# Study on the effect of processing methods and storage conditions on starch functional properties and pectin content in cassava roots

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# ABSTRACT

This study investigates the effects of processing methods on pectin content and starch functional properties. To do so, 8 different cassava genotypes harvested on Centro Internacional de Agricultura Tropical (CIAT, Palmira, Colombia) in 2023 were subjected to different treatments. First of all, samples were peeled and then cut into pieces that were directly dried by two different methods: freeze-drying and oven-drying. Other samples from the same roots were cut into pieces, grounded and then dried using the aforementioned methods resulting in four different types of samples: Oven-dried cubes (OC), oven-dried grated samples (OG), Freeze-dried cubes (FC) and Freeze-dried grated samples (FG). After that, all samples were milled to obtain flours that where therefore stored at -20°C and -80°C in order to determine the pectin content at different times of storage (0, 15, 30, 60, 90 and 120 days).

Findings from this study may provide valuable insights in order to determine the best cassava processing for determining physicochemical properties without significantly affecting their compounds. The selected processing could be then implemented for determination of postharvest quality of cassava in the frame of RTB Breeding project.

**Key Words: cassava, starch, pectin, cooking properties, RTB Breeding Project, texture,**

# 1 INTRODUCTION

Cassava (*Manihot esculenta*) is a major staple crop in tropical regions. It is widely used as food in different forms (boiled cassava, gari/eba, fufu, etc) due to its high carbohydrate content, mainly starch. Cooking ability of cassava and functional properties of its derived products such as flours may depend on starch properties as well as other complex polysaccharides present in the cell walls as pectins. Thus, understanding and controlling how these two components are affected during processing is very key for improving cassava's cooking characteristics and functional properties that may impact consumer preferences and culinary applications.

The method employed for the characterization of cassava flours can influence the functional properties of starch and the quantitative determination of pectins. Such artifacts in measurements may lead to incorrect decision-making or erroneous conclusions. In the case of post-harvest quality characterization of cassava, it is crucial that the properties determined are representative of the final product obtained and that they are not influenced by the measurement method or any transformations applied to the sample prior to the method. With this in mind, the main objective of this study is to determine the influence of the sample preparation method on the determined starch properties and pectin content. The information obtained may prove useful in the future, as it will enable the selection of the sample treatment method that best preserves both starch properties and pectin content determination.

# 2 MATERIAL AND METHODS

## 2.1 Sampling

Two cassava roots were treated by genotype, and a total of 8 genotypes were analysed. Each root was cut longitudinally, and its inner fibre was removed. Each half-cylinder was then cut again longitudinally to obtain 4 quarter-cylinders per root. Each quarter-cylinder was assigned to a different process: 1) cut into cubes and dried in an oven at 45°C until constant weight (~ 60 h, OC); 2) cut into cubes, frozen at -80°C, and then freeze-dried (FC); 3) grated and dried at 45°C until constant weight (30 h, OG); 4) grated, frozen at -80°C, and then freeze-dried (FG). Once the dehydration process was completed, all samples were milled in a ball mill for subsequent physicochemical analyses. The obtained flours from 2 different roots were mixed in order to homogenize the samples.

All different samples were characterised by means of total and methylated pectins. Moreover, OG and FG type of samples were analysed at time 0 and at 15, 30, 60, 90, and 120 days for total pectins and methylated pectins under two storage conditions: -20°C and -80°C.

## 2.2 Pectin content determination

Pectin content of cassava flours was determined according to a standard method following the RTBfoods SOP (1). They were first extracted at intermediate temperature (55°C) to facilitate their extraction while limiting their chemical and enzymatic degradation and the extraction of starch (temperature below starch gelatinization). Two different solvents were used: pH 10 to extract highly methylated pectin (MP) and pH 10 + EDTA to extract most pectin (TP).

## 2.3 Starch functional properties

Please, see Annex 1.

