

Accepted Manuscript

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

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id="fn1"><ce:note-para id="npar0005">These authors
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PII: S0144-8617(13)00695-4
DOI: <http://dx.doi.org/doi:10.1016/j.carbpol.2013.07.012>
Reference: CARP 7910

To appear in:

Received date: 4-4-2013
Revised date: 7-6-2013
Accepted date: 3-7-2013

Please cite this article as: Alvarado, P. M., Grosmaire, L., Dufour, D., Toro, A. G., Sanchez, T., Calle, F., Santander, M. A. M., Ceballos, H., Delarbre, J. L., & Tran, T., Combined effect of fermentation, sun-drying and genotype on breadmaking ability of sour cassava starch, *Carbohydrate Polymers* (2013), <http://dx.doi.org/10.1016/j.carbpol.2013.07.012>

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1 **Combined effect of fermentation, sun-drying and genotype on breadmaking**
2 **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. Supramolecular granule structure influenced sensitivity to fermentation.

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

54 **1. Introduction**

55 The Composite flour Program of FAO established in 1964 (Kent, 1985) financed
56 research in tropical countries to substitute wheat flour with cassava flour. Consumer
57 acceptability decreased in mixtures with > 30% cassava flour, due to noticeable
58 color, texture, and flavor differences (FAO, 2004). In South America a traditional
59 alternative to flour is the fermentation of cassava starch combined with sun-drying,
60 which has excellent breadmaking capacity (Cereda, 1973 and Westby and Cereda,
61 1994, in Brazil and Cárdenas and de Buckle, 1980 and Zakhia, Dufour, Chuzel &
62 Griffon, 1996, in Colombia). These authors described the process to obtain
63 fermented or sour starch (“Polvilho azedo” in Brazil or “Almidón agrio” in Colombia).
64 In this process, the main steps are starch extraction, natural fermentation for about
65 30 days and final sun-drying for 12 hours. The sour starch is used locally for bread
66 and pastry production. There are also opportunities to use sour cassava starch as
67 adjuvant for breadmaking or as the main ingredient for gluten-free breads. It is
68 important, therefore, to understand better the mechanisms giving breadmaking ability
69 of sour cassava starch.

70

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

79 starch paste thermomechanical properties (Bertolini, Mestres, Lourdin, Valle &
80 Colonna, et al., 2001a). Evidence from these studies suggests that a degradation
81 mechanism of starch occurs during fermentation and that sun-drying plays a key role
82 in the breadmaking properties. However, the nature of the changes are not fully
83 elucidated (Dufour, Brabet, Zakhia & Chuzel, 1995). Two hypotheses have been
84 suggested to explain the link between starch degradation and breadmaking ability:
85 firstly at molecular level, free-radical formation results in starch oxidation and
86 depolymerisation (Bertolini, Mestres & Colonna, 2000; Bertolini, Mestres, Raffi,
87 Buléon, Lerner & Colonna, 2001b; Demiate, Dupuy, Huvenne, Cereda & Wosiacki,
88 2000; Guerra Dias et al., 2011; Vatanasuchart, Naivikul, Charoenrein & Siroth, 2005)
89 or secondly at supramolecular level, the structure of the starch granules is altered
90 and results in modified gelatinization behaviour (Camargo, Colonna, Buleon &
91 Richard-Molard, 1988; Gomes, Mendes da Silva & Ricardo, 2005; Onitilo, Sanni,
92 Oyewole & Maziya-Dixon, 2007).

93
94 In addition, few publications explored the influence of cassava genotype on
95 breadmaking ability of sour starch (Escobar, Dufour, Sanchez, Giraldo & Dufour,
96 2009; Onitilo, Sanni, Oyewole & Maziya-Dixon, 2007) in contrast with most works
97 which used only one or two genotypes and therefore did not take the into
98 consideration genetic differences. Escobar et al. (2009) in particular, found that the
99 altitude above sea level at which cassava is cultivated appears to be another
100 determining factor for the breadmaking ability. Farmers traditional knowledge has
101 often indicated that genetic and climatic factors influence breadmaking ability.

