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Evaluation of the Production of Starch from Bitter Cassava (*Manihot utilissima*) using Different Methodologies

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Cassava is a shrub belonging to euphorbiaceous family, widely cultivated in South America, Africa and the Pacific because of its roots with starches of high nutritional value. There is a variety called *Manihot utilissima* or bitter cassava, which contains high concentrations of cyanogenic elements that make it unusable and poisonous raw material which avoided for human consumption, while the high concentrations of carbohydrates place it as a potential source of starch mainly for industrial use. The wet extraction method was used to get starch from bitter cassava (*Manihot utilissima*). A factorial (2x3) design was implemented for the experimental set up, with 2 levels of time and mincer speed and 2 for the temperature of H_2O, with 2 independent replicates. The comparison with the dry extraction method was also studied, this method consists in drying the bitter cassava over night at 50ºC. The yield of starch obtained in wet method ranged from 17.2 to 39.4 g of starch (mean of 26.6 g) obtained from the original samples of 250 g of wet bitter cassava, yielding yields ranging from 6.88 to 15.76% (average 10.64%) of the dry mass. The results were then analysed using PAST software v3.16 for ANOVA statistical evaluation, trend and Pareto were used to determine optimal conditions for the extraction of starch by wet and dry methods. Once the assumptions for analysis of variance (ANOVA) were made, it was concluded that a higher yield of starch is obtained from lower speeds and time, whereas the temperature of H_2O is not significant for the process, giving as optimal value for wet method = 0.0678762 for 1/x of the yield of starch obtained, with a starch purity of 64.90% ± 1.21% ranging up to 85.37% ± 1.42%. The extraction of bitter cassava starch has demonstrated its potential for the use of this variety of cassava which generates an added value to the product.

1. Introduction

Cassava is a shrub belonging to euphorbiaceous family, widely cultivated in South America, Africa and the Pacific because of its roots with starches of high nutritional value. It is considered a functional component of food due to the health benefits it confers following its consumption (Ogbo and Okafor, 2015). There is a cassava variety called *Manihot utilissima* or bitter cassava, which contains high concentrations of cyanogenic elements that make it poisonous and thus unusable as raw material for human consumption. For this reason, bitter cassava cultivars have been employed mainly as an emergency famine food (Tumwesigye et al., 2017).
However, its high carbohydrates concentrations place it as a potential source of starch mainly for industrial use. Starch obtained from cassava and bitter cassava, has numerous applications in the paper, textile, pharmaceutical (as excipient), adhesives, food (as thickener), water treatment (as coagulant), and polymer industries (Hernandez-Carmona et al., 2017).
Starch and chemically modified starch based films have drawn considerable attention on food packaging owing to their attractive combination of price, environmental friendliness, and abundance (Owi et al., 2017). Natural biodegradable polymers can be obtained directly from starch rich agricultural product (like corn, potato, wheat, cassava, barley, and rice) and wastes, using different processes such as: extraction and plastification of agricultural materials rich in cellulose and starch; microbial production; chemical synthesis of

source monomers; and chemical synthesis of synthetic monomers (Hernandez-Carmona et al., 2017). Starch from cassava has been used to obtain biopolymeric materials such as bio-derived films and food packaging film (Tumwesigye et al., 2017), green nanocomposites (Owi et al., 2017), thermoplastic starch blends with other biodegradable polymers (Fidelis et al., 2017), and starch/polyurethane dispersion blends for surface sizing agents (Rusman et al., 2017), among other applications. In this paper, we evaluate the experimental conditions (temperature, time and mincer speed) that increase the starch production from bitter cassava using the wet extraction method.

2. Methodology

2.1 Sample collection and preparation

The bitter cassava or industrial cassava (*Manihot utilissima*) was collected in the rural area of San Jacinto town, department of Bolívar (North Coast of Colombia). For this research approximately 12 kg of cassava were collected and only the ones that did not present malformations or physical damages were used.

2.2 Crude starch extraction procedure

The starch extraction was carried out by two methodologies, called "dry" and "wet" with the objective of comparing their performance (Hernández-Carmona et al., 2017). The wet process (Figure 1) includes the following stages: root reception, washing, chopping and crushing, extraction, sedimentation, and drying:

• Roots reception: bitter cassava was collected and transported immediately after harvest in order to avoid physiological and/or microbial deterioration.