## 2.4 Statistical analysis of data

The data were statistically analysed to determine if there were significant differences between samples from different processes or samples at different storage times. First, the Shapiro-Wilk test was conducted to assess the normality of the data, followed by the Levene's test to check for the homogeneity of variances. Based on these results, the Kruskal-Wallis test was applied, as the assumptions for parametric tests were not met. This analysis was conducted using XLStats version 2021.4.1.

# 3 RESULTS

## 3.1 Effect of the processing method on pectin content of samples

Figure 1 shows the boxplots related to the values of pectin extracted using a pH equal to 10 (PM) and with a pH equal to 10 using also EDTA (PT) for the 8 genotypes under different methods FC, FG, OC and OG. Statistical analysis indicated that the processing method significantly influenced the pectin content (PM and PT) of the samples being the ones obtained after freeze drying (in cubes or grounded) the ones that shown a highest level of pectins, Table 1. No significant different of size (cubes of grounded form) were obtained between groups of samples obtained after freeze drying, however, for MP obtained from oven dried samples a significant effect of size was observed indicating a higher value for those oven dried in cubes.

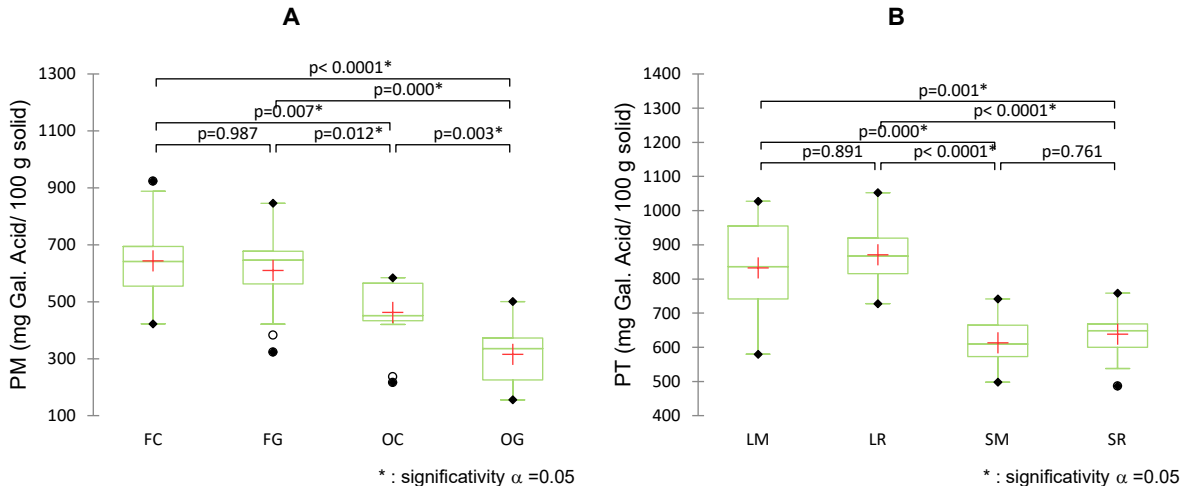


**Figure 1: Values of pectin content after extraction at pH=10 (A) or pH=10 + EDTA (B) under different processing methods: Freeze dried cubes (FC), Freeze dried grounded samples (FG), Oven dried cubes (OC) and oven dried grounded samples (OG).**

These results clearly indicate that drying method significantly affect the determination of pectin content on cassava samples. As freeze drying shown the highest values it led to the conclusion that in order to determine the real quantity of pectin of samples this method should be chosen. The different values obtained from both methods maybe due to some enzymatic activity that could take place during oven drying that would be avoided during freeze drying because of the low used temperatures. However, further studies should be carried out in order to confirm this hypothesis.

