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Eric E. Rondet, Loyal Dahdouh, Andrès Escobar, Bernard Cuq, Michèle Delalonde. Development of a physicochemical method to quantify the extracellular liquid volume: Application to the transformation of cassava into gari. *LWT - Food Science and Technology*, 2019, 108, pp.1-5. 10.1016/j.lwt.2019.03.068 . hal-02096559

HAL Id: hal-02096559

<https://institut-agro-montpellier.hal.science/hal-02096559>

Submitted on 11 Apr 2019

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PII: S0023-6438(19)30263-4

DOI: <https://doi.org/10.1016/j.lwt.2019.03.068>

Reference: YFSTL 7966

To appear in: *LWT - Food Science and Technology*

Received Date: 7 January 2019

Revised Date: 12 March 2019

Accepted Date: 22 March 2019

Please cite this article as: Rondet, E., Dahdouh, L., Escobar, Andrès., Ruiz, E., Cuq, B., Delalonde, Michèle., Development of a physicochemical method to quantify the extracellular liquid volume: Application to the transformation of cassava into gari, *LWT - Food Science and Technology* (2019), doi: <https://doi.org/10.1016/j.lwt.2019.03.068>.

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1 **Development of a physicochemical method to quantify the extracellular liquid volume:**
2 **Application to the transformation of cassava into gari**

3
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16 Declaration of interest statement: 'Declarations of interest: none'.
17

18 **Keywords:**

19 Liquid volume localisation, Extracellular liquid, Cassava processing, poly(4-styrenesulfonate) sodium
20 salt
21

22 **Abstract**

23 In fresh vegetal materials, liquid is mostly located in cells. During processing, this liquid is distributed
24 in the entire vegetal matrix volume including extracellular volume. According to its location, it will be
25 more or less available for physico-chemical mechanisms that takes place during processing of vegetal
26 matrices. This availability greatly influences the quality of the final products. This work consisted in
27 developing a method for determining the quantity of extracellular liquid in a cell-component food
28 matrix. The method developed is spectrophotometric-based and consists in adding a known mass of

29 polymer (poly(4-styrenesulfonate) sodium salt, PSS) to a rasped vegetal material and defining its
30 concentration in the supernatant separated from the solid phase. The PSS molar mass prevents it from
31 penetrating into plant cells. The PSS concentration measured makes it possible to calculate the
32 extracellular liquid volume. The method validation and its application to rasped and processed
33 (fermentation, pressing) cassava makes it possible to obtain results in agreement with those obtained
34 with other methods.

35

36 **1. Introduction**

37 During processing of plant or animal raw materials into food, cell-component food matrices can be
38 subjected to liquid flows at cell element level. The understanding and the quantification of this
39 phenomenon represents a major challenge in understanding mechanisms and controlling the products
40 elaboration (Sehy, Banks, Ackerman & Neil, 2002). With regard to meat matrices, much work has
41 been devoted to the post-mortem evolution of intra- and extracellular spaces in relation to liquid
42 movements in the muscle and with water holding capacity (Offer & Knight, 1988). Meat juiciness is
43 assumed to be directly related to the progressive intracellular water efflux occurring during the
44 acidification of postmortem muscle, a change in good agreement with the parallel increase in the
45 extracellular space. These movements of intracellular liquid towards the extracellular space are
46 assumed to be due to pH decrease close to the pHi of myofibrillar proteins that induces a release in
47 bound water (Guignot, Vignon & Monin, 1993; Ouali *et al.*, 2006).

48 During apoptosis, cells swell up with liquid to the point where lysis of their plasmic membrane occurs.
49 It is a genuine cellular explosion which leads to the release of the cytoplasmic content in the
50 surrounding medium. Liquid availability also plays a fundamental role in manufacturing or value-
51 adding processes (such as agglomeration, cooking, drying) of food and non-food plant matrices. This
52 availability is closely linked to the histological structure of the plant walls (Lima *et al.*, 2013). Plant cell
53 walls are complex structures with central roles in plant form, growth, development and responses to
54 environmental stresses (Sørensen, Domozych, & Willats, 2010). Composed of polysaccharides,
55 proteins and phenolic compounds, cell walls generally belong to either primary or secondary cell wall
56 categories. Primary cell walls are composed of cellulose, pectins, hemicelluloses, and protein or
57 phenolic compounds. They generate turgor pressure (resisting to tensile forces), accommodate cell
58 expansion, mediate cell adhesion, and occur at the surface of most plant cells. Secondary cell walls are

59 restricted to specific types of differentiated cells. They are produced after the cessation of cell
60 expansion in certain specialized cells. They tend to be thicker than primary walls, and resist to
61 compressive forces. These two wall types are composed of cellulose and hemicelluloses and are often
62 impregnated with lignin (Cosgrove, 1993). Pectins are involved in the control of cell wall porosity and
63 are the major adhesive material between cells (Willats, McCartney, Mackie & Knox, 2001).
64 Hemicelluloses form strong hydrogen-bonded complexes with cellulose fibers. The most important
65 biological role of hemicelluloses is to strengthen the cell wall.