• Washing: the dust and dirt were removed from the surface with water; the cassava was then dried on adsorbent paper.

• Chopping and crushing: the husks and roots were removed, the pulps (about 250 g) were manually minced at an average length of 3 cm, and then crushed with 250 ml water at different temperatures (25 and 40°C).

• Extraction: the excess fibre or bagasse was separated with a sieve from the liquid phase, which contains the starch.

• Decanting: the liquid phase was left to rest, and the starch was separated by density differences with the water in a decanter. Decanting time varied from 6 to 8 hours at room temperature (25ºC). The starch phase was then vacuum filtrated with filter paper (Whatman®) to remove the excess water for about 20 min until a semi-solid tablet was partially observed.

• Drying: the starch was dried in a conventional laboratory oven at 40°C for 8 hours.

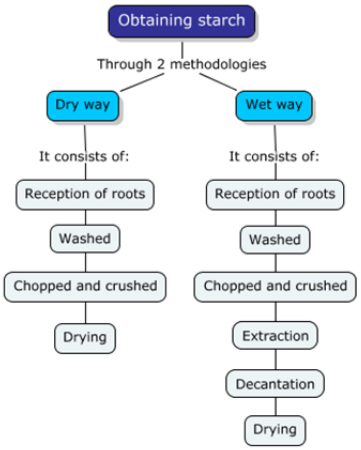


Figure 1: Scheme of the bitter cassava starch extraction wet and dry procedures.

2.3 Experimental Design

The starch yield (w/w%) from bitter cassava was selected as the dependent variable while the crushing velocity (crushing machine), crushing time and temperature of the experiment were selected as the independent ones. A balanced 2^3 factorial design (Table 1) was used for experimental planning process, with 2 crushing levels (low and high speed), 2 crushing time levels (2 and 6 min), and 3 independent replications taken at each of the 3×2 treatment combinations. The design size was N=2×4×3=24 (Montgomery, 2009).

The results were later analysed with PAST v3.14 and STATGRAPHICS CENTURION XVI software (Hammer et al., 2001; Reyes et al., 2013) for statistical evaluation. A multifactor-way ANOVA test was performed to evaluate whether the crushing velocity, crushing time and the temperature affect the starch yield and if there are interactions between them.

Table 1: 2^3 Factorial design.

Factor	Experimental factor	Levels	Coded variable
A	Crushing velocity	Low / High	- / +
B	Crushing time	2 min / 6 min	- / +
C	Temperature	25ºC / 40ºC	- / +

2.4 Product characterization

2.4.1 Determination of starch purity and amylose/amylopectin ratio

The Lane-Eynon volumetric method was used, based on the determination of the volume required to completely reduce a known volume of alkaline copper reagent. Methylene blue indicator was used to determine the final point (Storz and Steffens, 2004; Blanco et al., 2000).

2.4.2 Iodine test

The Lugol solution was prepared with 5 g of I_2 and 10 g of KI diluted with 100 mL distilled water, giving a brown solution with total iodine concentration of 150 mg/mL.

2.4.3 Colour determination

The cassava starch samples were compared with a standard starch forming rectangles (2.5-5.0 cm length and 1.6-3.5 cm height) with a spatula on a sheet of white paper, pressing the samples with a clean and fine paper to equalize the upper surface, and comparing the colour (Grace, 1977).

2.4.4 Apparent density

The starch samples were added with a spatula into a 250 ml graduated cylinder previously dried and weighed, until the total volume was freely completed, and then weighed again in order to calculate the density as the relationship between the sample mass an volume.

2.2.5 Gelatinization temperature

A starch suspension (10 g/100 mL) was prepared in cold water and doubled boiled at 85ºC, measuring the temperature until paste formation (Grace, 1977).

2.2.6 pH

A suspension was prepared with 20 g starch and 100 mL previously boiled distilled water. After 15 minutes the mixture was filtered through a Whatman® filter paper and the pH was measured to the liquid phase with a HANNA pH-meter.

2.2.7 Ashes

Ashes content was measured by incineration at 550°C during 3.5 hours.