**Table 1: Values of pectin content after extraction at pH=10 (A) or pH=10 + EDTA (B) under different processing methods: Freeze dried cubes (FC), Freeze dried grounded samples (FG), Oven dried cubes (OC) and oven dried grounded samples (OG).**

| Group of samples | PM (mg Gal. Acid/ 100 g solid) | PT (mg Gal. Acid/ 100 g solid) |
|---|---|---|
| **OC** | 463±112[a] | 613±75[a] |
| **OG** | 315±106[a] | 638±72[a] |
| **FC** | 643±135[b] | 832±136[b] |
| **FG** | 610±141[b] | 871±88[b] |

Data is presented as mean ± standard deviation. Data value of each gour of samples with different superscript letters in columns is significantly different Steel-Dwass-Critchlow-Fligner.

## 3.2 Effect of storage conditions and time on pectin content of cassava samples

Oven dried and freeze-dried grounded samples were stored at -20°C and -80°C in order to evaluate the pectin content each month up to 4 months of storage.

The statistical analysis showed that for the freeze-dried ground samples, Table 2, and for oven-dried ground samples, Table 3, there was no significant effect of storage temperature (-20 or -80°C). However, in both cases the effect of storage time was significant for the determination of methylated pectins. After 90 days of storage, the methylated pectin content was significantly higher. This result cannot be explained by PME enzymatic activity, as in that case, the amount of methylated pectins would have decreased (2), but rather by the degradation of other components in the sample, which could facilitate better extraction of the non-methylated pectin using the same buffer solution. In any case, further studies should be conducted to validate or refute this hypothesis. In light of the results, the recommendation would be to determine the methylated pectins in the samples within a maximum period of 60 days of storage at -20°C or -80°C. Regarding the total pectin content, no significant differences were observed between the different storage days.

**Table 2: Values of pectin content after extraction at pH=10 (PM) or pH=10 + EDTA (PT) of freeze-dried grounded samples (FG) stored at different temperatures at different storage times.**

| Type of sample | Storage temperature (°C) | Storage time (days) | PM (mg Gal. Acid/ 100 g solid) | PT (mg Gal. Acid/ 100 g solid) |
|---|---|---|---|---|
| **FG** | -20 | 0 | 610±141[a] | 871±75[a] |
| **FG** | -20 | 15 | 600±130[a] | 864±72[a] |
| **FG** | -20 | 30 | 617±134[a] | 849±136[a] |
| **FG** | -20 | 90 | 767±99[b] | 846±88[a] |
| **FG** | -20 | 120 | 754±76[b] | 838±67[a] |
| **FG** | -80 | 0 | 610±141[a] | 871±88[a] |
| **FG** | -80 | 15 | 623±131[a] | 882±61[a] |
| **FG** | -80 | 30 | 622±158[a] | 876±118[a] |
| **FG** | -80 | 90 | 777±74[b] | 845±57[a] |
| **FG** | -80 | 120 | 733±65[b] | 832±64[a] |

Data is presented as mean ± standard deviation. Data value of each gour of samples with different superscript letters in columns is significantly different Steel-Dwass-Critchlow-Fligner.

**Table 3: Values of pectin content after extraction at pH=10 (PM) or pH=10 + EDTA (PT) of oven dried grounded samples (oG) stored at different temperatures at different storage times.**

| Type of sample | Storage temperature (°C) | Storage time (days) | PM (mg Gal. Acid/ 100 g solid) | PT (mg Gal. Acid/ 100 g solid) |
|---|---|---|---|---|
| **OG** | -20 | 0 | 315±106[a] | 638±71[a] |
| **OG** | -20 | 15 | 339±102[a] | 763±68[a] |
| **OG** | -20 | 30 | 350±107[a] | 697±105[a] |
| **OG** | -20 | 90 | 586±118[b] | 790±62[a] |
| **OG** | -20 | 120 | 539±111[b] | 741±100[a] |
| **OG** | -80 | 0 | 315±106[a] | 638±72[a] |
| **OG** | -80 | 15 | 325±98[a] | 757±53[a] |
| **OG** | -80 | 30 | 328±101[a] | 654±77[a] |
| **OG** | -80 | 90 | 580±107[b] | 758±76[a] |
| **OG** | -80 | 120 | 542±114[b] | 750±55[a] |

Data is presented as mean ± standard deviation. Data value of each gour of samples with different superscript letters in columns is significantly different Steel-Dwass-Critchlow-Fligner.

