Effects of vacuum packaging storage of minimally processed cassava roots at various temperatures on microflora, tissue structure, starch extraction by wet milling and granule quality

Martin Odoch[a,b], Elna M. Buys[a] and John R.N. Taylor[a*]

[*]*Correspondence to: Prof J.R.N. Taylor, Department of Consumer and Food Sciences and Institute for Food, Nutrition and Well-being, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa, E-mail: john.taylor@up.ac.za*

[a]*Department of Consumer and Food Sciences, University of Pretoria, Pretoria, South Africa*

[b]*Department of Food Science and Post-Harvest Technology, Gulu University, Gulu, Uganda*

ABSTRACT

BACKGROUND: Vacuum package storage is commonly applied to reduce post-harvest deterioration in minimally processed cassava roots. However, the influence of vacuum packaging conditions on root end-use quality is poorly understood. Hence, the effects of vacuum packaged storage at ambient, refrigerated and freezing temperatures on microflora, cassava tissue structure and starch extraction by wet milling were studied.

RESULTS: Vacuum packaged storage temperature strongly affected cassava root quality. Minimal adverse effects were obtained with frozen storage. With refrigerated storage, there was negligible microbial growth but some disruption of the parenchyma cell wall structure suggestive of chilling injury. With ambient temperature storage, there was considerable *Lactobacilli* dominated fermentation. This caused substantial cell degradation, probably due to their production of extracellular cellulolytic and other cell wall degrading enzymes. A benefit of this cell wall breakdown was that it substantially improved starch extraction with wet milling from the stored cassava pieces; by 18% with pieces that had been ambient vacuum packaged and wet milled using a 2,000 μm opening screen. However, ambient temperature storage resulted in some starch granule pitting due to the action of extracellular amylases from the fermenting microorganisms.

CONCLUSION: The best vacuum packaging storage conditions for minimally processed cassava depends on application and cost. For short-term storage, refrigeration would be best for vegetable-type products. For several months storage, freezing is best. For wet milling applications, this could be combined with subsequent short-term ambient temperature storage as it improves starch extraction efficiency and could reduce distribution energy costs.

Keywords: Cassava, Cell walls, Starch extraction, Storage temperature, Vacuum packaging

INTRODUCTION

Cassava is the most important starchy root crop in terms of production and a major food staple in tropical Africa and Latin America[1]. It is also the second most important source of starch for industry after maize. However, utilisation of cassava is constrained by the rapid postharvest deterioration of the roots[2]. There are two main causes: physiological and microbiological[3]. Physiological deterioration is a complex and incompletely understood oxidative wounding response process involving numerous enzymes, including catalase, peroxidase and polyphenol oxidase[2,4,5]. It is characterised by dark discoloration of the vascular parenchyma tissue and can render the roots unpalatable within 24-72 h. Microbiological (also called secondary) deterioration causes rotting[3]. Under aerobic conditions, various fungi, including species of *Aspergillus, Penicillium* and *Rhizopus* are responsible for dry rot of both the roots and intermediate products like chipped cassava pieces. Under anaerobic conditions, bacteria ferment the roots, producing acid and softening the tissue.

Numerous technologies have been investigated to retard the deterioration of cassava roots and moist intermediate products. Non-chemical preservation technologies generally utilise the principle of exclusion of air to slow down the oxidative deteriorative reactions and prevent growth of spoilage fungi and aerobic bacteria. These methods range from storage in air-tight plastic containers or plastic bags to coating individual roots with paraffin wax[2]. Low temperature[3] and frozen storage[6] have also been found to be effective.

There is a need to extend the shelf-life of minimally processed cassava roots, which have been simply peeled, washed and cut into smaller pieces[7]. Minimal processing of cassava should involve limited physical treatments to the roots without modification of their sensory characteristics[8]. Minimally processed cassava is utilised as is after cooking or to make fried

potato-like chips, or further processed into food products like *gari* or wet milled to extract starch. It has been shown that vacuum packaged storage at 3ºC is an effective way of storing minimally processed cassava as it retarded physiological deterioration and inhibited the growth of yeasts, moulds and aerobic bacteria through the removal of air[7]. However, a significant gap in our knowledge concerns the effects of different storage temperatures on end-use quality attributes of minimally processed cassava under vacuum packaging. It was hypothesised that there would be appropriate vacuum packaged storage temperature conditions for different end-use applications for the cassava. Hence, this work investigated the effects of vacuum packaged storage of minimally processed cassava roots at various temperatures on their microbial stability, microflora, root tissue structure, starch extraction by wet milling and extracted starch granule quality.