102

103 The originality of this work is to study in detail the genetic effect on breadmaking
104 ability of sour cassava starch using 13 Colombian genotypes, cultivated in exactly the
105 same conditions at two locations with low-intermediate (1000 meters above sea level,
106 m.a.s.l.) or high (1700 m.a.s.l.) altitudes and referred to respectively as lowland and
107 highland. The effect of post-harvest treatments (fermentation and sun-drying of the
108 13 native starches), was analyzed through characterization of both native and
109 modified starches. Physicochemical parameters, pasting and thermal properties were
110 quantified in order to provide information on the degradation mechanism(s) of sour
111 starch. The aim of this work was to contribute to a better understanding of sour
112 cassava starch properties, and to highlight the effects of genotype, altitude and
113 process (fermented versus native starches) parameters on the breadmaking ability.

114

115 **2. Materials and methods**

116

117 **2.1. Genotypes and growing conditions**

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

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

132

133 **2.2. Treatments experimental conditions:**

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

148

149 **2.3. Breadmaking test**

150 Bread dough was prepared according to CIAT protocol (CIAT, unpublished protocol):
151 125 g of starch sample (12-13% moisture content), 75 g of "Costeño" cheese and
152 18.7 g of sunflower oil were hand mixed on a stainless steel plate. Secondly pre-

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

163

164 **2.4. Gelatinization temperature and amylose content**

165 The methodology reported by Mestres and Rouau (1997) was used. DSC analyses
166 were performed on a Perkin-Elmer DSC 7 device (Perkin-Elmer, Norwalk, VA) using
167 sealed stainless-steel pans. The sample pan (10 - 11 mg of starch and 50 μ L of lyso-
168 phospholipid (SIGMA, 2% w/v in water) and the reference pan (empty) were heated
169 from 25 to 160°C at 10°C.min⁻¹, held at 160°C for 2 min, and then cooled to 60°C at
170 10°C.min⁻¹. The temperatures of gelatinization (T_o , T_p and T_c) and enthalpies (ΔH) of
171 each sample were determined on the thermograms. The gelatinization temperature
172 range (ΔT) was calculated as ($T_c - T_o$) as described by Cavallini and Franco (2010).
173 Amylose content was also measured from the energy of amylose-lysophospholipid
174 complex formation. The analyses were performed in duplicate, and mean values
175 were calculated.

176

177

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 Supphantharika 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 cm³) 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

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

207

208 **2.7. Intrinsic Viscosity**

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

218

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

227

228 **2.8. Statistical analysis**

229 Statistically significant differences between samples means were determined using
230 analysis of variance (ANOVA) followed by a Fisher's test at 95% confidence level.
231 When relevant, a multivariate cluster analysis was performed to identify groups of
232 samples with similar characteristics, using the "average linkage method" based on
233 the calculation of the distances between the averages of samples (Sokal & Michener,
234 1958) and groups thereof using Statistica v7.1 software (StatSoft, Maison-Alfort,
235 FRANCE). The Principal Component Analysis (PCA) was carried out to identify
236 groups of samples with similar characteristics and performed with The Unscrambler X
237 10.2 software (Camo).

238

239 **Results and discussion**

240

241 **3.1. Breadmaking ability**

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

252

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.

253

254 Highland genotypes displayed higher significant average values of loaf expansion

255 than lowland's, regardless of the treatments. NO, FO, NS and FS treatments of

256 highland genotypes were 16.8, 18.9, 30.2 and 19.1% significantly higher than those

257 of lowland's, respectively. In lowland genotypes only FS improved breadmaking

258 (214% significantly higher than NO). In highland genotypes both NS and FS

259 improved breadmaking (40 and 223% significantly higher than NO, respectively). In