66 A recent study conducted on the processing of gari, a powdered food generated from cassava and very
67 widely consumed in west Africa, highlighted the major role played by intracellular liquid mobilized
68 during the manufacturing process on the final product quality (Escobar *et al.*, 2018). Some of the final
69 quality descriptors of gari (compactness, diameter distribution, or gelatinization rate) are highly
70 dependent on liquid availability during processing. Liquid distribution in the total available volume
71 can be described in two compartments: (i) the liquid volume present between the tissue fragments
72 generated after the preliminary step of rasping of the cassava root, (ii) the liquid volume inside the
73 tissue fragments (in tight intercellular junctions and inside the cells *i.e.* in the cytoplasm or vacuole).
74 Depending on this distribution, liquid is more or less available to establish capillary bridges between
75 the tissue fragments during the subsequent steps of the manufacturing process (such as pressing or
76 sieving) thereby modulating agglomeration phenomenon. Similarly, liquid availability might greatly
77 affect the gelatinization process occurring during gari roasting insofar as it is highly dependent on the
78 hydrothermal conditions. The hydrottextural approach developed to track the processing pathway from
79 raw material to end product describes the liquid and air proportions within the solid matrices (Ruiz,
80 Delalonde, Bataille, Baylac, Dupuy de Crescenzo, 2005; Escobar *et al.*, 2018). However, the liquid
81 contents considered are only overall values, which do not take into consideration the different
82 locations (outside the tissue fragments or inside the cell) of the liquid.

83 In the field of human or animal physiology, measurement of compartment volumes is of major
84 importance. Quantification methods of intracellular or extracellular liquid volumes by using
85 radioactive tracers (tritiated water, deuterium, ^{18}O) or ionic elements (bromide) are often employed
86 (Fielding *et al.*, 2003; Simpson *et al.*, 2001). In the field of health, dual-frequency impedance
87 measurement methods have also been used to determine the amount of intracellular or extracellular
88 liquids (Ellis & Wong, 1998; Moreno, 2007). At low frequencies (1 - 5 kHz) the capacitive nature of the
89 cell membranes does not allow the current to penetrate the cell, so impedance is only related to the

90 extracellular compartment. At high frequencies (200 - 500 kHz) the current penetrates the cell
91 membranes and the recorded impedance is a measure of the combined intracellular and extracellular
92 spaces. Recently, these impedance measuring methods were used in the food sector to detect the
93 lesions sustained by membrane cells of potatoes (Ando, Mizutani & Wakatsuki, 2014) and carrot
94 (Ando *et al.*, 2016) during drying and freezing processes. While these two studies were able to identify
95 the presence of liquid flows *via* variation in impedance value, to our knowledge no works in the
96 literature have quantified extracellular and intracellular liquid volumes.

97 The objective of this work was to develop an easily-deployable physico-chemical method (low-tech,
98 smart tech), able to quantify the evolution in the proportion of extracellular liquid during processing of
99 cell-component food matrices. This method is developed in the specific framework of cassava
100 processing into gari and is based on the use of a soluble polymer whose molar mass prevents it from
101 diffusing in the cell through the membrane. The method is first validated and used for the
102 measurement of the extracellular liquid in different cassava pulps (fermented or non-fermented,
103 before and after pressing). The new method for determining liquid location can be used to describe the
104 impact of unit operation on the spacial distribution of liquid in the vegetal tissues. This will result in a
105 better understanding of phenomenom occurring during processing as, for example, agglomeration
106 which has been observed during cassava pulp pressing-sieving (Escobar *et al.*, 2018).

107

108 **2. Materials and methods**

109 **2.1 Raw Material**

110 Cassava roots from Costa-Rica were purchased in a French supermarket for this study and
111 analyzed/processed within the following 24 h. They were first manually peeled and then rasped in a
112 kitchen robot (Thermomix®, Vorwerk, Germany) for 1 min at speed 6 so as to generate a pulp. After
113 rasping, the water content of the cassava pulps (1.88 ± 0.04 g water / g dry basis) was measured after
114 drying a 5 g sample in an oven at 105 °C for 24 h.

