Effect of Fermentation Time and Varietal Difference on the Pasting Properties and Bread-Making Ability of Cassava Starch (*Manihot esculenta*)

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Abstract

This research is to investigate the breadmaking ability of three cassava starch varieties (96/1414, TME15, and YARA) grown in Cameroon. To achieve this, starch samples from each variety are collected before and during fermentation to determine chemical, rheological, and bread-making properties. They are analyzed for pH, titratable acidity (TTA), lactic acid (LA), specific volume (SPV), and pasting properties using known method; These parameters are used to perform a principal component analysis (PCA). The morphological characteristics are studied by scanning electron microscope (SEM). Result shows that, there is a decrease in pH (6.21–3.8) with an increase in TTA (0.34–7.05) and LA (0.15–6.46) with fermentation time. In parallel, a decrease of pasting properties and an increase in SPV (1.15–2.82 $cm^3\ g^{-1}$) are observed. The PCA surrounds 96/1414 day 30, YARA day 30, and TME15 day 25 α 30 as the best samples, and the SEM shows a superficial degradation of the granules after fermentation. Thus, this study suggests that the optimal sour cassava starch for bread-making can be obtained from 96/1414 day 30, TME15 day 25 α 30, and YARA day 30. The variety 96/1414 appears to have the best bread-making ability while TME15 appears to be most suitable for short fermentation time.

1 Introduction

In recent years, bread has become one of the most important foods in our diet due to its ever-increasing consumption. It is often made from wheat, which is distinguished from other plants by its properties, such as the viscoelasticity necessary to retain the CO_2 produced during fermentation.[1] However, several problems are associated with the use of wheat flour alone in bread-making like gluten intolerance and the unavailability of this commodity in several countries that depend entirely on import. The Covid-19 pandemic has disrupted global supply chains and had long-term effects on livelihoods. In addition, the conflict between Russia and Ukraine, two of the world's largest producers of agricultural commodities have a significant effect on the food security of many African countries.[2] Indeed, Russia and Ukraine together account for more than 10% of global wheat production and exports, and many African countries rely on imported grains from Russia and Ukraine to meet their consumption needs.[3] To remedy this situation, the use of locally available flours has proven to be very interesting. The use of locally available flours helps reduce the dependence on wheat imports and the development gluten-free foods.[4] The local products generally used for this purpose are cereals, legumes, and tubers, including sweet potatoes, yams, potatoes, and cassava. The most successful results have been observed with cassava, whose starch is most capable of mimicking the properties of wheat, and its functional properties, such as clarity, swelling, water retention, and high viscosity, make it a popular ingredient in the food industry.[5] It forms better films and has a higher yield point than other starches, which makes it an alternative to wheat flour. This is the case in South America where wheat flour was substituted with cassava starch modified by a 30 days fermentation and UV irradiation of the sun known as sour starch.[6] However, the bread-making ability of sour cassava starch remains erratic, and its use in bakery products remains empirical and regional. Several factors, including genetic and geographical factors, influence the bread-making ability of cassava starch. Thus, the mastery of the bread-making ability acquisition of sour cassava starch in different area can promote its bread-making usage. It is in this interest that[7] studied the cassava varieties' effects on the physicochemical and functional properties of sour cassava starch[8]; studied the effects of fermentation length and varietal difference on the pasting properties of sour cassava starch[6]; studied the effect of genetic factors on the bread-making capacity of cassava sour starch. However, to the best of our knowledge there is little or no literature on bread-making ability of sour cassava starch from cassava varieties grown in Cameroon. Hence, the aim of this study was to study the influence of fermentation and varietal difference (96/1414, TME15, and YARA) on the bread-making ability of cassava starch (*Manihot esculenta*) grown in Cameroon.

2 Experimental Section

2.1 Materials

Cassava tubers collected in a peasants' farm in the eastern Cameroon were used as plant material, three local varieties (96/1414, Yara, and TME 15) were chosen based on availability. Baking ingredients consisted of starch, water, cheese (Mozzarella), and salt (white diamant). The cheese and the salt were purchased from the local supermarket and all the chemical reagent used in this study were of analytical grade and was purchased from Sigma–Aldrich Pty. Ltd. (Johannesburg, South Africa) it included lactic acid (LA) solution, iron III chloride,

potassium hydroxide (KOH), hydrogen chloride (HCl), sodium hydroxide (NaOH), phenolphthalein, iodine (I_2), potassium iodide (KI), and distilled water (H_2O).

2.2 Methods

2.2.1 Starch Extraction and Sampling

The cassava starch was extracted using a wet process, where the cleaned and peeled cassava tubers were transformed into a fine paste by a rotating and coordinated grinding process known as rapping. The fine paste was then conveyed to a centrifugal sieve for separation. The starch milk obtained was transferred to sedimentation tanks. Just after extraction, a sample of 300 g starch milk (unfermented starch) was taken and kept throughout the fermentation period (5, 10, 15, 25, 30, days of fermentation). Before each withdrawal, a grid was drawn on the surface of the fermentation tank, and the sample was taken from the center of each square. All samples taken at the same time of fermentation were mixed to obtain a homogeneous sample, representative of the entire fermentation basin at that moment. The collected samples were stored in a freezer and were later on subjected to the same amount of sunlight for drying. All the samples studied were sun-dried on black plastic sheets for about 12 hours, similar to the traditional conditions for drying sour starch in Colombia.[9]

2.2.2 Chemical Properties

The obtained samples were forwarded to the research unit of medical plants where analyses of pH and titratable acidity (TTA) were performed using the method proposed by.[10] The LA content was determined using the method described by,[11] where a calibration curve was established by preparing a series of LA solutions by the double dilution method on the basis of the standard solution of concentration 89 g L^{-1}. Then 100 µm of an LA solution of appropriate concentration was mixed with 2 mL of a 0.2% iron III chloride solution. For the measurement of LA content in the starch, a 10% w/v aqueous suspension of starch was shaken at room temperature for 30 min and then centrifuged at 5000 × *g* for 15 min. Of the resulting supernatant 100 µL was added to 2 mL of a 0.2% iron III chloride solution and mixed. The optical density of the color solutions obtained was measured using a spectrophotometer at a wavelength of 390 nm; using a 0.2% iron III chloride solution as control. The amylose and amylopectin content were determined following the method described by[12] where a calibration curve was established as presented in **Table** 1. For the measurement or determination of the total starch and amylose in the starch, 5 mL of 1 n KOH were added to 0.1 g of starch and the solution was carefully homogenized at room temperature before being neutralized with 5 mL of 1 n HCl. A pH meter was used to ensure that the solution was neutral. The mixture was then boiled in a water bath for 15 min and its volume readjusted to 10 mL. The mixture was centrifuged and the supernatant was collected. This was latter filtered and used for the starch assay as presented in **Table** 2. Using these measurements, the proportions of total starch, amylose, and amylopectin in the samples were calculated. The amylopectin content was obtained by difference as follows:

$$\% \text{ Amylopectin} = \% \text{ Total starch} - \% \text{ amylose} \quad (1)$$

Table 1. Establishment of the calibration curve.