3. Results

3.1 Starch yield from the wet extraction method

Considering the experimental design (Table 1) 24 starch samples were obtained. The crude starch yield is presented in Table 2, ranging from 6.88% to 15.76%.

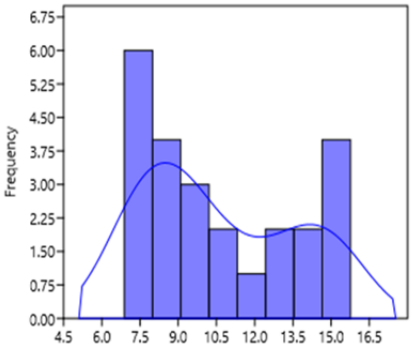
3.2 Statistical summary about data distribution

The descriptive statistical analysis and the variance analysis (multifactorial ANOVA) were implemented to the production of bitter cassava starch with the statistical software package PAST v3.16 to evaluate if the variables affect the starch yield and if there are interactions between them. Additional tests were carried out to verify the assumptions of data independence, data normality and homoscedasticity (Hammer et al., 2001; Montgomery, 2009).

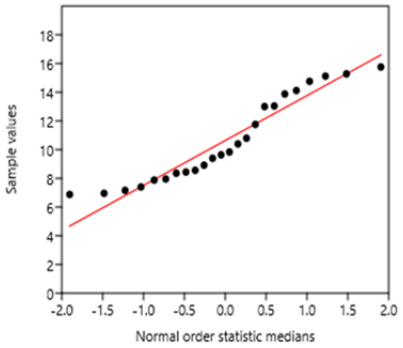
Table 2: Summary of performance in wet methodology

Crushing speed	Crushing time (min)	Temperature (ºC)	Crude starch yield (w/w%)			
			Block 1	Block 2	Block 3	Mean
Low	2	25	9.84	13.88	15.12	12.95
Low	2	40	8.36	13.04	14.76	12.05
Low	6	25	10.4	11.76	10.8	10.99
Low	6	40	8.56	7.96	14.12	10.21
High	2	25	13.0	6.96	7.88	9.28
High	2	40	15.28	9.64	8.44	11.12
High	6	25	9.4	7.4	6.88	7.89
High	6	40	15.76	8.92	7.16	10.61

	All
N	24
Min	6,88
Max	15,76
Sum	255,32
Mean	10,63833
Std. error	0,6103764
Variance	8,941423
Stand. dev	2,990221
Median	9,74
25 prcntil	8,06
75 prcntil	13,67
Skewness	0,4259054
Kurtosis	-1,318146
Geom. mean	10,24865
Coeff. var	28,10799
Shapiro-Wilk W	0,9026
p(normal)	0,02448



Starch yield histogram



Probability plot for residuals

Figure 2: Statistical results summary.

A summary of the statistical results is presented in Figure 2, where it can be seen that the values of standardized bias and standardized kurtosis are within the range -2 to +2, which indicates that the data have a normal distribution. The value of Shapiro-Wilk must be within the acceptance zone (ZA) for the null hypothesis (H0), which must be formed by all the values of the test statistic W_{exp} which are lower than the expected or tabulated value $W_{(1-\alpha;n)}$. ZA = $W_{exp} < W_{(1-\alpha;\, n)}$. Since the value of W_{exp}=0.9026 is lower than the expected value $W_{(0.95;\, 24)}$=0.916, the null hypothesis is accepted, concluding that there is a 95% confidence that the starch yield variable is not normally distributed. This could also be confirmed with the *p*(normal) value (*p*-value=0.02448) which is lower than the level of significance (α=0.05), confirming that the distribution is not normal. Therefore, it is not possible to use tests that consider standard deviations until it is stabilized, which is done by transforming the dependent variable with either its neperian logarithm, base 10 logarithm, its inverse or its square root (Table 3).

Table 3: Correlation coefficients of transformations in order to normalize the data

Coefficient	Value
Normal	0.9613
Ln X	0.9725
Log10 X	0.9721
1/X	0.9753
√X	0.9675

Analysing the results obtained with the Shapiro-Wilk test, histogram and normal probability graph (Figure 2), it is necessary to stabilize (transform) the data resulting from the starch yield, which was done by applying the inverse of each of the data obtained in order to approximate it as much as possible to a normal distribution. This decision was taken according to the results from Table 3, once transformed.