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

267
268 Cluster analysis and a Fisher test (not shown) carried out on the FS samples
269 confirmed the high variability between samples in terms of breadmaking ability. A
270 clear genotype effect was found, and two main groups were identified as follows. The
271 high breadmaking ability group includes 4 genotypes: Cumbre 3, SM707-17,
272 SM1495-5 and Tambo 4 ranging from 7.49mL/g to 8.58 mL/g. The intermediate and
273 lower breadmaking ability group was composed of 9 genotypes further separated into
274 two subgroups: the intermediate breadmaking ability of 4 genotypes: CM4574-7,
275 SM1498-4a, CM7138-7 and SM1058-13 ranging from 5.29 mL/g to 6.59 mL/g and
276 the lower breadmaking ability consisting of 5 genotypes: HMC-1, CM6438-14,
277 CM7436-7; CM7438-14 and CM7591-5 ranging from 3.05mL/g to 4.47mL/g. Altitude
278 was not a definite factor for predicting breadmaking ability, with one lowland
279 genotype having good breadmaking ability (CM4574-7) and one highland genotype
280 having poor breadmaking ability (SM7591-5). Hence both genetic and environmental
281 factors appeared important in the control of breadmaking ability, in addition to the
282 drying and fermentation processes. Other factors, such as the bread recipe
283 containing lipids, may also contribute explain the observed differences in
284 breadmaking ability among samples.

285

286 **3.2. Thermal properties**

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

293

294 The gelatinization temperatures (T_o , T_p and T_c), gelatinization temperature range (ΔT)
295 and enthalpy (ΔH) were comparable to values reported in the literature (Table 2)
296 (Bertolini et al. 2001a; Garcia, Colonna, Bouchet & Gallant, 1997; Marcon et al.,
297 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 (°C)	T_p (°C)	T_c (°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)

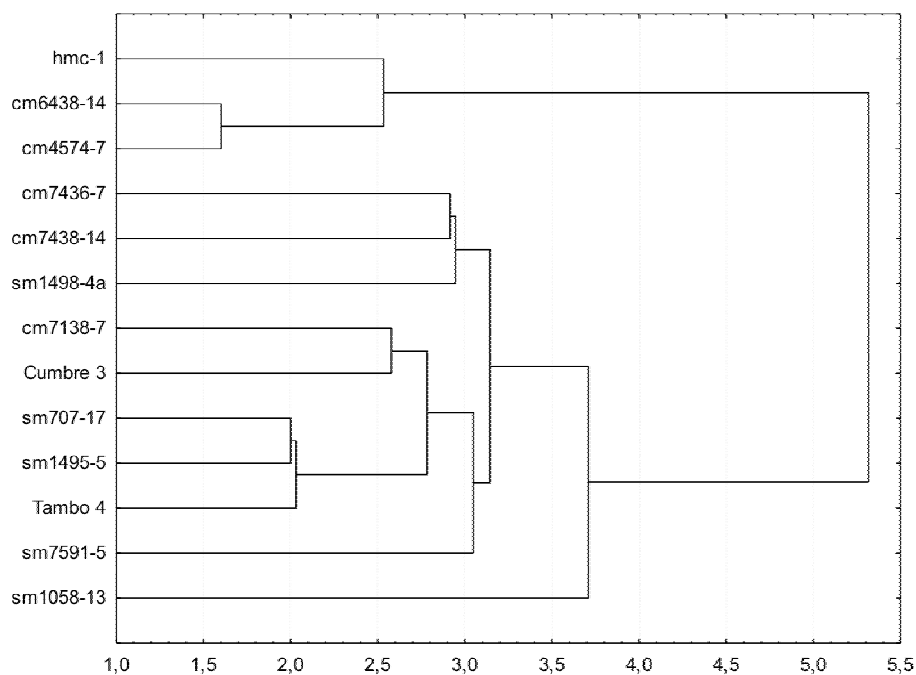
^y Values obtained from DSC: T_o : onset gelatinization temperature ; T_p : peak gelatinization temperature; T_c : conclusion temperature.

^{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

308 previous studies which found a broader gelatinization temperature range of
 309 approximately 20°C ($T_o = 61.35^\circ\text{C}$ and $T_c = 81.77^\circ\text{C}$) (Putri, Haryadi & Marseno and
 310 Cahyanto, 2011).

311



312
 313

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

316

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

321

322 Figure 1 also shows that the best five breadmaking genotypes (CM7138-7, Cumbre
 323 3, SM707-17, SM1495-5, Tambo 4) were clustered in two neighboring groups.
 324 Hence, information on breadmaking ability might be contained within DSC
 325 parameters, although more data would be needed to establish a more conclusive
 326 correlation.

327

328 **3.3. Amylose Content**

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

335

336 Altitude appeared to be a determining factor for amylose content, with the lowland
337 genotypes average (21.2%) being significantly higher than highland genotypes
338 (18.0%). A possible explanation could be a difference in starch biosynthesis at
339 different altitudes linked to different enzyme activities (probably related to the
340 differences in temperature at the two locations), as well as genetic diversity.

