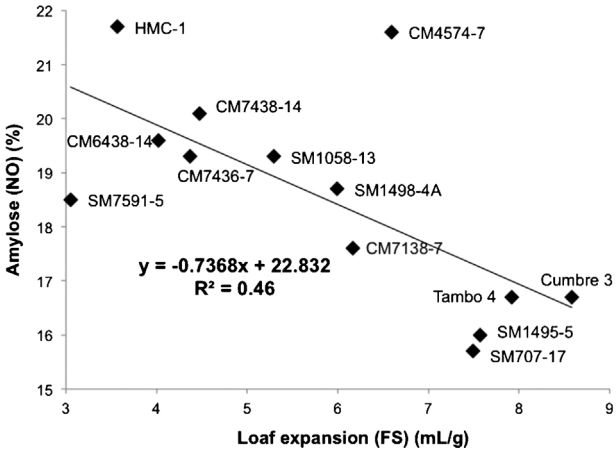


**Fig. 2.** Negative association ($R^2 = 0.46$) between loaf expansion of fermented, sun-dried starches (FS) and amylose content of non-fermented, oven-dried (NO) starches.

### 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 (Table 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 et al., 1999).

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 (Table 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.

**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] |

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

**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)$^{b}$ |

$^{a–d}$ Different letters, within column, indicate significant differences at $p < 0.05$ (Fisher). Standard deviations are given within brackets.
[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.

### 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 (Table 5).

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

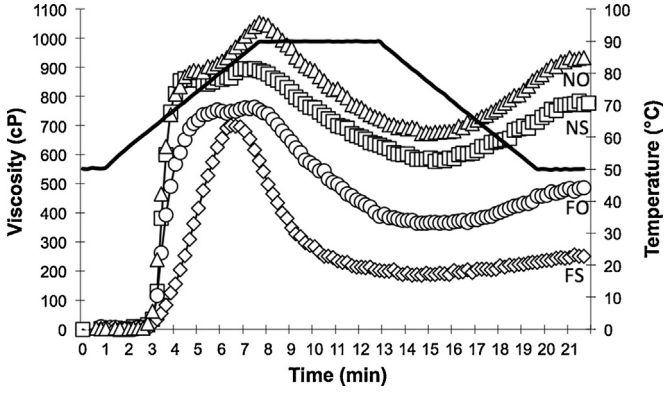


**Fig. 3.** Viscoamylograms of one cassava starch sample (SM 1495–5) after 4 treatments: NO (open black triangles), NS (open black squares), FO (open black circles) and FS (open black diamonds).

Statistically significant differences between lowland and highland genotypes were found, for all treatments, in pasting temperature and peak viscosity. PT was 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.

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

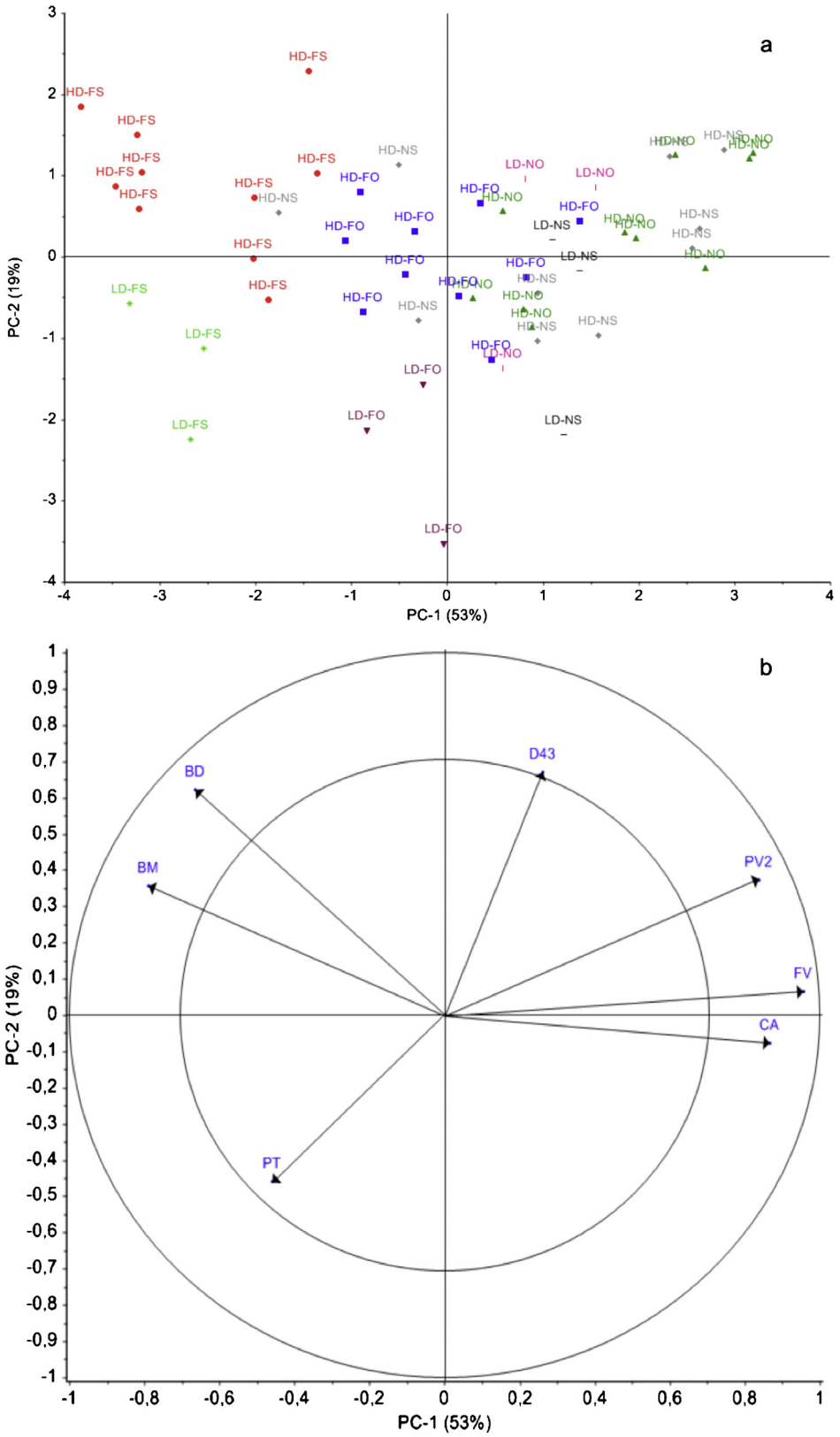


**Fig. 4.** Score plot (a) and loading plot (b) of principal components 1 and 2, describing the variation of the samples. HD = highland and LD = lowland. NO = non fermented, oven-dried; NS = non-fermented, sun-dried; FO = fermented, oven-dried; FS = fermented, sun-dried. BM = breadmaking ability, BD = 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

**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] |

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

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, 2001; Bertolini, Mestres, Raffi, et al., 2001; 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.

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

## 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 (Bertolini et al., 2000; Bertolini, Mestres, Lourdin, et al., 2001; Marcon et al., 2009; Mestres et al., 1997; 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, & 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 $CO_2$ 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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