MATERIALS AND METHODS

Materials

Mature cassava roots (sweet variety '*South Africa'*) were harvested from eleven month old plants grown in a single field at Tonga, Mpumalanga Province, South Africa.

Cassava root transport, preparation and vacuum packaging

Directly after harvesting, the cassava roots were placed in polystyrene insulated boxes (20-23ºC) and transported to the University of Pretoria. On arrival, the roots were minimally processed, which involved peeling, washing the peeled roots in running tap water and cutting them into approx. 9 cm^3 pieces. The entire process from harvest to vacuum packaged storage was completed within 18 h to minimise post-harvest deterioration.

Determination of the effects of vacuum packaged storage of cassava pieces at different temperatures on starch extraction, tissue and starch granule integrity

Cassava pieces (2.4 kg) were vacuum packed into multi-layered vacuum bags (polyethylene 55 µm, ionomer resin 10 µm and polyamide 15 µm) (Plastikon, Pretoria, South Africa) and stored for 14 days. Three storage temperatures were studied: -20^{o}C (frozen), 4^{o}C (refrigerated) and 25^{o}C (ambient). Frozen and refrigerated conditions were obtained using blast air chilling/freezing-equipped thermometric controlled insulated coldroom storage.

At completion of storage under the three conditions, the frozen cassava pieces were thawed at 25^{o}C for 2 h prior to starch extraction. Additionally, a sample from a further batch of fresh cassava was analysed. Samples of the four treatments: -20^{o}C vacuum packaged stored, 4^{o}C vacuum packaged stored; 25^{o}C vacuum packaged stored; and fresh cassava (the control) were ground using a Trespade electric mincer (Turin, Italy) fitted with an 8 mm opening plate. The ground cassava (400 g) was mixed with 400 ml distilled water and pulverised in a Waring blender for 1 min, 30 s at low speed and 30 s at high speed. The pulp was suspended in 5x its volume distilled water and wet milled using a Retsch EZ200 wet mill (Haan, Germany) fitted with a 500 µm opening screen size.

Determination of the effects of -20^{o}C storage of cassava root pieces followed by 25^{o}C storage under vacuum packaged conditions on starch extraction and microflora

Fresh cassava pieces were placed in high density polyethylene plastic bins, tightly sealed and kept frozen at -20^{o}C for three months. The pieces were then thawed out over 24 h. At the same

time, another batch of fresh (not frozen) cassava pieces was prepared. The frozen then thawed and the fresh cassava pieces were vacuum packaged into multi-layered bags and stored at 25^{o}C for 14 days, as described above. Additionally, a sample of the thawed frozen cassava pieces and of a further batch of fresh cassava were analysed.

Samples of the four treatments: Frozen then thawed and vacuum packaged stored; Not frozen and vacuum packaged stored, Frozen then thawed and not further stored; and Fresh cassava (the control) were then wet milled as described above, but using a larger, 2,000 µm, opening screen size.

Starch isolation

After wet milling, starch was isolated from the cassava root slurries by sieving on a 106 µm mesh sieve into a fibre-rich residue fraction that remained on the sieve and a starch-rich fraction (the filtrate), as previously reported[9]. The two fractions were dried in a forced draught oven at 50°C for 72 h and then milled using a micro hammer mill (Janke and Kunkel, Staufen, Germany) fitted with a 500 µm opening screen. The milled samples were stored in tightly sealed polyethylene bags at 4^{o}C until assayed.

Analyses

pH and titratable acidity (TA)

The pH and TA of the ground cassava pieces were determined after diluting the pulp 1:2 with distilled water and thoroughly mixing. The pH was read directly using a pH meter. TA was determined by a potentiometric method and expressed in percent lactic acid equivalents[10].

Fibre-rich residue fraction particle size

The particle size distribution of the fibre-rich residue fraction was determined by sieving 10 g of the wet fraction through a 250 µm opening sieve using 1 L distilled water. Retained particles and the filtrate were dried separately by AACC Method 44-15A[11].

Scanning electron microscopy (SEM)

SEM was used to examine the integrity of the cassava cell walls from the fibre-rich residue fraction and liberated starch granules from the starch-rich fraction produced by wet milling. Prior to examining the cell walls, the fibre-rich residue fraction was de-starched as described[12] in order to aid visualisation. The de-starched cassava cell walls were freeze dried. For SEM, this freeze dried fibre-rich residue fraction and the liberated starch granules were mounted onto aluminium stubs, carbon coated (<0.5 nm thickness) and viewed using a JEOL JEM-8700 SEM (Tokyo, Japan), magnification 4,500 x and constant voltage 1.0 kV. For each replicate treatment, three specimens were collected randomly and mounted and five locations per specimen viewed.