# 4 CONCLUSIONS

Two main conclusions can be drawn from this study. First, the sample processing method significantly affects the determination of pectin content in the samples. Freeze drying, due to it is a very low-temperature process preceded by freezing, appears to be the most suitable pretreatment for the subsequent determination of pectin content. On the other hand, oven drying has the drawback of being a lengthy pretreatment (several hours or days) that, despite being at a relatively low temperature, can promote enzymatic degradation of the samples, which may subsequently affect the pectin content and the functional properties of the starch. Regarding the storage effect, the results allow us to conclude that samples should be stored at low temperatures to preserve the different components of interest. However, the different trends observed after 3 months of storage, which are difficult to explain from an empirical standpoint, suggest that the samples should be analyzed within that time frame to avoid any degradation or inaccurate results.

Taking this into account, the main recommendations for the subsequent characterization of pectin content in cassava within the framework of the project would be the use of freeze drying as the sample pretreatment method (either in pieces or ground), followed by the analysis of pectin content within a maximum period of 3 months of storage at -20°C or -80°C.

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# APPENDICES

## 5.1 Outlines of proposed paper on study of processing methods effects on starch functional properties

The objective of this study was to evaluate the effect of drying on the chemical composition, physical characteristics, functional properties, and morphological characteristics of cassava flours. To this end, eight cassava cultivars were selected and subjected to two drying methods: freeze-drying and forced-air oven drying, to produce flour.

The data obtained within the framework of the RTB Breeding project show significant differences in the functional properties of starch, depending on the drying method used (freeze-drying versus oven-drying). These results could have a considerable impact on the scientific community, so the following guidelines for publication are proposed.

**1. Introduction**

**2. Materials and methods**

*2.1. Sample preparation (flour production)*

Eight cassava genotypes were used, which were washed, peeled, and processed. A 250-gram sample of grated pulp was taken from each genotype and subjected to different drying methods: freeze-drying, oven-drying, oven-drying with silver nitrate at a concentration of 5 mM, and oven-drying with mercuric chloride at a concentration of 5 mM. Silver nitrate and mercuric chloride were used as enzyme inhibitors.

For the freeze-dried samples, they were stored at -80°C before proceeding with the freeze-drying process. Oven-drying was carried out at 50°C for 48 hours. Once dried, the samples were ground and stored at -80°C.

*2.2. Characterization of flours*

2.2.1. Dry matter determination of samples

One g of flour was weighed and dried in an oven at 105°C for 24 hours. Dry matter was expressed as a percentage of dry weight relative to fresh weight (AOAC, 2004).

2.2.2. Total starch content

Total starch content was determined according to the Megazyme by NEOGEN method (McCleary et al., 2019) with some modifications. Total starch content was determined by enzymatic hydrolysis (α-amylase and amyloglucosidase), followed by the GOD-POD reaction and colorimetry at 510 nm.

2.2.3. Differential Scanning Calorimetry (DSC) and Amylose Content.

The methodology reported by Mestres et al. (29) 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 2% w/V in water) and the reference pan (empty) were heated from 25 to 160 °C at 10 °C $min^{-1}$, holding at 160 °C for 2 min, and then cooling to 60 °C at 10 °C $min^{-1}$. The onset temperatures (GT) of each sample were determined on the thermograms. Amylose content was also measured from the energy of amylose-lysophospholipid complex formation using the DSC.