Reagents/tubes	T_0 (blank)	T_1	T_2	T_3	T_4	T_5
Starch (5 mg mL^{-1}) [mL]	0	0.02	0.04	0.06	0.08	0.1
H_2O [mL]	4.9	4.88	4.86	4.84	4.82	4.80
I2/KI reagent [mL]	0.1	0.1	0.1	0.1	0.1	0.1
Calculation [starch] [mg mL^{-1}]						
Incubation time		10 min				
$D0_1$ (580 nm)						
$D0_2$ (720 nm)						

For starch: DO = 1.8366Q − 0.0005 with Q = amount of starch; R^2 = 0.9923. For amylose: DO = 1.2009Q − 0.0123 with Q = amount of amylose; R^2 = 0.9834.

Table 2. Determination of starch, amylose, and amylopectin in starch.

Reagent/sample	Blank [mL]	Sample 1 [mL]	Sample 2 [mL]
Sample	0	0.05	0.05
H_2O	4.90	4.85	4.85
I_2/KI reagent	0.1	0.1	0.1
Incubation time	10 min before DO readings		
Avg. DO (580 nm)			
Avg. DO (720 nm)			

2.2.3 Pasting Properties

The viscous behavior of starch at various fermentation times and varieties was measured using a Rapid Visco Analyzer (RVA) model RVA-4 Series (Newport Scientific, Warriewood, Australia) as described by.[10] 2.5 g (dry basis) flour was dispersed in 22.5 g water. The following temperature profile was used to measure the viscosity: holding at 50 °C for 1 min, heating from 50 to 90 °C at 6 °C min^{-1}, holding at 90 °C for 5 min, and then cooling down to 50 °C at 6 °C min^{-1} with continuous stirring at 960 rpm for 10 s and then at 160 rpm for the rest of the experiment. The visco-amylogram yielded the following seven parameters: peak viscosity (PV), minimum viscosity (MV), breakdown (BD), final viscosity (FV), setback (SB), peak time (PT), and pasting temperature (PT C). The analyses were performed in triplicate and mean values were calculated

2.2.4 Specific Volume

The method developed by[6] was used. Briefly, 46 g of sour starch was combined with 54 g of cheese (Mozzarella) in a Hobart mixer for 1 min on low speed (165 rpm). A certain amount of water was added to obtain a total volume of 35 mL. The dough was then kneaded for 2 min at medium speed (300 rpm) and afterwards formed into balls. These dough pieces were baked at 250 °C for 30 min before cooling at room temperature for 2 h. The loaves' specific volume (SPV) was determined using the repeated displacement method,[10] where 5 L of a vat was filled up with sesame grains, then part of the sesame grains contained in the vat was replaced by the bread and the vat was filled to the level again with the grains of sesame. The remaining sesame grains correspond to the volume and the SPV was obtained by dividing the volume by the bread masse.

2.2.5 Principal Component Analysis (PCA)

A PCA was performed to determine the correlation between the variables and their contribution to the bread-making ability acquisition. It was equally used to determine the best fermentation time for each variety based on certain parameters. The following parameters were used: pH, TTA, LA content, rheological properties (PT C, PV, MV, BD, FV, and SB) and bread-making ability (SPV). The RVA parameters predict the behavior of the dough during baking, each parameter gives information about the bread-making ability, hence they were all used for the PCA.

2.2.6 Morphological Characteristics

Morphological characteristics of native and modified cassava starches were studied by scanning electron microscope (SEM) (Tescan Vega3). Here, starch granules were placed on SEM stub using double-sided cellophane tape and then coated with gold. An accelerated potential of 20 kV was used during micrographic examination.

2.2.7 Statistical Assessment

The results of the triplicate analyses were expressed as standard deviations and calculated using Excel software version 2013, with statistical analysis performed using SPSS software version 23.

3 Results and Discussion

3.1 pH, Titratable Acidity, and Lactic Acid Content

A decrease in pH and an increase in TTA (**Figure 1**a) was observed for the three varieties with fermentation time as well as an increase in the LA content (**Figure 2**a). The TME15 variety had the highest pH (6.21–4.1), lowest TTA (6.54 × 10–5 mol g^{-1}), and LA content (6.16 × 10—5 mol g^{-1}) while variety 96/1414 had the lowest pH (3.8), highest acidity level (7.05 × 10–5 mol g^{-1}), and LA content (6.16 × 10–5 mol g^{-1}). The increase in TTA in the milieu explains the decreasing pH. These findings are consistent with those of,[8] who observed a decrease in pH from 6 to 4 and an increase in total acidity from 1.5 to 2 during fermentation of cassava starches. Similarly,[13] observed an increase in acidity from 1.5% to 5% and the decrease in pH

from 6.4 to 4 with fermentation of cassava starch. The fermentation process is divided into three phases (Figure 1); the first phase characterized by rapid acidification of the milieu with a drop in pH from 6.4 to 4.6 and a parallel increase in acidity from 0.55 to 3.7. This is characterized by a decrease in oxygen concentration in the milieu associated with an attack of amylolytic enzymes on granular starch, providing a carbon source for fermentation agent metabolism.[14] The second phase characterized by a gradual increase in acidity which is critical for the production of high-quality sour starch because it introduces more demanding microorganisms that produce acids and gases.[15] The third phase characterized by a rapid increase in total acidity which results in a pH drop from 4.5 to 3. Indeed, the fermentative microflora present during cassava starch fermentation produce organic acids, particularly LA which contributes to the bread-making ability acquisition.[16]