341

342 A high variability in amylose content was found between genotypes. Hence,
343 genotype effect was noticeable as confirmed by a Fisher test (Tab. 3) and a cluster
344 analysis (not shown). These tests showed three groups: (i) a low amylose group
345 including 4 genotypes (Cumbre 3, SM707-17, SM1495-5 and Tambo 4) ranging from
346 15.7 to 16.7%, (ii) an intermediate amylose group including 7 genotypes (CM6438-
347 14, CM7436-7, CM7438-14, SM1498-4a, CM7138-7, SM7591-5 and SM1058-13)
348 ranging from 17.6 to 20.1% and (iii) a high amylose group with 2 genotypes (HMC-1
349 and CM4574-7) at 21.6 and 21.7%. The four genotypes in the low amylose group
350 matched those in the high breadmaking ability group. Hence, lower amylose contents
351 appeared to improve breadmaking ability, which may be related to the formation of

352 less amylose-lipid complexes in low-amylose starches (Tester & Morrison, 1992). A
 353 weak negative correlation of $R^2 = 0.46$ was observed between the breadmaking
 354 ability of fermented, sun-dried starches (FS, both lowland and highland) and the
 355 amylose content (measured on non-fermented, oven-dried starches, NO) (Fig. 2).
 356 When one outlier (lowland CM4574-7) was removed, the correlation improved with R^2
 357 = 0.71, which tends to confirm the link between amylose content and breadmaking
 358 ability. Mestres et al. (2000) and Shirai et al. (2007) also reported a link between
 359 amylose content and loaf expansion, with low expansion for oxidized normal corn
 360 starch, and high expansion for oxidized waxy corn starch, comparable to sour
 361 cassava starch or oxidized cassava starch.
 362

Table 3

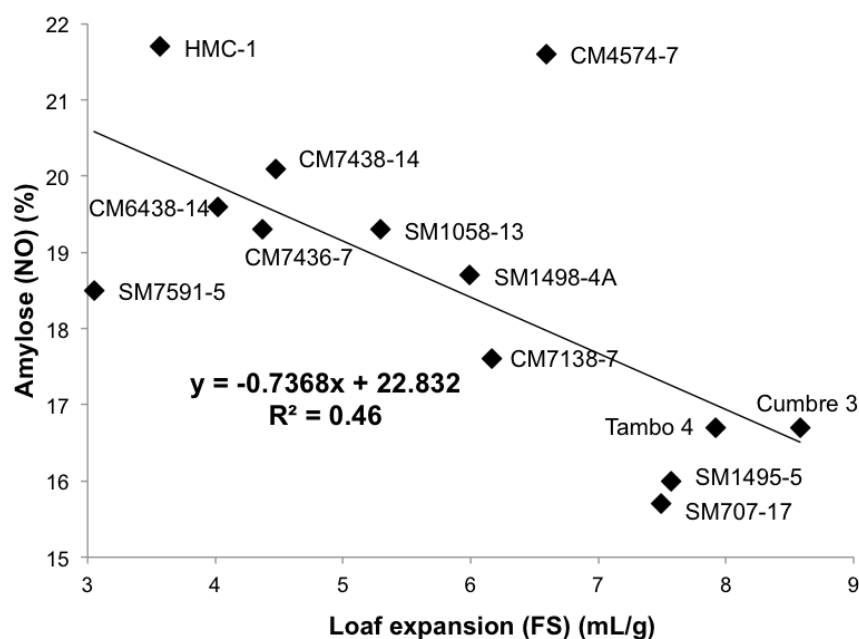
Amylose content of native cassava starches (NO).

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 measurements.^{a-g} Different letter indicate significant differences at

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

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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

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372

373

374

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; Siroth, 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.