115 The poly(4-styrenesulfonate) sodium salt (PSS) is a water soluble polymer with an average molecular
116 weight of 70 kDa. It is a strong polyelectrolyte completely dissociated from pH 3 (Balastre, Persello,
117 Foissy & Argillier, 1999). It was purchased from Alfa Aesar (USA). A PSS solution of 70 g/L was
118 generated and maintained under stirring until complete dissolution (approx. 24 h at 25 °C). Successive

119 dilutions were used to generate solutions at different PSS concentration (9.4, 18.2, 27.3, 40.1, 55.3, and
120 60.0 g/L).

121 **2.2 Methods**

122 ***2.2.1 Determination of the extracellular liquid volume by UV spectrophotometry of the*** 123 ***PSS concentration***

124 A known quantity of PSS was added into the cassava pulp. The high molar mass of PSS prevents it
125 from diffusing in the cells. Thus, the PSS concentration determination in the extracellular liquid by UV
126 spectrophotometry makes it possible to deduce the extracellular liquid volume. The
127 spectrophotometric measurements were performed using a spectrophotometer (Shimadzu UV 2450,
128 Japan) in Fischer FB 55923 tanks. The maximal absorption wavelength (λ_{\max}) of PSS in solution is 225
129 nm (Dejeu, 2007) but the extracellular liquid contained in the cassava pulp also present UV absorption
130 properties (Fig. 1). In order to maximize the UV absorption difference (ΔDO_{\max}) between the
131 compounds naturally present in the extracellular liquid and the added PSS solution,
132 spectrophotometric measurements were made at a wavelength of 229 nm. The PSS calibration curve
133 was built at 229 nm, and characterized by the equation $[PSS] = 0.0303 \times \Delta DO$, where [PSS] represents
134 the concentration of PSS (g/L) and ΔDO the optical density difference between the extracellular liquid
135 without PSS and the extracellular liquid enriched with PSS. So this method involves, for each
136 measurement made in the presence of PSS, to compare it to the equivalent control without PSS.

137 ***2.2.2 Validation of the method***

138 The validation of the method involves the appreciation of the amplitude of a number of phenomena
139 that could interfere with the measurement. Depending on their interference with the measurement,
140 these phenomena may be neglected or not. In the case of a notable interference, they will have to be
141 taken into account in the calculation of the extracellular volume. It is thus necessary to assess: (i) the
142 increase in osmotic pressure generated at the extracellular level by the addition of PSS which is a
143 strong polyelectrolyte (a significant rise could lead to plasmolysis that artificially increase the
144 extracellular volume), (ii) the amount of PSS sorbed on plant tissues that changed its concentration in
145 extracellular spaces, (iii) the negligible effect of centrifugation (necessary for liquid solid separation)
146 on intracellular fluid drainage. Since this method is to be used to evaluate how fermentation and
147 pressing operations can affect the quantity of extracellular liquid, it will be tested and validated also on
148 the products resulting from these operations. For the study of the impact of sorption phenomenon,

149 osmotic effects and centrifugation, 200 g of freshly rasped cassava pulp were introduced into two
150 separate crystallizers. In one of them, 2 ml of PSS solution (i) at 70 g/L (for the quantification of
151 osmotic and centrifugation effects) and (ii) at different concentrations: 9.4, 18.2, 27.3, 40.1, 55.3, 60,
152 or 70 g/L (for the quantification of sorption effect) were introduced and distributed homogeneously
153 (mixed with a spatula for 2 min). After homogenization, 5 g samples of each crystallizers were collected
154 immediately for quantification of sorption effect or at different times for appreciation of osmotic
155 effects (1.5, 3, 4.5, 6, 8, 9.5, 12, 29 and 70 min). These 5 g were introduced into 50 mL polypropylene
156 centrifuge tubes. Concerning the study of centrifugation effect (iii), 2 samples of 5 g of pulp were
157 collected after homogenization from each crystallizer and introduced into two 50 mL polypropylene
158 centrifuge tubes for an immediate centrifugation. Two other 5 g samples from each crystallizer were
159 also collected into 50 mL polypropylene centrifuge tubes and immediately filtered on a woven filter
160 with 150 μm mesh size (VWR, Fontenay-sous-Bois, France).