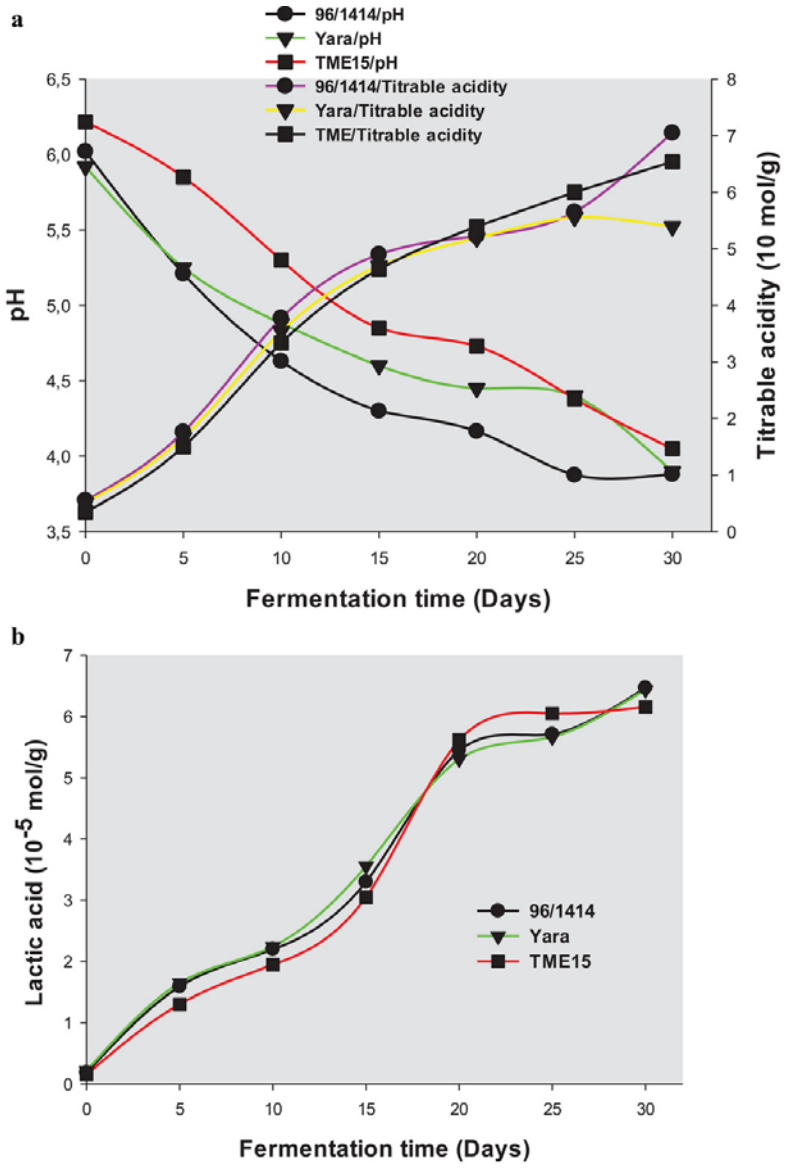

Figure 1. a) Evolution of pH and titratable acidity during fermentation, b) evolution of the lactic acid content during fermentation.

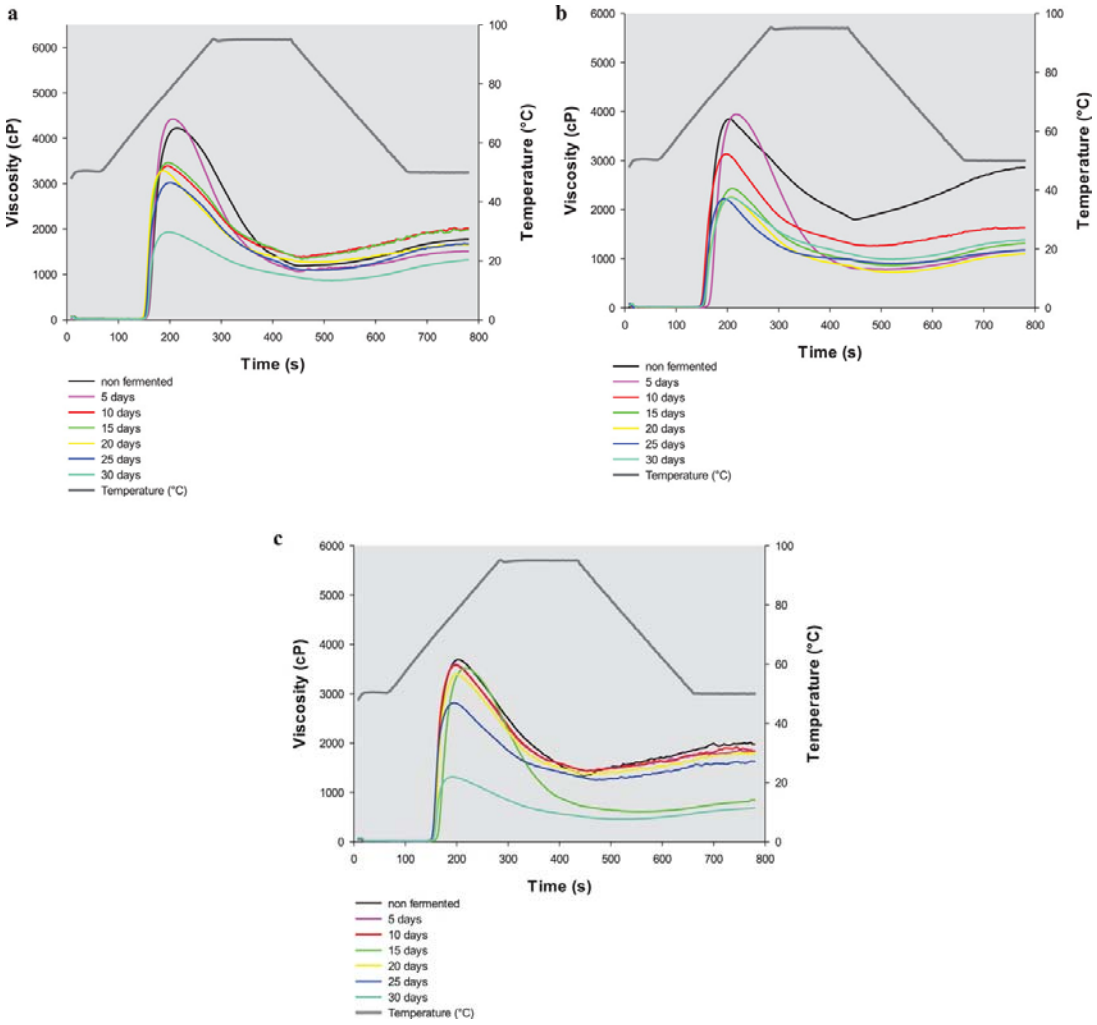

Figure 2. a) Viscoamylogram of the variety 96/1414 during fermentation, b) viscoamylogram of the variety TME15 during fermentation, c) viscoamylogram of the variety YARA during fermentation.

3.2 Pasting Properties

The understanding of the different modifications that occurs during gelatinization and retrogradation of a specific starch is critical for a better prediction of the functional properties of processed starch.[17] Figure 2a,b,c show the viscoamylogram of cassava starch of the three varieties at different fermentation times (0, 5, 10, 15, 20, and 30 days). We can draw from these figures that there is an increase of the viscosity to a maximum which is followed by a minimum decreased as the granules break off. Then the viscosity increases again to a final value with the decrease. Also, there is a general decrease of the viscosity with the fermentation time. The decreased in peak viscosity with fermentation time can be related to the degradation of starches into simple molecules and the depolymerization during fermentation.[18] These observations are similar to those of,[19-21] in their study on the effect of fermentation and sun drying on the physical and technological properties of cassava starch. However, we observed a low viscosity decrease of the variety TME15 with fermentation compared to the others which correlate the low morphological modification observed (Figure 7c,d).