	All
N	24
Min	0,06345178
Max	0,1453488
Sum	2,428176
Mean	0,101174
Std. error	0,00555799
Variance	0,00074139
Stand. dev	0,02722848
Median	0,1026802
25 prcntil	0,07320636
75 prcntil	0,1241254
Skewness	0,09723115
Kurtosis	-1,336276
Geom. mean	0,09757386
Coeff. var	26,91253
Shapiro-Wilk W	0,9292
p(normal)	0,09364

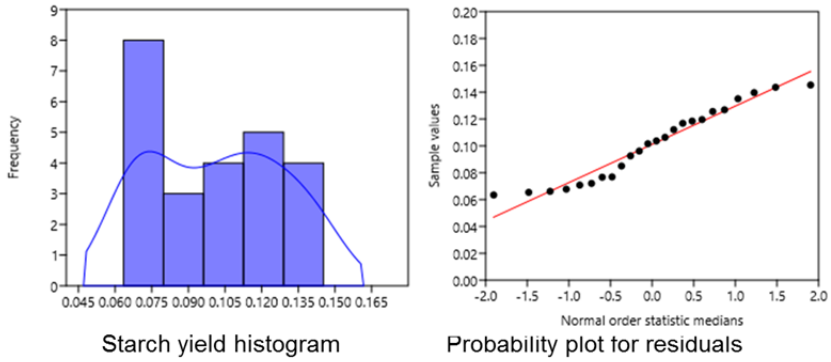


Figure 3: Statistical summary of the data after (1/X) transformation.

In the analysis of variance (ANOVA) for the response variable (Figure 3), 1/X yield of the starch obtained, each variable is analysed statistically and its effect on the model explaining the variation of each of them in the response. As shown in Table 4 there are, in this case, two effects that have a p-value of less than 0.05. This indicates that they are significantly different from zero with a confidence level of 95%, so they have a high impact on starch yield, while the other variables and/or their combinations do not have a significant effect on the response variable.

Table 4: Multifactor ANOVA test for starch yield from bitter cassava

Variable Source	Square Summation	Degrees freedom	ofMedium Squares	F	p-Values
A	0.010412	1	0.010412	45.36	0.0000
B	0.001473	1	0.001473	6.41	0.0222
C	0.000528	1	0.000528	2.30	0.1488
AB	0.000370	1	0.000370	1.61	0.2225
AC	0.000498	1	0.000498	2.17	0.1601
BC	0.000021	1	0.000021	0.09	0.7653
ABC	0.000076	1	0.000076	0.33	0.5721
Error	0.003673	16	0.000230	-	-
TOTAL	0.017052	23	-	-	-

Since the p-value of these is less than α, it can be said that the differences between some of the means are statistically significant, so the null hypothesis is rejected and it is concluded that not all the population means are equal. The statistical value r^2 of the model, thus adjusted, gives a value of 78.46% of the variability in performance. This is adjusted in a great way since it is higher than the 69.03% which is the most suitable in models with different number of independent variables.
Equation 1 shows the optimal model of the variable (1/X):

$$\frac{1}{X}(Yield) = -0{,}0137996 + 0{,}0638877\boldsymbol{A} + 0{,}0135389\boldsymbol{B} + 0{,}00129068\boldsymbol{C} - 0{,}00582842\boldsymbol{AB} - 0{,}00108332\boldsymbol{AC} - 0{,}00017528\boldsymbol{BC} + 0{,}000118942\boldsymbol{ABC} \quad (1)$$

3.3 Product characterization

Table 5: Properties comparisons

Properties	Bitter cassava	Cassava starch
Density (g/mL)	0.572	1.560
pH	4.42	4.5 - 5.5
Gelatinization temperature (ºC)	70	57.5 - 70
% Amount of ashes	0.13	-

The amount of amylose obtained varied between 66-69% while amylopectin was found between 30-33%. The starch obtained from bitter cassava contains high values in the content of amylose which favours a greater

solubility, higher viscosity, better clarity of the paste and greater tendency to the retrogradation of the gels. The starch sample produced an intense blue colour corresponding to the starch according to the Iodine test. In addition, by the qualitative colour determination, neutral coloration (white) also corresponds to starch. The only significant difference, when compared to standard starch, is that the standard starch is much finer than the starch of bitter cassava. The product characterizacion is shown in Table 5, the density and the pH results are minimal lower in relation with cassava starch, the low density can be explained with the regular compaction of the starch.