Starch content

Starch contents of the liberated starch-rich and fibre-rich residue fractions were determined using the Megazyme Total Starch Assay procedure (Amyloglucosidase / α-Amylase Method)[13].

Microbiological analyses

At 0 h and at 48 h intervals during storage, root pieces (25 g) were suspended in 225 ml Maximum Recovery Diluent (MRD) containing 0.1% (w/v) peptone and 0.85% (w/v) NaCl. The suspension was homogenised in a Stomacher for 30 s and from appropriate 10-fold dilutions,

inocula were acquired. Lactic acid bacteria (LAB) were enumerated on De Man, Rogosa and Sharpe agar[14] (Biolab, Johannesburg, South Africa) after incubation at 30^{o}C for 48 h. Yeasts and moulds were enumerated on Potato Dextrose Agar (Biolab) amended with 30 µg/ml chloramphenicol after incubation at 25°C for 96 h.

For the not frozen and the frozen then thawed vacuum packaged cassava pieces stored at 25^{o}C for 14 days, isolates of presumptive LAB, yeasts and moulds were identified. Five colonies from the highest dilution were purified. Purified isolates of presumptive lactic acid bacteria, yeasts and moulds were transferred onto a Microflex LT^{TM} Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) target plate (Bruker, Bremen, Germany) and overlaid with α-cyano-4-hydroxycinnamic acid matrix. Identification analysis was by using the Biotyper automation software and library scores.

Statistical analyses

All experiments were performed twice. Data were analysed by STATISTICA v10 software (StatSoft Southern Africa, Sandton, South Africa) using one-way analysis of variance. Fisher's Least Significant Difference test was used to determine significant differences between treatment means.

RESULTS AND DISCUSSION

Effects of vacuum packaged storage at different temperatures on starch extraction, tissue and starch granule integrity

A storage period of 14 days was chosen as had been found to be the shelf life of vacuum packaged minimally processed cassava stored at 3°C[7]. Here, it was found that storage of vacuum packaged cassava pieces at 25°C for 14 days resulted in a statistically significant reduction in pH from 6.4 to 3.9 with a concomitant increase in TA to 0.6% lactic acid equivalents (Table 1). In contrast, with storage at 4°C there was only a very slight reduction in pH to 6.2 and increase in TA to 0.1%, and with storage -20°C there was no change in pH and TA. This indicates that at 4°C and -20°C, LAB growth was almost or totally suppressed, respectively, which indicates that the observed maximum shelf life of 14 days storage at 3°C[7] was probably due to physiological deterioration and not microbiological deterioration. For the cassava pieces stored at 25°C, the reduction in pH and increase in TA were due to the dominant presence of *Lactobacilli* species; *Lactobacillus plantarum* (63.3%), *L. pentosus* (15.0%) and *L. paraplantarum* (12.5%) (Table 2).

Vacuum packaged storage at 4°C and -20°C resulted in a significant reduction ($p<0.05$) in the proportion of the starch-rich fraction produced by wet milling with a consequent 13-15% reduction in starch yield compared to the control fresh cassava pieces (Table 1). At the same time, there was only a small reduction in the proportion of large particles (> 250 μm) in the fibre-rich residue fraction with storage at 4°C and none with storage at -20°C. SEM of the fibre-rich fractions after treatment with α-amylase to remove the trapped starch granules revealed that the parenchyma cells remain intact with little distortion in their shape after -20°C storage (Fig. 1B), when compared to fresh roots (Fig. 1A). However, after 4°C storage, there was greater distortion of the cells with some disruption of the cell walls (Fig. 1C). Since negligible microbial

fermentation took place with vacuum package storage at 4ºC (Table 1), these structural changes are suggestive of chilling injury. Cassava roots are sensitive to chilling injury if stored at temperatures below 5-8ºC, which exhibits as internal tissue breakdown, increased water loss and decay[15]. With specific regard to post-harvest physiological deterioration effects on the cassava parenchyma cell walls, the accumulation of the acidic, water-soluble type of polysaccharides (pectins) has been observed[16]. However, sample preparation effects cannot be ruled out as the cause of the cell wall distortion and disruption, notwithstanding the fact that all the treatments were subjected to the same preparation procedures throughout.

The fact that there was a reduction in starch yield with vacuum packaged storage at 4ºC and -20ºC (Table 1) is likely to be due to the fact that cassava starch granules are large in size, 10-15 μm diameter (Fig. 2). As a consequence, many starch granules would have remained trapped in the physically intact cells or became entangled in the large particles of cell walls, which constituted the fibre-rich fractions in these treatments (Fig. 1).