Tables 3 and 4 show the pasting parameters of native and modified starches of the three varieties. It appears that the PV, MV, BD, FV, and SB differed significantly ($p < 0.05$) for the native and the sour cassava starch and between the three varieties. There is no significant difference ($p < 0.05$) in the PT and PT°C of the native, fermented cassava or between the varieties. In its native state, the variety 96/1414 had the highest PV and BD values, while YARA had the lowest.[22] TME15 achieved the highest MV and FV, while 96/1414 had the lowest. In terms of SB, variety YARA had the highest value and TME15 the lowest. Nonetheless, in the sour state, TME15 had the highest PV, BD, MV, and FV values, while YARA had the lowest. YARA had the highest SB value, followed by 96/1414 and TME15, with the lowest value.

Table 3. Pasting properties of native cassava starch.

Pasting properties	Native starch		
	96/1414	TME15	YARA
PV [cP]	4221 ± 30.36^{Aa}	3852.5 ± 28.92^{Ba}	3692.5 ± 11.28^{Ca}
MV [cP]	1229 ± 31.08^{Ca}	1878.5 ± 2.84^{Aa}	1398.5 ± 82.68^{Ba}
BD [cP]	2992 ± 0.6^{Aa}	1974 ± 50.88^{Ca}	2294 ± 93.96^{Ba}
FV [cP]	1773.5 ± 0^{Ca}	2858 ± 42.36^{Aa}	1977 ± 9.84^{Ba}
SB [cP]	544.5 ± 26.76^{Ca}	994.5 ± 13.32^{Ba}	1715.5 ± 21.12^{Ba}
PT [s]	212 ± 0.00^{Aa}	208 ± 0.00^{Ba}	204 ± 0.00^{Ca}
PT°C [cP]	49.95 ± 0.00^{Aa}	48.95 ± 0.00^{Ba}	48.95 ± 0.00^{Ba}

Values with different letters are significantly different at $p < 0.05$. Letters in majuscule indicate differences between varieties for the same fermentation time and letters in lowercase indicate differences between fermentation times for the same variety. BD, breakdown; FV, final viscosity; MV, minimum viscosity; PT, peak time; PT°C, pasting temperature; PV, peak viscosity; SB, setback.

Table 4. Pasting properties of sour cassava starch.

Pasting properties	Sour starch		
	96/14	TME15	YARA
PV [cP]	1930 ± 15.48^{Bb}	2248.5 ± 22.56^{Ab}	1309.5 ± 15.48^{Cb}
MV [cP]	965 ± 128.64^{Cb}	1111 ± 25.44^{Ab}	522 ± 1.32^{Bb}
BD [cP]	965 ± 144.24^{Bb}	1137.5 ± 48^{Ab}	787.5 ± 14.04^{Cb}
FV [cP]	1315.5 ± 236.88^{Bb}	1385 ± 92.52^{Ab}	677.5 ± 0.6^{Cb}
SB [cP]	350.5 ± 252^{Bb}	274 ± 11.2^{Cb}	632 ± 16.2^{Ab}
PT [s]	200 ± 0.00^{Bb}	208 ± 0.00^{Ab}	192 ± 0.00^{Cb}
PT°C [cP]	49.25 ± 0.00^{Ab}	48.95 ± 0.00^{Aa}	48.95 ± 0.00^{Aa}

Values with different letters are significantly different at $p < 0.05$. Letters in majuscule indicate differences between varieties for the same fermentation time and letters in lowercase indicate differences between fermentation times for the same variety. BD, breakdown; FV, final viscosity; MV, minimum viscosity; SB, setback, PT, peak time; PT°C, pasting temperature; PV, peak viscosity.

The PV is observed when starch granules swell and increase in viscosity with increasing temperature until the maximum viscosity is reached.[23] It indicates the water-binding capacity of the starch and the ease with which the starch granules are disintegrated.[17] The high PV of 96/1414 and the lowest PV of YARA can be explained by their amylopectin content which is high in 96/1414 and low in YARA. The decreased of PV value with fermentation can be related to the solubilization and the disintegration of the granules that occurred during the fermentation which contributed to this decrease of PV value.[24] Indeed there is a hydrolyzation of the starch during the fermentation resulting in a lower PV.[24] It has also been proven that native starch swells slowly, whereas starch modified by fermentation and solar irradiation swells faster, resulting in an early PV.[25] This increases the starch molecules' resistance to pressure for a high viscosity opposes the push of water vapor bubbles leading to an increase of the bread making ability. As for the MV which indicates the granules rapture,[17] there is a decreased from 1229 ± 31.08 to 1878.5 ± 2.84, 1398.5 ± 82.68 to 965 ± 128.64, and 1111 ± 25.44 to 522 ± 1.32 for 96/1414, TME15, and YARA, respectively. The decrease in viscosity of starch granules explains the decrease in MV. Also, there is a decreased of BD from 2992 ± 0.6 to 1974 ± 50.88, 2294 ± 93.96 to 965 ± 144.24, and 1137.5 ± 48 to 787.5 ± 14.04 for 96/1414, TME15, and YARA, respectively. It has to be highlighted that BD measures the degree of disintegration or stability of the dough[26] and it is influenced by the size of the granules and the extent of recrystallization.[27] Hence, the differences between the variety. The decrease in BD value can be related to the differences in molecular packing after fermentation which affects structural strength to granules and melting of crystallites.[24] A low BD indicates better stability of the dough at high temperatures.[6] Thus, sour starch is more stable at high temperatures than native starch. As for the FV is the most commonly used parameter for analyzing starch and determining the ability of a material to evaporate after cooking.[29] Here, the FV decreased from 1773.5 ± 0.00;

2858 ± 42.36 and 1977 ± 9.84 to 1315.5 ± 236.88; 1385 ± 92.52 and 677.5 ± 0.6 for 96/141, TME15, and YARA, respectively. A low final viscosity is adequate for bread making because it allows a better film formation.[28] In fact, the fermentation of cassava starch improves its ability to form films however it has the disadvantage of not being able to evaporate at the end of the cooking process. Concerning the SB is a measure of amylose retrogradation during cooling.[14] This retrogradation phenomenon is related to the re-association of amylose molecules via hydrogen bond formation between hydroxyl groups which is positively correlated with amylose content.[6] The highest SB obtained with YARA compared with other variety is related to its high amylose content.[22] The decrease in SB from 544.5 ± 26.76; 994.5 ± 13.32 and 1715.5 ± 21.12 to 350.5 ± 25.2; 274 ± 11.2 and 632 ± 16.2 for 96/1414, TME15, and YARA respectively is related to the differences in the rearrangement or the recrystallization of amylose before and after the fermentation.[24] These decreases indicate that sour cassava starch retrograde less compared to native starch. These results corroborate with previous work of [30] and [21] where there was significant decrease in SB with fermentation. As for the PT°C, or the temperature at which the first variation in viscosity is observed, is not affected by the fermentation and is relatively low for all the samples. This mean that all starches have a short cooking time which allow energy saving.[20] Observed similar results in their study on the effect of starch modification on the rheological properties. The production of organic acids during fermentation and the enzymatic actions on the granules affected the structural strength of the starch granule, the bonding between starch chains, the rearrangement and recrystallization of the molecules resulting in a reduction of the pasting properties.