161 For all the assays, 7 mL of distilled water were added to each 50 mL polypropylene centrifuge tubes in
162 order to increase the quantity of supernatant for subsequent spectrophotometric analysis. The 50 mL
163 polypropylene centrifuge tubes were vortexed and centrifuged (Eppendorf Model 5810 R centrifuge,
164 Hamburg, Germany) at 18,000 g for 30 min (excepted that collected to study the impact of
165 centrifugation that are immediately filtered). For all the assays, the liquid phase (supernatant or
166 filtrate) was collected and re-centrifuged (18,000 g for 30 min), to enable sedimentation of the
167 smallest particles. Upon centrifugation operation, the supernatants were diluted to 1/20th, and their
168 respective optical densities were measured at 229 nm. It was possible to determine the ΔDO value, (*i.e.*
169 the optical density difference measured between samples with and without PSS collected at each
170 times) and the PSS concentration (g/L) in the extracellular fluid. The calibration curve equation was
171 used to assess the PSS concentration (g/L) in the extracellular fluid after correcting the dilution factor
172 to 1/20th and considering the introduction of 7 mL of distilled water into the 50 mL polypropylene
173 centrifuge tube.

174 **2.2.3 Impact of fermentation or pressing on the extracellular fluid volume**

175 A quantity corresponding to 180 g of the pulp remaining in each crystallizer was then either (i) left to
176 ferment for 24 h in an oven at 37 °C, or (ii) introduced into a compression cell (IFTS, Foulayronnes,
177 France) and pressed under 300 kPa for 1 h and the drained juice was collected under the compression

178 cell using a test tube. This drained liquid corresponds to extracellular fluid, whose collected volume
179 can be compared to the volume of extracellular fluid measured before pressing.

180 5 g of each of the fermented pulp masses (with or without PSS) were introduced into 50 mL
181 polypropylene centrifuge tubes. The methodology was the same as that described to quantify the
182 proportion of the extracellular liquid in which the PSS was dissolved within the fermented matrix. This
183 value is compared to that previously determined in the matrix before fermentation.

184

185 **3. Results and discussion**

186 **3.1 Method validation**

187 ***3.1.1 Impact of osmotic effects related to PSS introduction in the extracellular liquid***

188 PSS is a salted polymer (sodium salt), with a high molar mass (70 kg/mol) and whose monomer unit
189 has a molar mass of 206 g/mol. One mole of PSS contains $70000/206 = 339$ monomer units. When
190 pH is higher than 3 in solution, each sulfone function is salified and (the dissociation coefficient is 1
191 which is the worst case for our purposes) each monomers releases one Na^+ ion. After complete
192 dissociation in the solution, 1 mole of PSS releases 339 moles of Na^+ and 1 mole of polymer chain (*i.e.*
193 340 osmoles). The PSS solution prepared in the present study to determine the extracellular volume
194 was concentrated at 70.02 g/L (*i.e.* 10^{-3} mol/L). A volume of 2 ml of this solution (containing $2 \cdot 10^{-6}$
195 moles) was introduced into 200 g of cassava pulp. Assuming a dissociation coefficient of 1, the $2 \cdot 10^{-6}$
196 moles released $680 \cdot 10^{-6}$ osmoles into solution. Since the molecular mass of PSS prevents it from
197 penetrating into the cell, it was located solely in the extracellular space. Bearing in mind that the water
198 content of cassava is 63 g water / 100 g wet cassava, if 20% of the liquid was in the extracellular space
199 (the worst case of those measured subsequently), the $680 \cdot 10^{-6}$ osmoles of PSS incorporated into 200 g
200 of wet cassava pulp should be diluted in $0.2 \times 0.63 \times 200 = 25.2$ mL. The increase in osmolarity of
201 the extracellular space induced by the addition of PSS would then be:
202 $C = n/V = 680 \cdot 10^{-6} / 25.2 \cdot 10^{-3} = 0.027$ osmol /L. Since the osmolarity of a plant cell is in the same
203 range of values as an animal cell (0.280 osmol/L) (Durand-Smet *et al.*, 2014), this osmolarity increase
204 represents approximately 10 % of the osmolarity of the initial medium. The diffusive flow of liquid
205 generated from the intracellular to the extracellular space should therefore be negligible. Fig. 2
206 represents the evolution of ΔDO measured experimentally between pulp samples with or without PSS
207 over time steps ranging from 1.5 to 70 min after introduction of PSS into the medium. This represents

208 an experimental validation of the previous hypotheses concerning the impact of PSS introduction on
209 osmotic effects. In the event of a diffusive flow of intracellular fluid to the extracellular space, the PSS
210 concentration of the extracellular fluid should decrease thereby leading to a reduction in the ΔDO
211 value. As Fig. 2 shows, the ΔDO value does not vary with time. This demonstrates the absence of
212 diffusive flow of intracellular liquid to the extracellular space. Therefore, osmotic effects under the
213 conditions of our study can be neglected.