In contrast, with vacuum-package storage at 25ºC there was essentially no difference in the proportion of the starch-rich fraction and starch yield with wet milling compared to the fresh root pieces (Table 1). However, there was a considerable reduction (by approx. 38%) in the proportion of large size (> 250 μm) particles in the fibre-rich residue fraction. Furthermore, the cellular tissue structure of the fibre-rich residue fraction had been completely disrupted and some of the cell walls had partially been degraded into fibrils (Fig. 1D). Spontaneous fermentation of wet milled cassava has been similarly found to result in a reduction in the proportion of large size fibre particles and more specifically in the partial degradation of the hemicellulose component of the cassava cell walls[17]. Moreover, strains of *L. plantarum,* the dominant microbial species in the not frozen and vacuum packaged cassava stored at 25ºC (Table 2), have been

found to produce extracellular cellulase when fermenting cassava roots[18]. The mould *Penicillium dierckxii,* the probable presence of which was also indicated, has similarly been found to exhibit polygalacturonase (pectinase) activity when isolated from cassava[19]. Thus, the complete disruption of cellular structure and reduction in cell wall material particle size were presumably responsible for the significantly higher starch yield compared to 4ºC and -20ºC vacuum-packaged storage treatments. Disruption of the cassava parenchyma cells has been found to free the starch granules[9] and the smaller cell wall particle size would presumably result in fewer starch granules being entangled.

Regarding the quality of the starch granules extracted by wet milling from the vacuum packaged cassava root pieces, SEM showed that the granules from the pieces stored at -20ºC and 4ºC were unchanged in appearance when compared to those from fresh cassava (Fig. 2A-C). However, the granules from roots stored at 25ºC showed clear evidence of surface pitting (Fig. 2D). This pitting was similar to that in a report of indentations on the surface of cassava starch granules isolated from fermented roots[20]. Starch granule pitting occurs when the susceptible amylose fraction is more degraded by α-amylase than its neighbouring crystalline amylopectin[21]. As strains of *L. plantarum* isolated from spontaneous fermentation of cassava have been found to exhibit high α-amylase activity[22], it is likely that *L. plantarum* was responsible for the starch granule pitting as this LAB dominated the microflora of not-frozen and vacuum-packaged cassava stored at 25ºC (Table 2).

In summary, this work revealed that least cell tissue damage was caused by -20ºC vacuum packaged storage. However, with 25ºC vacuum packaged storage, the highest starch extraction rate was obtained, which was probably primarily as a consequence of cell wall degradation by extracellular cellulolytic enzymes produced by the fermenting *Lactobacilli*.

Effects of -20ºC storage followed by 25ºC temperature storage under vacuum packaged conditions on starch extraction and microflora

Frozen storage of cassava roots may be applied commercially to extend their shelf life due to post-harvest storage deterioration at ambient and refrigerated temperatures[23]. However, frozen storage distribution (-20ºC cold chain) of this type of perishable commodity is very costly, especially in tropical developing countries. This is due to the fact that there is an inverse relationship between energy consumption (and consequently cost) versus storage temperature in food supply chains[24]. For this reason, it has been recommended that mixed temperature alternatives are investigated in order to optimise product quality versus energy cost[24].

Thus, in this work the effect of frozen storage followed by vacuum packaged storage at 25ºC was investigated. Further, a larger opening size wet mill screen was used, 2000 μm as opposed to 500 μm, as it was hypothesised that the combined freezing and vacuum packaged storage treatment would improve starch extraction, hence reducing the fine milling requirement.

Vacuum packaged storage at 25ºC for 14 days of the frozen then thawed and of the not frozen cassava pieces resulted in a significant ($p<0.05$) increase in the proportion of the starch-rich fraction produced by wet milling using a 2,000 μm opening screen compared to the fresh (control) and the frozen then thawed but not further stored cassava, with 7% and 18-19% increases in starch yield, respectively (Table 3). Notably, there was no real difference in starch-rich fraction purity with less fine wet milling using the 2,000 μm opening screen (Table 3) in comparison to the 500 μm opening screen (Table 1). In both cases, the average starch-rich fraction purity was approximately 900 g starch kg^{-1} dry basis. The increases in starch yield were as a result of substantial reductions in the proportion of large (> 250 μm) particles in the fibre-

rich residue fractions (Table 3), indicative of better destruction of parenchyma cell integrity. The starch yield findings with 25ºC vacuum packaged storage followed by coarse grinding using the 2000 μm screen are in slight contrast to those with finer grinding using the 500 μm opening screen where there was no increase in starch extraction with 25ºC vacuum packaged storage compared to the fresh cassava control (Table 1). It is likely that the finer grinding negated the positive effect of 25ºC storage on cell degradation.