3.3 Total Starch, Amylose, and Amylopectin Content

The total starch, the amylose, and the amylopectin contents were determined only on the native starch. Results showed that the variety 96/1414 had the highest total starch and amylopectin, and the lowest amylose content followed by TME15. The amylose content of the different starch explained the pasting properties and the SPV obtained below. The variety 96/1414 which had the lowest amylose content also showed a higher SPV. Indeed, the amylose content is negatively correlate with the pasting properties and positively correlated with the SPV. This negative correlation between the amylose content and the SPV is due the complex formed by amylose and lipids which inhibits the swelling power of the starch. This was also observed by[6] in his study on the effect of genetic factors on the bread-making capacity of sour cassava starch (**Figure** 3).

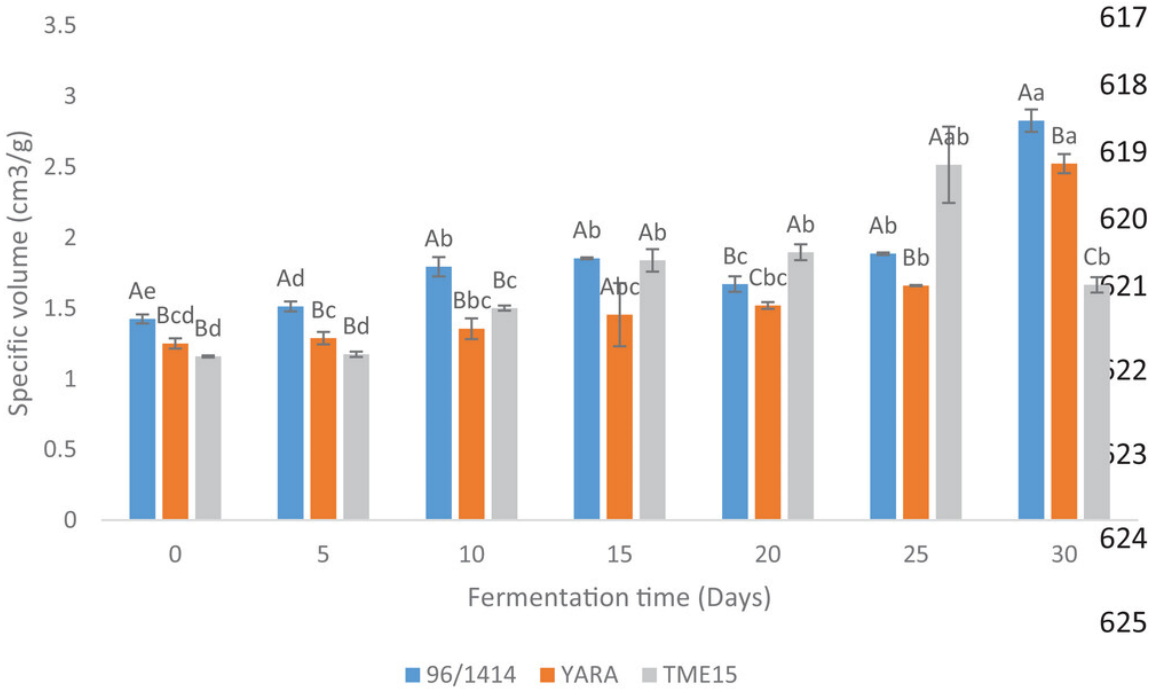

Figure 3. Evolution of the specific volume of varieties 96/1414, Yara, and TME15 during fermentation. Values with different letters are significantly different at $p < 0.05$. Letters in majuscule indicate differences between varieties for the same fermentation time and letters in lowercase indicate differences between fermentation times for the same variety.

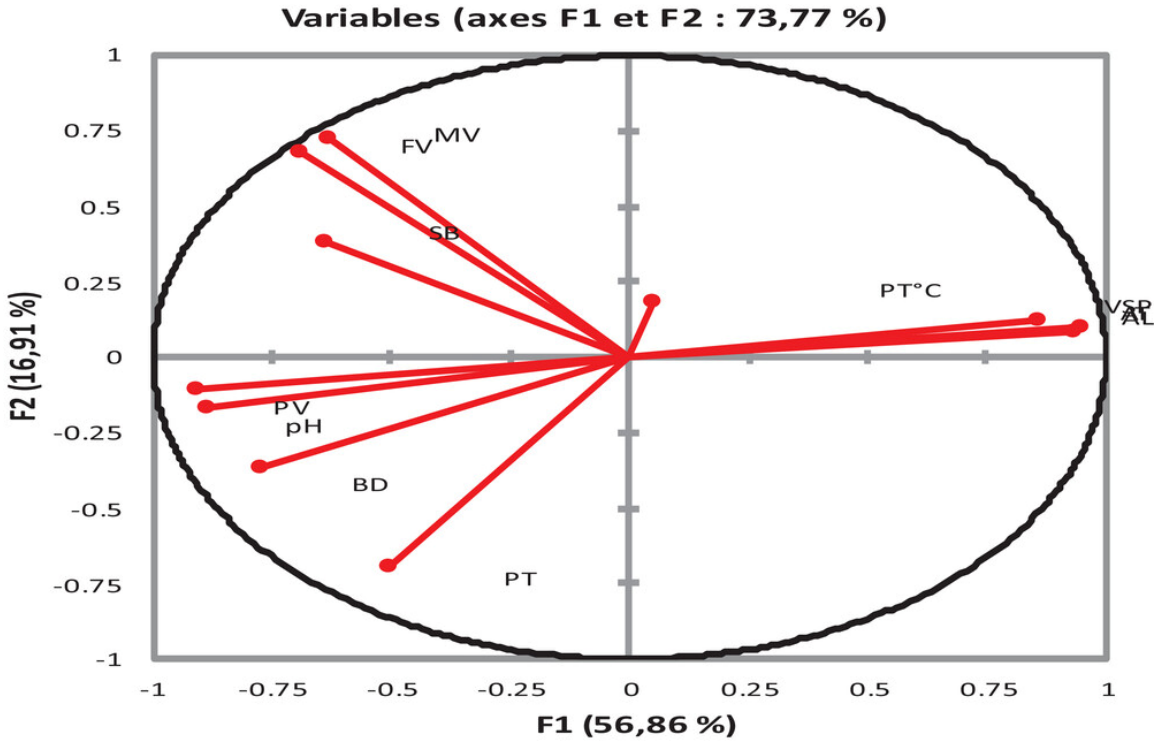

Figure 4. Correlation between variables during fermentation. BK, breakdown; FV, final viscosity; HV, holding viscosity; LA, lactic acid; PT, peak time; PT C, pasting temperature; PV, peak viscosity; SB, setback; SPV, specific volume; TTA, titratable acidity.