375
376
377 A small difference was observed in the average granular sizes of non-modified (NO)
378 lowland and highland genotypes, which subsided after fermentation and drying
379 treatments. A more significant difference between lowland and highland genotypes
380 was observed in the decrease in granular size caused by the NS, FO and FS
381 treatments (Tab. 4), as confirmed by Fisher tests. In lowland genotypes NS, FO and
382 FS treatments decreased granule size by 4.6, 9.1 and 8.9 % respectively compared
383 to NO, whereas, in highland genotypes, these treatments had no influence on the
384 granular size. To explain this, it is hypothesized that different granular structures in
385 lowland and highland genotypes resulted in varied levels of sensitivity to treatments.
386 In lowland genotypes, NS, FO and FS treatments only shaved off the outer layers of
387 the granules, leading to smaller granules with mostly intact cores. In contrast, in
388 highland genotypes, granules were attacked more homogeneously throughout their
389 structure during fermentation, resulting in weakened granules but with a non-
390 significant reduction in granule average diameter. The RVA and intrinsic viscosities
391 results tended to support this hypothesis, as discussed below.

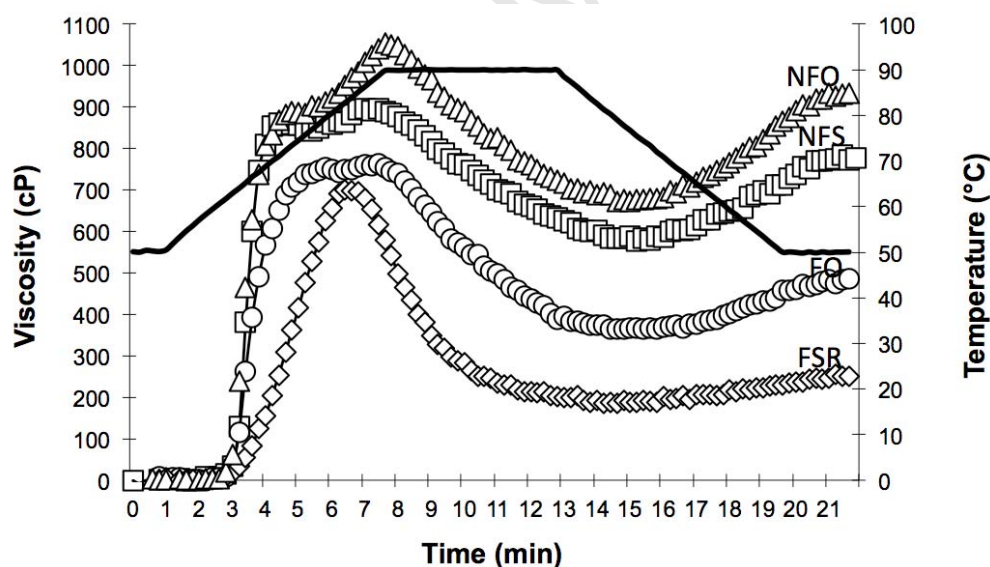
392

393 **3.5. Pasting behavior**

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

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

412
 413



414
 415 **Fig. 3.** Viscoamylograms of one cassava starch sample (sm 1495-5) after 4 treatments: NO (open
 416 black triangles), NS (open black squares), FO (open black circles) and FS (open black diamonds).
 417

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

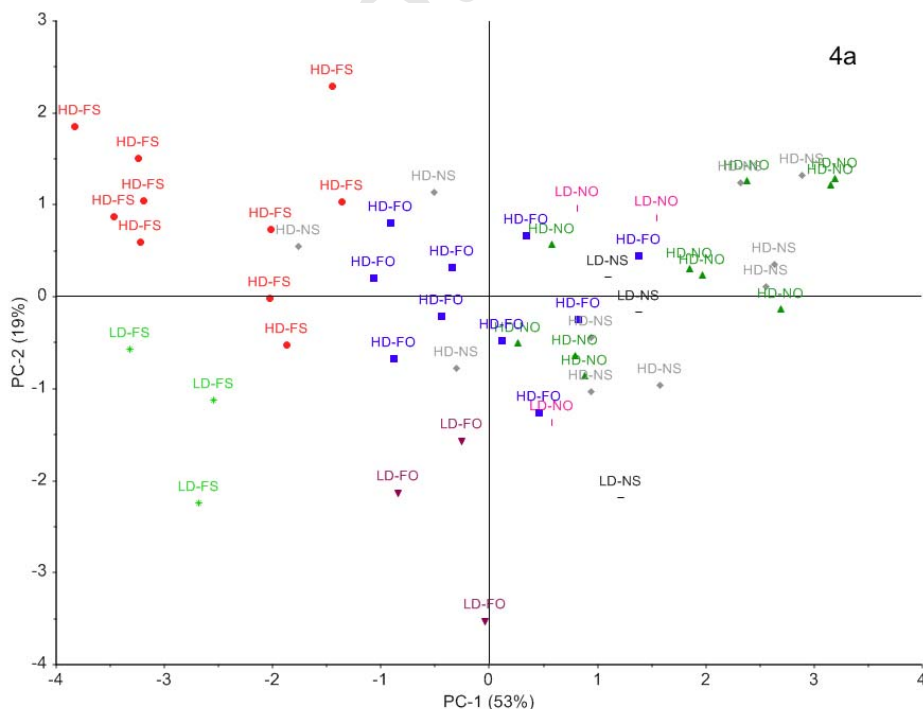
429 Statistically significant differences between lowland and highland genotypes were
430 found, for all treatments, in pasting temperature and peak viscosity. PT was
431 consistently 3.5°C lower, and PV1 was consistently higher (81-168 cP higher),