Vacuum packaged storage at 25ºC for 14 days of the frozen then thawed and of the not frozen cassava pieces resulted in a substantial reduction in pH from 6.4 to 3.8 and 4.1, respectively (Table 3), with concomitant increases in TA to 0.6% and 0.4% lactic acid equiv. As observed earlier, a substantial decline in cassava pH is associated with endogenous LAB fermentation. Hence, freezing the minimally processed cassava roots appears not to kill the microflora responsible for LAB fermentation. The fact that there was no reduction in pH with the frozen storage alone treatment (Table 1) was due to the absence of the subsequent 25ºC storage period, which could have enabled microflora to grow. For both the frozen then thawed and the not frozen vacuum packaged storage at 25ºC treatments, pH declined rapidly within 48 h of storage at 25ºC, followed by a 12-day period of gradual further decline (Fig. 3A). Expectedly, TA mirrored the changes in the pH trends. However, the decrease in pH from 6 days storage onwards and the increase in TA from 2 days storage onwards were significantly greater ($p<0.05$) with the frozen then thawed treatment. This was probably related to the fact that the types of microorganisms present in the two treatments differed somewhat. Although the LAB counts were similar in both treatments throughout the 14-day storage period, in the not frozen treatment, yeasts were present until day 6 but in the frozen then thawed treatment none were present (Fig. 3B). Presumably, the freezing treatment killed these eukaryotic microorganisms. The absence of

yeasts in the frozen then thawed treatment may have resulted in LAB activity being less hindered by antagonistic competition, which occurs among microorganisms associated with cassava fermentations[25].

With regard to the microflora in the frozen then thawed and vacuum packaged storage at 25^{o}C treatment, there was a considerably reduction in diversity (4 genera) in comparison with the not frozen vacuum packaged treatment (9 genera) (Table 2). This was presumably due to the adverse effect of freezing on some genera of microbes. However, the dominant species were still *L. plantarum*, *L. paraplantarum* and *L. pentosus.* As with vacuum packaged storage at 25^{o}C without freezing, the production of extracellular cellulolytic enzymes by *L. plantarum*[18] and polygalacturonase by *P. dierckxii*[19] was likely responsible for the loss in parenchyma cell wall integrity, which was reflected in the observed lower proportion of the large particle fibre-rich faction after wet milling and consequent higher starch yield compared to the fresh and the frozen then thawed and not further stored cassava (Table 3).

CONCLUSIONS

The temperature of vacuum packaged storage strongly affects the end-use quality of minimally processed cassava roots. Least adverse effects are obtained with frozen (-20^{o}C) storage. With refrigerated storage (4^{o}C), there is negligible microbial growth but some disruption of the parenchyma cell wall structure. With ambient temperature storage (25^{o}C) there is considerable *Lactobacilli* fermentation resulting in substantial cell degradation, which substantially improves starch extraction with wet milling. However, there may be some starch granule pitting.

Hence, choice of the most suitable vacuum packaging temperature storage conditions for minimally processed cassava depends on end-use application and cost. For short-term storage (approx. two weeks), refrigeration would be best for applications such as cassava vegetables or "potato" chips. For long-term storage (several months), freezing is best. For wet milling applications, this may be combined with subsequent short-term ambient temperature storage which improves starch extraction and could reduce distribution energy costs.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Figure Captions

Figure 1 Scanning electron micrographs showing the effect of vacuum packaged storage of cassava pieces for 14 days at various temperatures, followed by wet milling using a 500 µm opening screen on the root cell walls from the fibre-rich residue fraction.
(A) Fresh pieces (control), (B) -20^{o}C stored, (C) 4^{o}C stored, (D) 25^{o}C stored.
CWM = Cell wall material, **EC =** Empty cell, **F =** Fibrils.

Figure 2 Scanning electron micrographs showing the effect of vacuum packaged storage of cassava pieces for 14 days at various temperatures, followed by wet milling using a 500 µm opening screen on the liberated root starch granules from the starch-rich fraction.
(A) Fresh pieces (control), (B) -20^{o}C stored, (C) 4^{o}C stored, (D) 25^{o}C stored.
CWr = Cell wall remnants, **SGs =** Starch granules, **SGP =** Starch granule pitting.