3.4 Specific Volume of Bread

The graph (**Figure** 4) represents the SPV of bread at different times of fermentation for the three varieties. We observe an increase in the SPV of bread with fermentation time for the three varieties. Variety 96/1414 presents the highest SPV at day 30 (2.82 g cm^{-3}), but the highest volume is obtained at day 25 for the variety TME15 (2.51 g cm^{-3}) which is not significantly different from the SPV of YARA (2.52 g cm^{-3}) at day 30.

The evolution of the bread-making ability correlates with the increase in LA and the decrease in pH of the milieu. In fact, the increase in SPV of the bread with fermentation time may be due to the accumulation of LA as well as the degradation of the starch molecule during fermentation. During fermentation, a series of starch structure modifications occurs which is responsible for the acquisition of the bread-making ability observed. Therefore, the degradation of the starch structure would result to a change in the gelatinization behavior revealed by RVA. In addition, other factors such as the presence of the enzyme-amylase and some bacterial exopolysaccharides produced during fermentation could be responsible of the bread-making ability of sour cassava starch.[6] The fermentation of cassava starch produces LA and propionic acid, as well as carbonic gas, which is adsorbed by the starch granules. The presence of such organic acids coupled with the presence of alpha-amylase lead to slight modifications of the starch granule, which contributed to the acquisition of bread-making ability.[31] The expansion during cooking may be related to their desorption and to their expansion, similar to water vaporization.[32] During baking, the water in the dough is superheated and the vapor pressure is rapidly expanding cells rises due to increased heat transfer and temperature. The pressure of the gases retained inside the polymeric network of acid-modified amylase, amylopectin, and dextrins expands the mass, resulting in sour starch bake expansion properties.[16] Thus, the acidification observed during fermentation facilitates the adsorption of carbonic acids favoring the swelling capacity of the starch.

3.5 Principal Component Analysis (PCA)

The PCA analysis provides an overview of the similarities and differences between starch samples, as well as the relationships between chemical, rheological, and SPV (Figures 4 and 5). The first and second major components (F1 and F2) explain 73.77% of the total variation. Where F1 accounted for 56.86% of total variation, and F2 for 16.91% of total variation.

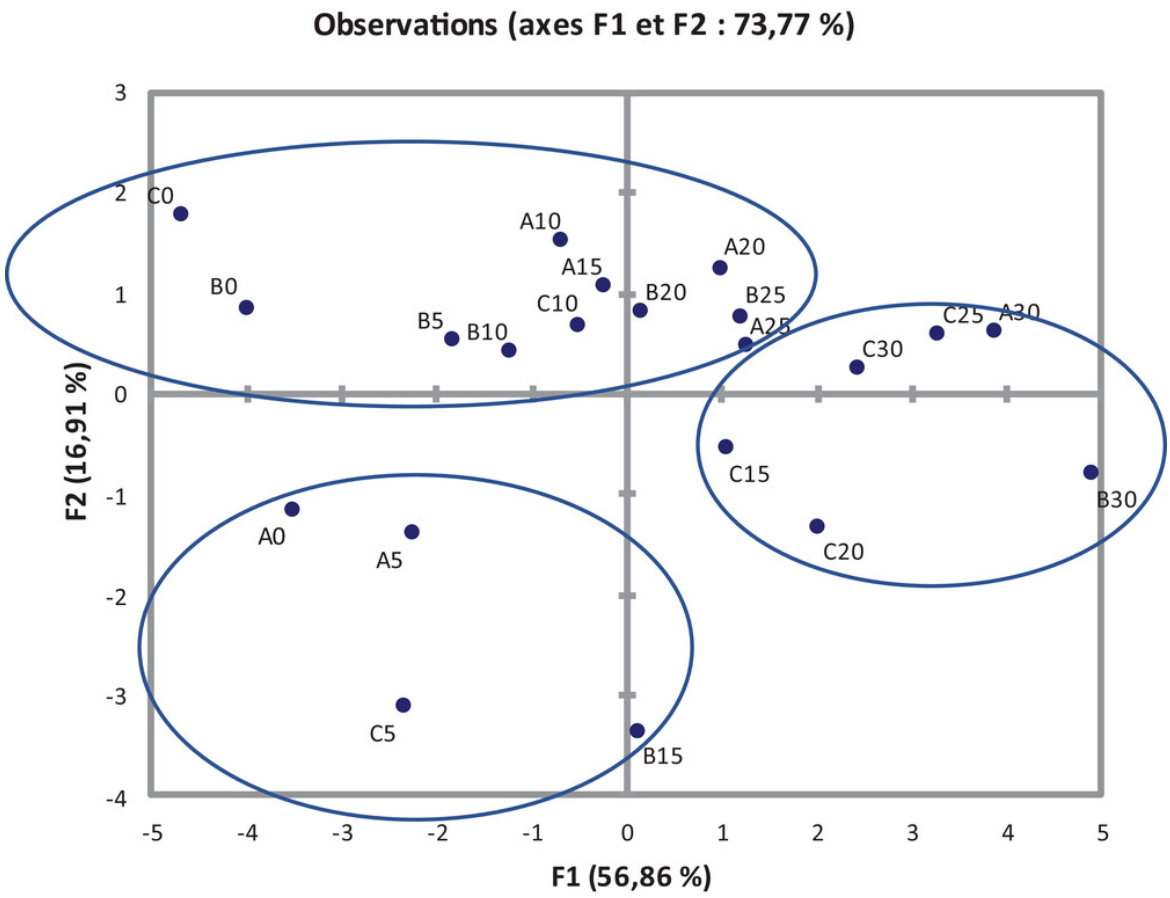

Figure 5. Principal component analysis (PCA) observation diagram of starch sample during fermentation. A0: 96/1414 days0, B0: Yara days0, C0: TME15 days0, A5: 96/1414 days5, B5: Yara days5, C5: TME15 days5, A10: 96/1414 days10, B10: Yara days10, C10: TME15 days10, A15: 96/1414 days15, B15: Yara days15, C15: TME15 days15, A20: 96/1414 days20, B20: Yara days20, C20: TME15 days20, A25: 96/1414 days25, B25: Yara days25, C25: TME15 days 25, A30:96/1414 days30, B30: Yara days30, C30: TME15 days30.