214 **3.1.2 Impact of PSS sorption**

215 Like any element in solution in contact with a solid phase, some of the PSS introduced into the
216 solution can exhibit a propensity for physical sorption on the solid compartment of the matrix. This
217 sorption phenomenon may occur on the external part of the cell walls, intact or not, or on any other
218 suspended solid elements (such as wall fragments, free starch granules, colloids). The sorbed PSS
219 molecules cannot be measured in the extracellular fluid. The PSS sorbed on the matrix was quantified
220 by introducing different amounts of PSS into the freshly rasped cassava pulp samples (Fig. 3).
221 Increasing the amount of added PSS led to a linear increase in the PSS concentration measured in the
222 extracellular fluid. The equation for the line is characterized by a non-zero intercept point at $3.16 \cdot$
223 $10^{-4} \text{ g PSS / g dry pulp}$. This value indicates the PSS content in the medium when the concentration
224 of PSS in the extracellular fluid is zero. Therefore it correspond to the quantity of PSS sorbed on the
225 matrix. When considering 1 ml of PSS solution at 70 g/L (*i.e.* 70 mg PSS mass), the PSS amount sorbed
226 on 100 g of fresh pulp (whose water content was approximately 1.9 g water / g dry matter) corresponds
227 to approximately 15 % of the PSS introduced into the medium. In view of this relatively high sorbed
228 PSS value, the subsequent calculations of the extracellular and intracellular volume would need to
229 consider this quantity of PSS inaccessible to experimental determination. This value is prone to
230 increase with increasing available surface area for PSS sorption, and therefore increasing rasping time
231 or intensity. Hence the utmost care must be taken with the rasping operation that must be performed
232 under the same condition from one test to another (see section 2.1).

233 **3.1.3 Impact of supernatant collection method**

234 Cell surface damage during centrifugation at 15,000 g was demonstrated to cause significant
235 reductions in bacteria (*E. coli*) viability compared to centrifugation at 5,000 g (Pembrey, Marshall &
236 Schneider, 1999). Concerning plant cells (Kalanchoe leaves), centrifugation and other forms of
237 mechanical stress can lead to cell damage and/or cell death (Pedroso & Durzan, 2000). In the present

238 study, a 18,000 g centrifugation step for 30 min is applied in order to separate the supernatant. It was
239 thus fundamental to verify whether centrifugation causes lysis of the vegetal cell membrane. Actually,
240 in the case of lysis, the centrifugation would induce the release of intracellular liquid into the medium
241 that would artefactually modify the volume of extracellular liquid in the matrix. Solid/liquid phase
242 separation by filtration on a 150 µm mesh size filter was tested in parallel to centrifugation, in order to
243 compare the PSS concentration in the liquid phase. It was assumed that the filtering method did not
244 cause any damage to the cell elements in the solid phase insofar as they are only submitted to
245 gravitation forces. The results obtained shows that the extracellular fluid separation method (filtering
246 *vs.* centrifugation) did not cause any significant modifications of the PSS concentration value
247 measured in the extracellular fluid: 0.31 ± 0.04 g/L and 0.32 ± 0.03 g/L for centrifugation and
248 filtration respectively. This demonstrates that centrifugation at 18,000 g does not cause any
249 membrane lesions of this cell. When compared to bacteria whose viability decrease above 15,000 g, the
250 vegetal cell resistance to centrifugation could be explained by the ability of vegetal cell wall to resist
251 compressive forces. There is thus no artifactual salting-out effect of intracellular fluid. As the
252 centrifugation separation method makes it possible to work at controlled temperature (20 °C) and to
253 separate the phases in several centrifuge tubes simultaneously, this method was selected for the
254 subsequent part of the study.

255 **3.2 Application to measurement of extracellular liquid before and after fermentation or** 256 **pressing of cassava pulp**

257 During gari processing, cassava pulp can undergo fermentation and/or pressing steps. These unit
258 operations could modify the liquid phase distributions (intracellular *vs.* extracellular liquid). The
259 proportion of extracellular liquid before and after fermentation on the one hand, and before and after
260 pressing on the other hand was assayed using PSS as an indicator compound. Fig. 4 shows that there is
261 no significant difference in the proportion of extracellular liquid in the matrix before and after
262 fermentation for 24 h at 37 °C. These results are in accordance with granulometric measurements
263 made on cassava pulp before and after fermentation by Escobar *et al.* (2018): the size, and therefore
264 the volume of intracellular liquid in pulp fragments remains constant during fermentation. The
265 enzyme action inherent to the fermentation step (Bainbridge, Harding, French, Kaping & Westby,
266 1998), if acting on the cell wall, does not seem to be sufficiently effective to cause cell lysis and
267 therefore salting-out of the intracellular liquid in the extracellular space.