Figure 3 Vacuum packaged storage at 25^{o}C for 14 days of fresh and -20^{o}C stored then thawed cassava pieces. (A) Effects on pH and titratable acidity, (B) Effects on populations of presumptive lactic acid bacteria (LAB), moulds and yeasts.
In graphs (A) and (B) at the same time interval and between time intervals, data points with different letters for the same parameter are significantly ($p<0.05$) different. Error bars indicate standard deviation, n = 3

Table 1 Effects of vacuum packaged storage of cassava pieces at -20ºC, 4ºC and 25ºC temperature conditions for 14 days[1] followed by wet milling using a 500 µm opening screen on pH, titratable acidity, starch extraction efficiency and particle size of the fibre-rich residue fraction

Vacuum packaged storage temp. (ºC)	pH	Titratable acidity (% lactic acid equiv.)	Fibre-rich fraction particles > 250 µm (g kg^{-1} dry basis)	Starch-rich fraction (g dry basis kg^{-1} cassava pieces as is basis)	Starch-rich fraction purity (g starch kg^{-1} dry basis)	Starch-rich fraction – Mass of starch (g dry basis kg^{-1} cassava pieces as is basis)	Starch yield relative to the control (%)
Fresh pieces (control)	6.4^{a}±0.0[1]	0.0^{c}±0.0	715^{b}±18	275^{b}±0	913^{a}±3	251^{a}±1	
-20	6.4^{a}±0.0 (0.0)	0.0^{c}±0.0 (0.0)	746^{a}±10 (+31)	240^{c}±2 (-35)	913^{a}±6 (0)	219^{b}±0 (-32)	87.3
4	6.2^{b}±0.0 (-0.2)	0.1^{b}±0.0 (+0.1)	636^{c}±15 (-79)	239^{c}±3 (-36)	898^{b}±4 (-15)	214^{b}±4 (-37)	85.3
25	3.9^{c}±0.0 (-2.5)	0.6^{a}±0.0 (+0.6)	442^{d}±20 (-273)	281^{a}±1 (+6)	906^{ab}±1 (-7)	254^{a}±0 (+3)	101.2

[1]The values are from samples taken at the conclusion of the 14 days storage period.
[2]Means± standard deviations of two independent experiments. For each analysis, means of values with different letters in the same column are significantly ($p<0.05$) different. For each analysis, values in parentheses are the arithmetic differences between means of storage temperature and the control, fresh cassava pieces.
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Table 2 Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) identification of dominant microflora isolated during storage of the not frozen and frozen then thawed vacuum packaged cassava roots at 25^{o}C for 14 days

		Not frozen cassava		Frozen then thawed cassava			
Genus	**Species**	**No. of Isolates**	**Fraction (%)**	**No. of Isolates**	**Fraction (%)**	**Possible cell wall degrading activities***	**Possible amylolytic activities***
Aromatoleum	*evansii*	1	0.8				
Arthrobacter	*chlorophenolicus*	1	0.8				
Burkholderia	*glumae*	1	0.8				
Flavobacterium	*flevense*	1	0.8	2	4.2		
Fusarium	*proliferatum*	1	0.8				
Lactobacillus	*delbrueckii*	1	0.8				
L.	*paraplantarum*	15	12.5	8	17.0		
L.	*pentosus*	18	15.0	5	10.6		
L.	*plantarum*	76	63.3	26	55.3	Cellulase (Ref.[18])*	Amylase (Ref.[23])*
L.	*reuteri*	1	0.8				
Penicillium	*dierckxii*	2	1.7	4	8.5	Polygalacturonase (Ref.[19])*	
Pseudomonas	*agarici*	1	0.8	2	4.2		
Sphingobium	*xenophagum*	1	0.8				
TOTAL		**65**	**100.0**	**47**	**100.0**		

*Only research with enzymatic activity for a given microorganism fermenting cassava roots is referenced.

Table 3 Effect of -20°C storage then thawing and vacuum packaged storage of cassava pieces at 25°C for 14 days[1] followed by wet milling using a 2,000 µm opening screen on pH, titratable acidity, starch extraction efficiency and particle size of the fibre-rich residue fraction