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

436

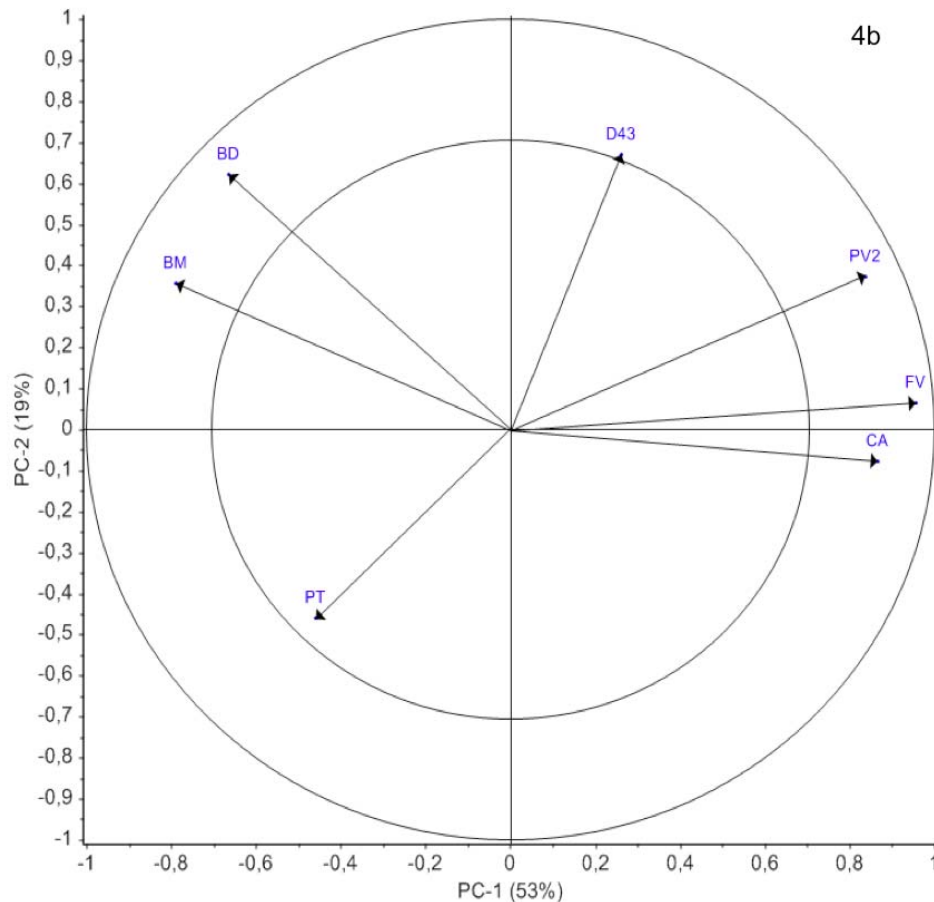
437 Additionally fermentation treatments (FO and FS) resulted in significantly higher
 438 breakdowns in highland genotypes compared to lowland genotypes (BD 115 and 101
 439 cP higher after FO and FS, respectively). This difference in breakdown may indicate
 440 that the granular structure was more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