Figure 4 depicts the correlation diagrams between the factors. It allows us to see the relationships between different variables and observations. The variables pH, TTA, LA, SPV, PV, and BD had a high load in contribution to F1 (chemical and breadmaking properties). TTA, LA, and SPV were highly correlated between them because they are in the same quadrant (Figure 5), while being negatively correlated with the variable pH, PV, and BD since they are in opposite quadrant (Figure 4). Although the variables MV, FV, SB, PD, and PT°C contributed more to the formation of F2 (pasting properties) (Figure 4) and were highly correlated between them because they were on the same side. Only two components are required to explain the majority of the variance, this confirmed the dependence between the eleven variables. PCA can transform a data set with inter-correlated variables into a new uncorrelated vector space (orthogonal axes). In the case of variables correlated with component 1, the circle of correlation (Figure 4) indicated that there was a tendency to increase in acidity and SPV when pH and rheological parameters decrease. This confirms that the acquisition of sour cassava starch bread-making ability required an increase in acidity and a decrease in rheological properties.

Figure 5 depicts a component-by-component analysis in which the various starch sample are treated as individuals. It is clear that along the axis F1 × F2, there is a demarcation of sample A30(96/1414 jour 30), C30(TME15 jour 30), C25(TME15 jour 25), C20(TME15 jour 20), C15(TME15 jour 15), and B30(YARA jour 30), compared to others sample. In addition, the hierarchical bottom-up clustering analysis grouped the observations into three classes (**Figure** 6). The first class consists A0, A5, C5, and B15; the second of B0, C0, B5, A10, B10, C10, A15, A20, B20, A25, and B25; and the third C15, C20, C25, A30, B30, and C30. The SPV, the TTA, and the LA are of greatest participation to the score representation of the third class (**Figure** 7). Indeed A30, C30, C25, C20, C15, and B30 are characterized on one hand by a high SPV, TTA, and LA content and on another hand by low values of pH and rheological parameters. We note that there are four fermentation times (days 15, 20, 25, and 30) for TME15 variety in the third class, which is representative of samples with high bread-making ability, instead of only one fermentation time (day 30) for varieties 96/1414 and YARA. Indeed, TME15 showed the highest SPV at the 25th day of fermentation heather than the 30th day like others, it also shows similar peak viscosity for 15, 20, 25, and 30 days of fermentations meanwhile the varieties 96/1414 and YARA had the lowest peak viscosity at the 30th day of fermentation which is significantly different from day 15, 20, and 25 the same for the SPV. This confirms the effect of varietal difference on the bread-making ability acquisition of sour cassava starch.

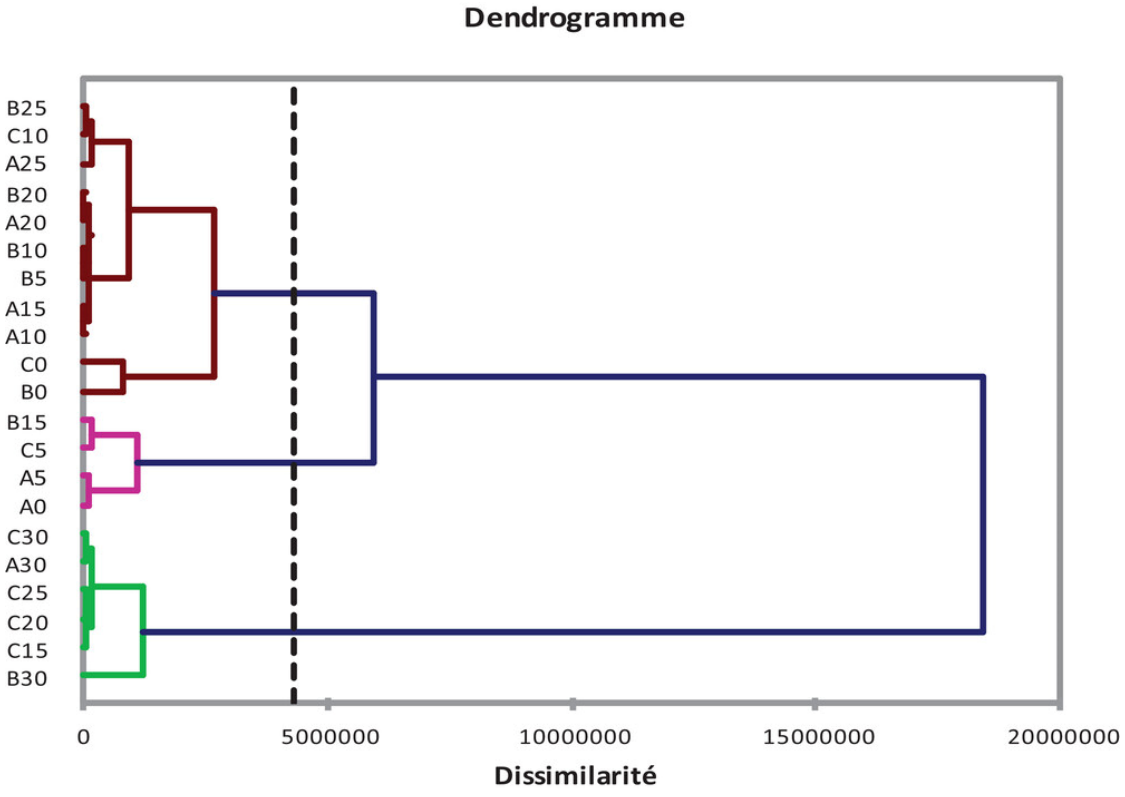

Figure 6. CAH dendrogram of observations of starch sample during fermentation. A0: 96/1414 days0, B0: Yara days0, C0: TME15 days0, A5: 96/1414 days5, B5: Yara days5, C5: TME15 days5, A10: 96/1414 days10, B10: Yara days10, C10: TME15 days10, A15: 96/1414 days15, B15: Yara days15, C15: TME15 days15, A20: 96/1414 days20, B20: Yara days20, C20: TME15 days20, A25: 96/1414 days25, B25: Yara days25, C25: TME15 days 25, A30: 96/1414 days30, B30: Yara days30, C30: TME15 days30.