Temperature and storage treatment	pH	Titratable acidity (% lactic acid equiv.)	Fibre-rich fraction particles > 250 µm (g kg^{-1} dry basis)	Starch-rich fraction (g dry basis kg^{-1} cassava pieces as is basis)	Starch-rich fraction purity (g starch kg^{-1} dry basis)	Starch-rich fraction – Mass of starch (g dry basis kg^{-1} cassava pieces as is basis)	Starch yield relative to the control (%)
Fresh cassava pieces (control)	6.4^{a}±0.0[1]	0.1^{c}±0.0	938^{a}±3	255^{c}±6	900^{b}±0	230^{c}±2	
-20°C storage, thawed and not further stored	6.4^{a}±0.0 (0.0)	0.1^{c}±0.0 (0.0)	850^{b}±11 (-88)	254^{c}±3 (-1)	901^{b}±0 (+1)	231^{c}±3 (+1)	100.4
Fresh cassava pieces, vacuum packaged stored at 25°C for 14 days	4.1^{b}±0.0 (-2.3)	0.4^{b}±0.0 (+0.3)	648^{d}±9 (-290)	309^{a}±1 (+54)	886^{c}±0 (-14)	273^{a}±1 (+43)	118.7
-20°C storage, thawed and vacuum packaged stored at 25°C for 14 days	3.8^{c}±0.0 (-2.6)	0.6^{a}±0.0 (+0.5)	793^{c}±8 (-145)	267^{b}±4 (+12)	925^{a}±1 (+25)	247^{b}±2 (+17)	107.4

[1]The values are from samples taken at the conclusion of the 14 days storage period.
[2]Means± standard deviations of two independent experiments. For each analysis, means of values with different letters in the same column are significantly ($p<0.05$) different. For each analysis, values in parentheses are arithmetic differences between means of different treatments and the control, fresh cassava pieces.

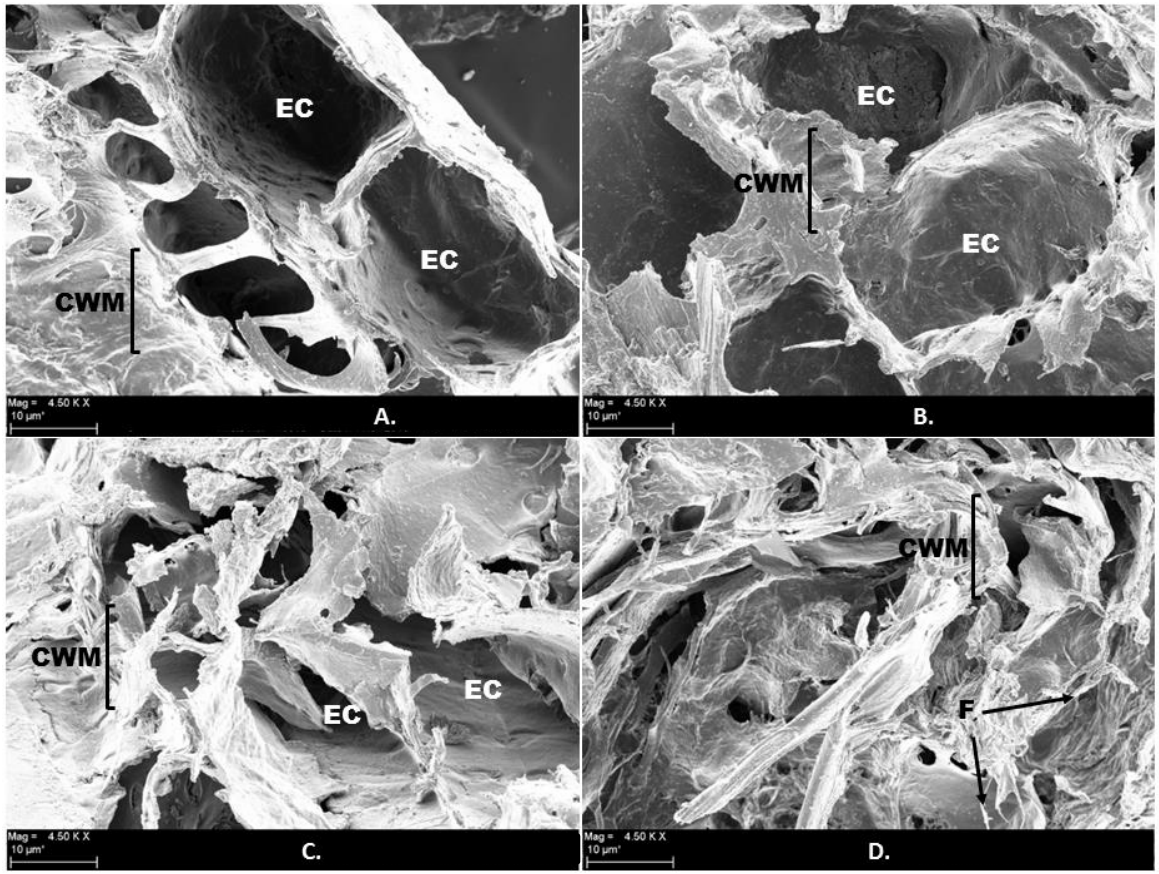

Figure 1 Scanning electron micrographs showing the effect of vacuum packaged storage of cassava pieces for 14 days at various temperatures, followed by wet milling using a 500 µm opening screen on the root cell walls from the fibre-rich residue fraction. (A) Fresh pieces (control), (B) -20ºC stored, (C) 4ºC stored, (D) 25ºC stored.