2.2.4. Organic acid and sugar content

A 0.5 g sample was weighed, and 10 mL of mobile phase (5 mM sulfuric acid) was added. The solution was homogenized in an Ultraturrax (Janke & Kukel, T25) for 30 seconds, then the sample was shaken in a shaker (Microplate VWR, Germany) for 30 minutes at 350 rpm. The suspension was centrifuged (8000 rpm, 10 minutes, and 25°C). The supernatant was filtered through a PVDF membrane (Millex-GV, 0.22 µm, Brazil). Organic acids and sugars were analyzed by HPLC using a Biorad Aminex HPX 87H column, equipped with a UV detector (MWD T 1365D for organic acids) set at 210 nm and connected in series with a refractive index detector (RID T1362A for sugars). The equipment features a quaternary injection valve. Samples were separated isocratically at a flow rate of 0.6 mL/min, 30°C, and an injection volume of 15 µL. Retention times and calibration curves were performed for the following sugars: glucose (Sigma-Aldrich, G7528), fructose (Sigma-Aldrich, F2543), sucrose (Sigma-Aldrich, ≥99.5% S7903), raffinose (Sigma-Aldrich, 99% R-0514), and for the following organic acids: oxalic acid (Sigma-Aldrich, 241172), citric acid (Sigma-Aldrich, CO759), malic acid (Sigma-Aldrich, 240179), trans-aconitic acid (Aldrich Chemical Company, Inc. 4023-65-8), succinic acid (SIGMA-Aldrich, ≥99%, S3674), and fumaric acid (Supel-Co Analytical, R412205). (Moreno-Alzate, 2011).

2.2.5. Total phenol content

Total polyphenol (TP) content was determined using the Folin-Ciocalteu method (Du et al., 2009) with some modifications. 0.5 g of flour was weighed into a 50 mL centrifuge tube, then 2 mL of acetone/water (70/30) was added, followed by horizontal shaking for 30 min at 300 rpm. The sample was centrifuged for 10 min at 6800 rpm. In a 2 mL Eppendorf tube, 790 µL of deionized water, 10 µL of flour extract, 50 µL of Folin-Ciocalteu reagent (1:1 with water) and 150 µL of sodium carbonate (20 g/100 mL) were added and mixed, vortexed for 20 s and allowed to stand at room temperature in the dark for 1 h. The absorbance was read at 750 nm and the total polyphenol concentration was calculated from a calibration curve, using gallic acid as a standard (0–1 mg/mL).

2.2.6. Pectin content

Total pectin content (as Galacturonic acid equivalent) was determined as described by Mestres et al., (2022). The sample was weighed (250 mg), then 10 mL of extraction buffer (pH10+EDTA) was added and dispersed in a vortex, the samples were incubated for one hour, then centrifuged at room temperature, 4000 rpm and 10 min. The supernatant was diluted 10 times with ultrapure water. 400 µL of the diluted supernatant (pectin extract) or the standard solutions (galacturonic acid), were added to glass tubes and then placed in an ice bath, then 2.4 mL of concentrated sulfuric acid (95-97%), it was shaken vigorously for a few seconds in a vortex, the sample was placed in a boiling water bath for 10 minutes, the sample was removed and cooled in an ice bath. 40 µL of 0.15% 3-phenylphenol solution (galacturonic acid measurement) or 40 µL of 125 mM NaOH solution (sample blank) were added. It was shaken vigorously and placed in a water bath at 35°C for 15 minutes, then the absorbance was measured at 520 nm.

2.2.7. Rapid Visco Analyser (RVA)

Viscoamylograms were obtained by analyzing the flours in water and in an inhibitor (0.002 M silver nitrate). To do this, 2 g of flour (dry basis) was dispersed in approximately 23 g of water or inhibitor solution to produce an 8% suspension, as previously described (Dufour et al., 2009). Five parameters were measured in the recorded viscoamylogram: pasting temperature (PT), pasting time (Pt), peak viscosity (PV), final viscosity (FV), and minimum viscosity (Tr), which occurred between the peak and final viscosity. Two additional parameters were subsequently calculated: breakdown (BD), estimated as the difference between PV and Tr, and setback (SB), estimated as the difference between FV and Tr.