268 Conversely, Fig. 5 shows that the pressing step has an impact on intracellular liquid drainage. Firstly,
269 measurement of PSS concentration in the extracellular liquid before pressing makes it possible to
270 calculate the proportion of extracellular liquid in 180 g of pulp (approximately 34 %). Then, these
271 180 g of pulp are introduced in compression cell (cf § 2.2.3) and pressed under 300 kPa for 1h. The
272 drained juice collected in a test tube corresponds to a proportion of approximately 55 % of the total
273 water contained in the pulp. Thus, during pressing, it seems obvious that a quantity of intracellular
274 liquid is drained to the extracellular space to top up the initial proportion of extracellular liquid. These
275 results support those obtained by Escobar *et al.* (2018) after granulometric measurement of the pulp
276 fragments before and after pressing. There was a clear decrease in fragment size, thereby revealing
277 drainage of intracellular liquid.

278

279 **4. Conclusion**

280 The objective of this work was to develop a spectrophotometric based method for determining the
281 quantity of extracellular liquid in a cell-component food matrix. This method consists in adding a
282 known mass of PSS to the rasped vegetal matrix (cassava pulp). PSS is distributed in the extracellular
283 liquid due to its high molar mass preventing its penetration into plant cells. After a time of contact, a
284 solid liquid separation by centrifugation is carried out and the PSS concentration is measured in the
285 supernatant. This concentration allows the calculation of the extracellular liquid volume. For the
286 validation of this method, several tests are implemented: the appreciation of the quantity of PSS
287 sorbed on the solid matrix, the appreciation of the osmotic effects and the potential effect of
288 centrifugation. These tests show that osmotic and centrifugation effects are negligible. The use of this
289 methodology makes it possible to show that fermentation did not cause significant transfer of
290 intracellular liquid into the extracellular space and pressing caused drainage of the cell contents to the
291 extracellular medium. These results are in good agreement with those of Escobar *et al.* (2018) which
292 showed that the volume of pulp fragments and damaged cells level remains constant during
293 fermentation, whereas the size of the pulp fragments decrease after pressing. One of the prospects of
294 this work would be to extend it to other food matrices (*e.g.* meat matrices) in order to track the effect
295 of enzyme complexes on salting-out of cell contents that has an impact on meat “juiciness”.

296

297 **Funding**

298 This work was supported by Roots, Tubercules and Bananas (RTB) Post Harvest Project (2013 – 2016)

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ACCEPTED MANUSCRIPT

**Development of a physicochemical method to quantify the extracellular liquid volume:
Application to the transformation of cassava into gari**

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ALL FIG.S IN GREY LEVELS

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Fig. 1: UV absorption spectrum (200 to 300 nm) of the 70 g/L PSS solution (bold line) and extracellular fluid (dashed line) contained in the rasped cassava pulp.

Fig. 2: Evolution of the ΔDO value as a function of the PSS residence time in the cassava pulp extracellular fluid. Each point is the result of a triplicate.

Fig. 3: Quantification of PSS sorbed on the solid matrix (intercept point) (in g (PSS)/g (dry pulp))

Fig. 4: Proportion of extracellular liquid in the matrix before pressing: comparison of not fermented *vs.* fermented product. Results from triplicate measurements.

Fig. 5. Comparison between the proportion of initial extracellular fluid in the pulp measured by assaying the PSS concentration before pressing and the proportion of liquid collected in the test tube after pressing. Results from triplicate measurements.