447



448

449



450 **Fig. 4.** Score plot (4a) and loading plot (4b) of principal components 1 and 2, describing the variation
 451 of the samples. HD = highland and LD = lowland. NO = non fermented, oven-dried; NS = Non-
 452 fermented, sun-dried; FO = Fermented, oven-dried; FR = Fermented, sun-dried. BM = Breadmaking
 453 ability, BD = RVA Breakdown, D43 = Average granule diameter, PV2 = Peak viscosity 2, FV = Final
 454 viscosity, CA = Cooking ability, PT = Pasting temperature. Colors correspond to samples grouped by
 455 altitude and post-harvest treatments.
 456
 457

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

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

477

478 **3.6. Intrinsic viscosity**

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

491 The altitude may also be a determinant factor: for highland genotypes, NS and FO
492 treatments decreased the average intrinsic viscosity by 23.8 % and 26.4 %
493 respectively compared to NO, in contrast to respectively 5.9 % and 4.5 % for lowland
494 genotypes. FS treatment reduced the intrinsic viscosity more effectively than other
495 treatments and this change was similar in lowland and highland genotypes (36.4 and
496 33.9% respectively), compared to NO. These results further support the hypothesis
497 that FO and NS treatments affected lowland genotypes only on the surface of the
498 starch granules, whereas in highland genotypes modifications occurred throughout
499 the granule structure. Thus, the core of starch granules in lowland genotypes could
500 remain mainly unmodified while the outer layers could be removed (as indicated by
501 the reduction in granule diameter discussed in section 3.4), resulting in high intrinsic
502 viscosity. In contrast, starch molecular weight of highland genotypes could be
503 reduced throughout the granules due to depolymerisation, resulting in lower intrinsic
504 viscosity.

505

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.

512

513

514

515

516 **4. Conclusion**

517 The influence of genotypes, altitude of cultivation and post-harvest treatments
518 (fermentation and sun-drying) on the breadmaking ability of 13 cassava starches
519 from Colombia was analyzed. Post-harvest treatments were prevailing factors in
520 improving breadmaking ability, while the genotype factor had a smaller influence.
521 Among post-harvest treatments, fermentation had a more pronounced effect than
522 sun-drying, in particular on starch granule structure as evidenced by granule size
523 analysis and RVA parameters such as PV1 and PV2. The combination of both
524 treatments was nevertheless necessary to obtain the highest dough expansion.
525 These results confirm several previous studies (Mestres et al. 1997, Bertolini et al.
526 2000, Bertolini et al. 2001a, Marcon et al. 2009, Guerra Dias et al. 2011), which
527 showed that fermentation and sun-drying cause oxidative depolymerization and
528 decrease RVA and intrinsic viscosities, and consequently increase loaf expansion.
529 However no definitive linear correlation was found between depolymerization
530 (measured by intrinsic viscosity) and expansion. One possible explanation,
531 suggested by our results, is that other factors are at play.

532

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

539

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

546

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

556

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

565

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

574

575

576 **Acknowledgments**

577 The authors want to acknowledge the following contributions: SENESCYT of Ecuador
578 government for providing funding for a doctoral fellowship. CIRAD, CIAT and UM1 for
579 providing funding and facilities for laboratory work. Agnès Rolland-Sabaté (INRA,
580 France), Sandra Hill (University of Nottingham, UK), Olivier Gibert and Christian
581 Mestres (CIRAD, France) for their contributions to scientific discussions on molecular
582 size, thermal analyses and granule structure. Julien Ricci and Françoise Matencio
583 (CIRAD, France) for technical support. Gustavo Jaramillo from the cassava breeding
584 project at CIAT for the development and availability of improved cassava germplasm
585 (both for highland and lowland environments).

586

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725 Highlights

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727 • Cassava starch breadmaking ability is enhanced by fermentation and sun-
728 drying.

729 • Amylose content decreased breadmaking ability of sour cassava starch.

730 • Fermentation/sun-drying decrease starch molecular size, as shown by RVA
731 and intrinsic viscosity.

732 • Edaphoclimatic conditions influence susceptibility of raw cassava starch to
733 hydrolysis.

734 • Sour cassava starch is promising for development of gluten-free ingredients.

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