2.2.8. Solubility and swelling power

Swelling power and solubility patterns (Mestres et al., 1997) were determined using 1.5% db (w/w) flour dispersions (0.42 g dm dispersed in 27.58 g of distilled water). Paste was prepared in RVA starting at 35 °C for 1 min, increasing temperatures at a 6 °C $min^{-1}$ rate, holding final temperatures at 90 °C for 2.5 min. The paste was immediately transferred to 50 $cm^3$ centrifuge tube. The supernatant and sediment after centrifugation for 5 min at 6000g at 25 °C were collected and weighed (Wsu and Wse, respectively) then dried at 105 °C for 24 and 24 h, respectively, and weighed (Dsu and Dse, respectively). Two parameters were calculated: concentration of soluble material in the
supernatant (solubility) and the swelling power.

solubility (%db) = 100Dsu/0.42
Swelling power= (Wse - Dse)/Dse

2.2.9. Quantification of Potential Hydrogen (pH)
2 grams of flour were weighed on a dry basis, 20 mL of deionized water (10% weight/volume) was added, and the mixture was stirred for 30 minutes. The solution was then centrifuged, and the supernatant was removed for analysis. The pH of the solution was determined at room temperature using a pH meter (Fischer Scientific, AB15, USA) (Gibert et al., 2009).

2.2.10. Enzymatic activity determination ($\alpha$ and $\beta$ amylase)
For the enzymatic activity of α-amylase and β-amylase, Megazyme by NEOGEN kits were used. For α-amylase, a substrate called BPNPG7 (benzylideneblocked p-nitrophenyl maltoheptaoside) was used. For β-amylase, a substrate called PNPβ-G3 (β-glucosidase and p-nitrophenyl-β-D-maltotrioside) was used.

2.2.11. Color measurements
The color of the flours was evaluated using a portable color reader (CR-410, Konica Minolta, Japan). The results were expressed according to the Hunter L*, a*, and b* system with illuminant C and a 2° viewing angle. Three monochromatic variables provided by the equipment were recorded: L* (0 = black and 100 = white); a∗ (−a∗ = greenness and +a∗ = redness) and b∗ (−b∗ = blueness and +b∗ = yellowness). Browning index and chroma were measured by the following formula (Salvador et al., 2007).

$$Chroma = \sqrt{a^{*2} + b^{*2}}$$

$$Browning\ index = \frac{100(x - 0.31)}{0.17}$$

Where x is obtained using the following formula:

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

2.2.12. Light and polarizing microscopy
The flours were placed on a slide using a spatula. A drop of deionized water was then added, and the mixture was homogenized. They were then observed using a light microscope (Optika B-510POL, Italy) with a 20x magnification lens. The sample was also examined using polarized light to observe the formation of the Maltese cross.

*2.3. <u>Characterization of dried flours with inhibitors</u>*

2.3.1. Starch content and pasting properties were determined following the methodologies described in Sections 2.2.2 and 2.2.7, respectively.

2.4. Statistical analysis

The results of the physicochemical characterization were expressed as the mean of the analyses performed in duplicate. These means were compared using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Pearson's correlation test was used to assess correlations between variables. Principal component analysis (PCA) was also used to identify the variables that explained most of the variability in the data. All statistical analyses were performed using JMP software (SAS Institute Inc., version 13 2.1).

## 3. Results and discussion

3.1. Physicochemical and functional properties of flour affected by the drying process.
3.2. Link between starch degradation and enzymatic activity of the samples.

## 4. Conclusions

Considering that the main consumers of cassava are developing countries, and that thermal processing is the most common preservation treatment, the effect of drying (freeze-drying versus oven-drying) on the physicochemical and functional properties of the flours was evaluated. According to the results obtained, it is observed that the properties of the flours vary depending on the type of drying used.

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