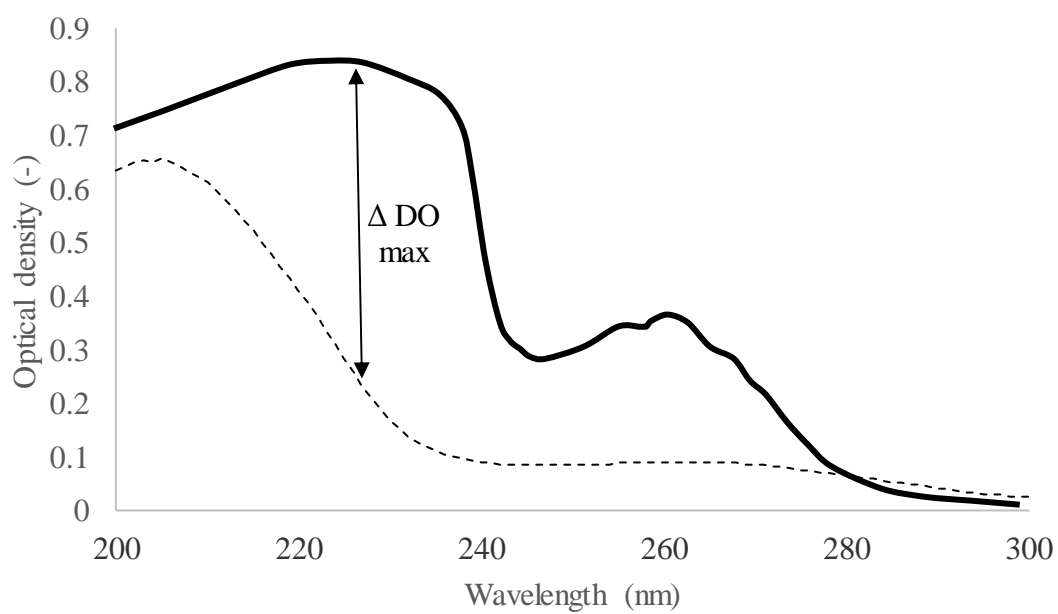


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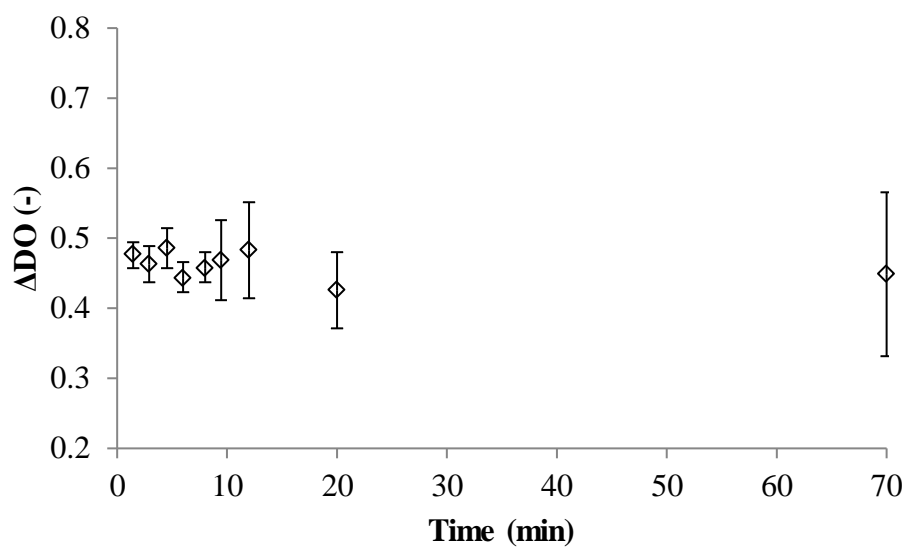


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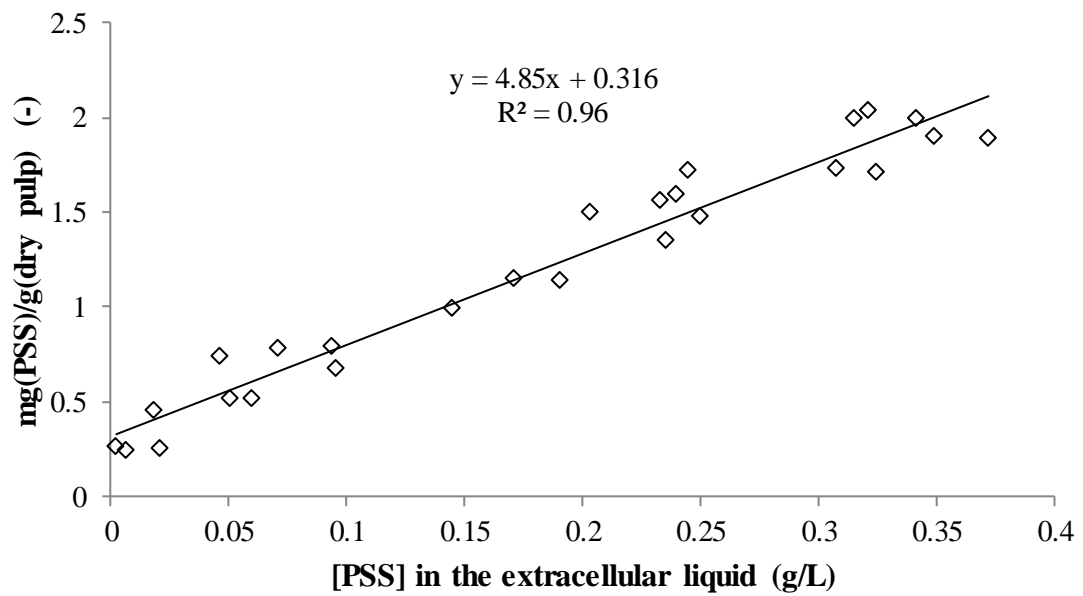