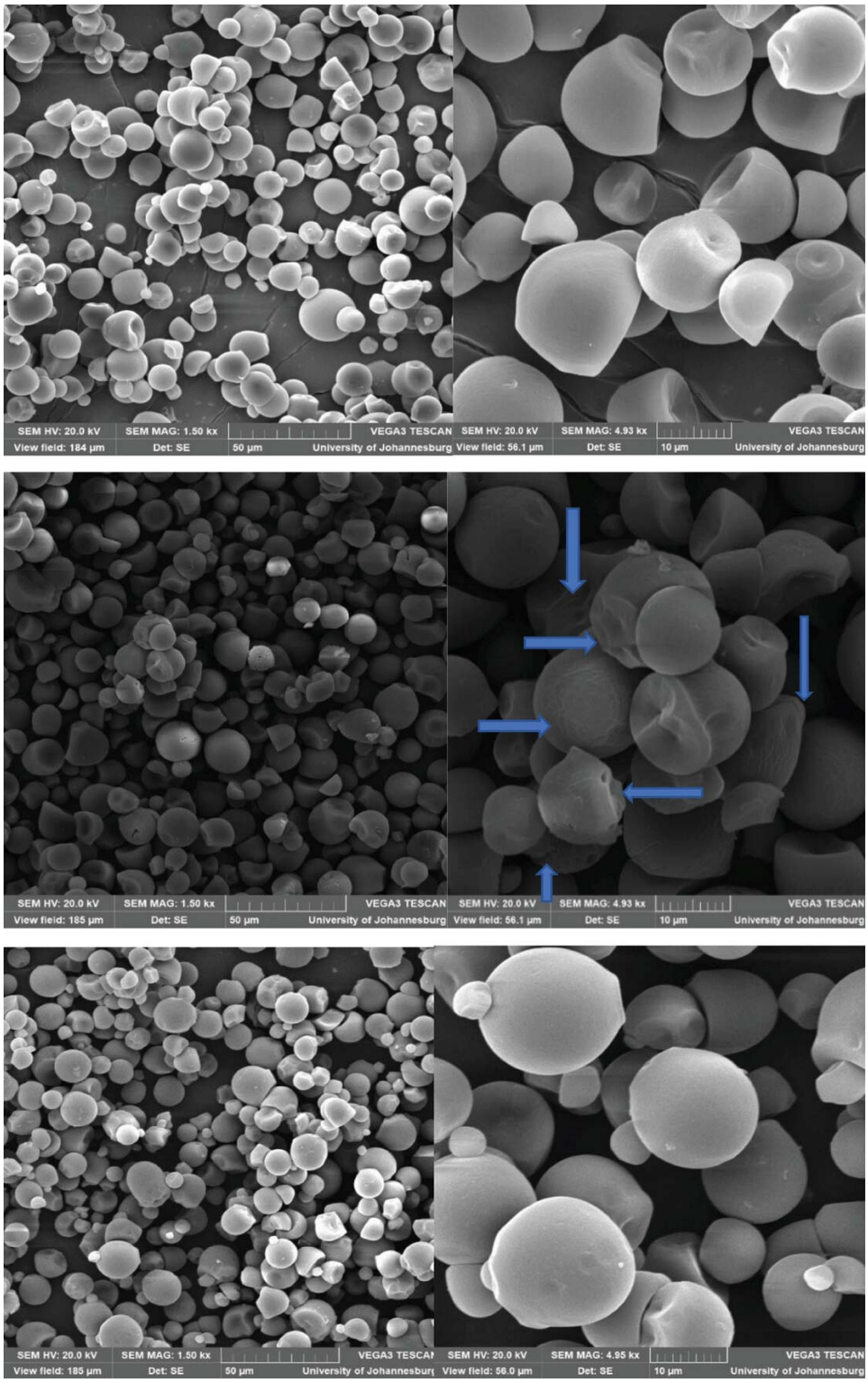

Figure 7. a) Scanning electron microscopy (SEM) image of native cassava starch of the variety Yara, b) SEM image of sour cassava starch of the variety Yara, c) SEM image of native cassava starch of the variety TME15, d) SEM image of sour cassava starch of the variety TME15, e) SEM image of native cassava starch of the variety 96/1414, f) SEM image of sour cassava starch of the variety 96/1414.

3.6 Scanning Electron Microscopy (SEM)

Figure 7a–f show the morphological structure of native and fermented starch for the three varieties. We can observe that cassava starch granules have a spherical and truncated shape with an apparently smooth, flat, and homogeneous surface as observe by authors.[6, 13] A superficial degradation of the sour cassava starch granules is observed with the appearance of certain cracks, pits and corrosion channels, in the sour starch for the three variety. This was also observed by[33] in their study on the physicochemical properties of fermented potatoes starches. However, YARA is most degraded, followed by 96/1414 and TME15 which is least degraded. Also, TME 15 had the smallest granules size which is between 3.44 and 19.46 µm followed by 96/1414 with value between 3.75 and 21.29 µm which can explain the low degradation of these ones compared to YARA which had the highest granules size (6.06–31.70 µm). Indeed, cassava starches granules with smaller size tend to be more resistant to enzymes action than those with bigger size.[34] These degradations confirm that there is not only an acid modification but also an enzymatic activity which occur during the natural fermentation of cassava starch. This enzymatic activity involves mainly α-amylase and amyloglucosidase and is characterized by the erosion and solubilization on the surface of the granules.[34] During fermentation, there is an erosions on the granule surfaces, which become deeper with increasing of the acidity.[13] Thus, the degradation caused by acidic fermentation occurred first on the starch granule surface, preferentially on amorphous regions. This may have caused the crystalline areas on these cassava starches to begin to degrade.[13]

4 Conclusion

The three-variety studied acquires bread-making ability after fermentation and solar radiation treatment. The PCA analysis showed that there is on one hand, a positive correlation between the SPV and the acidity and on the other hand a negative correlation between the SPV and the pasting properties. Thus, it can be said that the acquisition of bread-making ability is coupled with the increase of the acidification and the decrease of the rheological properties. Moreover, there is a superficial degradation of the starch granules which may also contribute to the bread-making ability. However, for better bread-making ability, 96/1414 is appropriate, whereas TME15 is suitable for short fermentation time. Thus, this analysis confirmed that sour cassava starches have a unique baking expansion property that is suitable for the development of gluten-free product.

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Conflict of Interest

The authors declared that they have no conflict of interest.

Author Contributions

M.M.N.N.: Conceptualization, methodology, resources, investigation, visualization, formal analysis, writing – original draft. J.M.K., B.F., F.N.Z., D.T.N., E.K., and E.M.M.: Conceptualization, methodology, project administration, supervision, review & editing. B.N., M.E.K.F., M.H.K.K., J.L.T.: Review and editing.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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