4. Conclusions

Starch extraction from bitter cassava (*Manihot utilissima*) was carried by wet extraction method. The optimal conditions were evaluated using 2^3 factorial design. The starch yield was selected as the dependent variable while the crushing speed, crushing time and the temperature were selected as the independent ones. The starch yield was varied between 6.88% and 15.76%. ANOVA analysis results shown the variables with the greatest influence on the starch yield are the crushing speed (low velocity) and crushing time (2 min). The characterization tests show that, when compared to standard cassava starch, the starch obtained from bitter cassava has similarity in colour, apparent density, gelatinization temperature and pH. The high content of amylose present in the extracted starch can favour properties such as solubility, viscosity, paste and retrogradation of the final biopolymer.

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Reference

Blanco M., Coello J., Iturriaga H., Maspoch S., González-Bañó R., 2000, On-line monitoring of starch enzymatic hydrolysis by near infrared spectroscopy, Analyst, 125, 749-752, DOI: 10.1039/A909248J

Fidelis J. et al. 2017, Development of biodegradable films of cassava starch and poly (butylene adipate co-terephthalate): effects of oregano essential oil and potassium sorbate in films characterization, Chemical Engineering Transactions, 57, 1969-1974, DOI: 10.3303/CET1757329

Grace M.R., 1977, Cassava preparation (in Spanish), Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy, 116 p.

Hammer O., Harper D.A.T., Ryan P.D., 2001, PAST: Paleontological statistics software package for education and data analysis, Palaeontologia Electronica, 4(1), 1-9, <https://folk.uio.no/ohammer/past/> accessed 10.6.2017

Hernández-Carmona F., Morales-Matos Y., Lambis-Miranda H., Pasqualino J., 2017, Starch extraction potential from plantain peel wastes, Journal of Environmental Chemical Engineering, 5(5), 4980-4985, DOI: 10.1016/j.jece.2017.09.034

Montgomery D.C., 2009, Design and Analysis of Experiments, 7th ed., Wiley. New Jersey, USA.

Ogbo F.C., Okafor E.N., 2015, The resistant starch content of some cassava based foods, Nigerian Food Journal, 33, 29–34, DOI:10.1016/j.nifoj.2015.04.007

Owi W.T., Lin O.H., Sam S.T., Villagracia A.R., Santos G.N.C., 2017, Tapioca starch based green nanocomposites with environmental friendly cross-linker, Chemical Engineering Transactions, 56, 463-468, DOI:10.3303/CET1756078

Reyes I.A., Patiño F., Flores M.U., Narayanan J., Calderón H., Pandiyan T., 2013, Use of ligand-based iron complexes for phenol degradation by Fenton modified process, Journal of the Mexican Chemical Society, 57(2), 96-104, <www.scielo.org.mx/pdf/jmcs/v57n2/v57n2a5.pdf> accessed 15.01.2017 (in Spanish)

Rusman R., Majid R.A., Wan Abd Rahman W.A., Low J.H., 2017, Carboxymethyl cassava starch/polyurethane dispersion blend as surface sizing agent, Chemical Engineering Transactions, 56, 1171-1176, DOI:10.3303/CET1756196

Storz E., Steffens K., 2004, Feasibility study for determination of the dextrose equivalent (DE) of starch hydrolysis products with near-infrared spectroscopy (NIRS), Starch/Stärke, 56, 58–62, DOI:10.1002/star.200300220

Tumwesigye K.S., Sousa A.R., Oliveira J.C., Sousa-Gallagher M.J., 2017, Evaluation of novel bitter cassava film for equilibrium modified atmosphere packaging of cherry tomatoes, Food Packaging and Shelf Life, 13, 1-14, DOI:10.1016/j.fpsl.2017.04.007