Fig. 3. Quantification of PSS sorbed on the solid matrix (intercept point) (in g (PSS)/g (dry pulp))

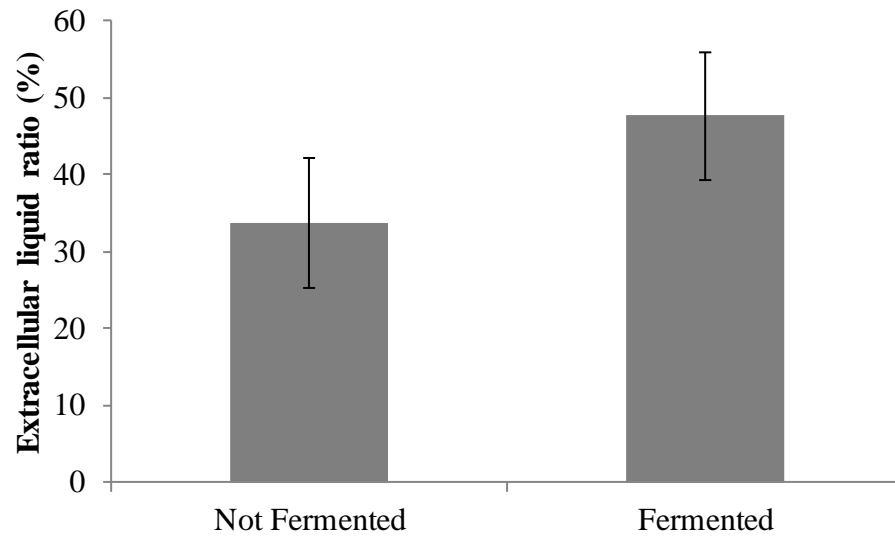


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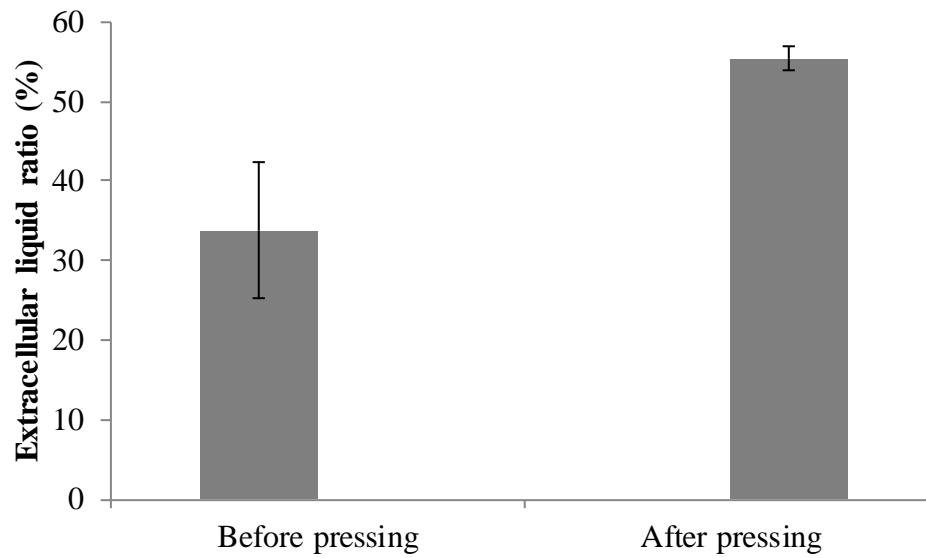


Fig. 5. Comparison between the proportion of initial extracellular liquid in the pulp measured by assaying the PSS concentration before pressing and the proportion of liquid collected in the test tube after pressing. Results from triplicate measurements.

Highlights

- A new method for extracellular liquid quantification has been developed and validated.
- Adding PSS to the extracellular liquid did not generate osmotic flows.
- The sorption phenomenon of the polymer on the solid surfaces is significant.
- The extracellular fluid extraction method by centrifugation does not alter PSS concentration.
- This method applied to cassava pulp gave results coherent to that obtained with other methods.