CWM = Cell wall material, **EC =** Empty cell, **F =** Fibrils.

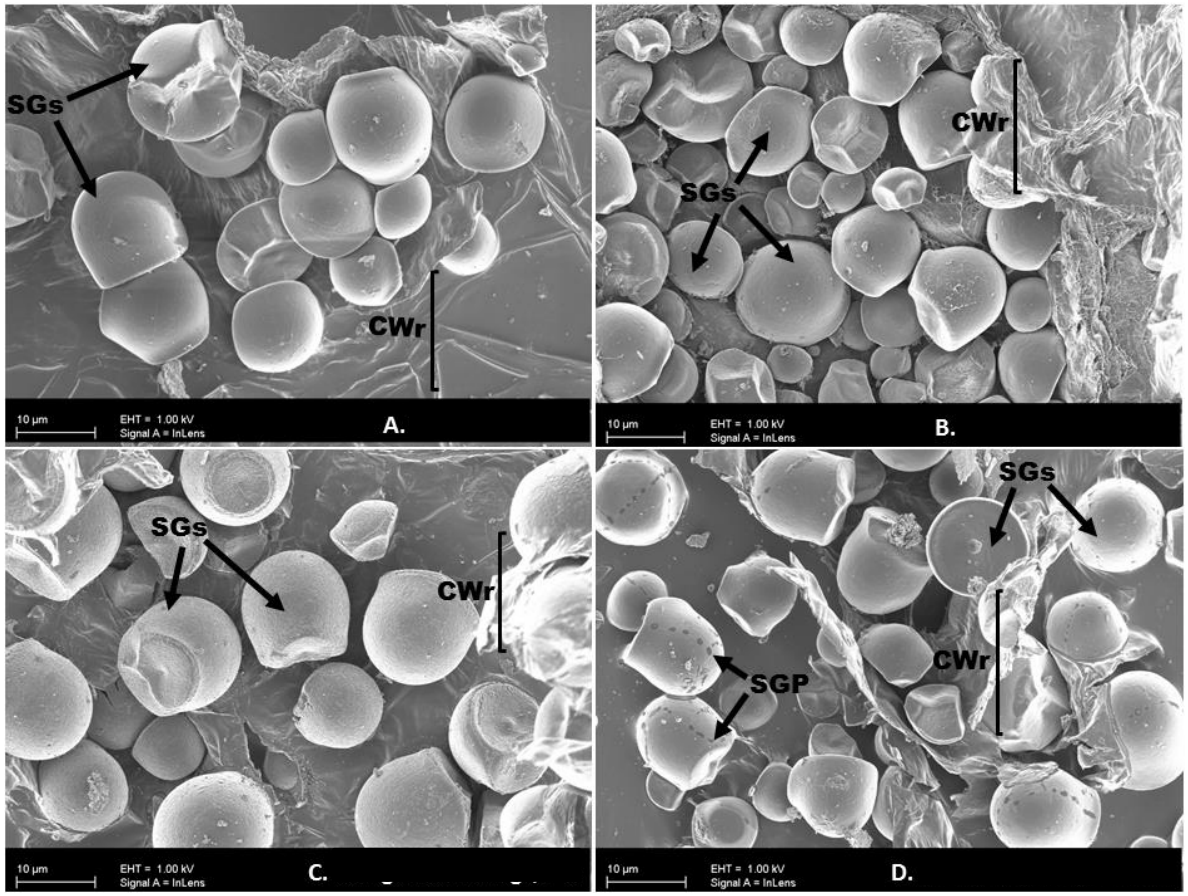

Figure 2 SEM micrographs showing the effect of vacuum packaged storage of cassava pieces for 14 days at various temperatures, followed by wet milling using a 500 µm opening screen on the liberated root starch granules from the starch-rich fraction.

(A) Fresh pieces (control), (B) -20ºC stored, (C) 4ºC stored, (D) 25ºC stored.

CWr = Cell wall remnants, **SGs =** Starch granules, **SGP =** Starch granule pitting.

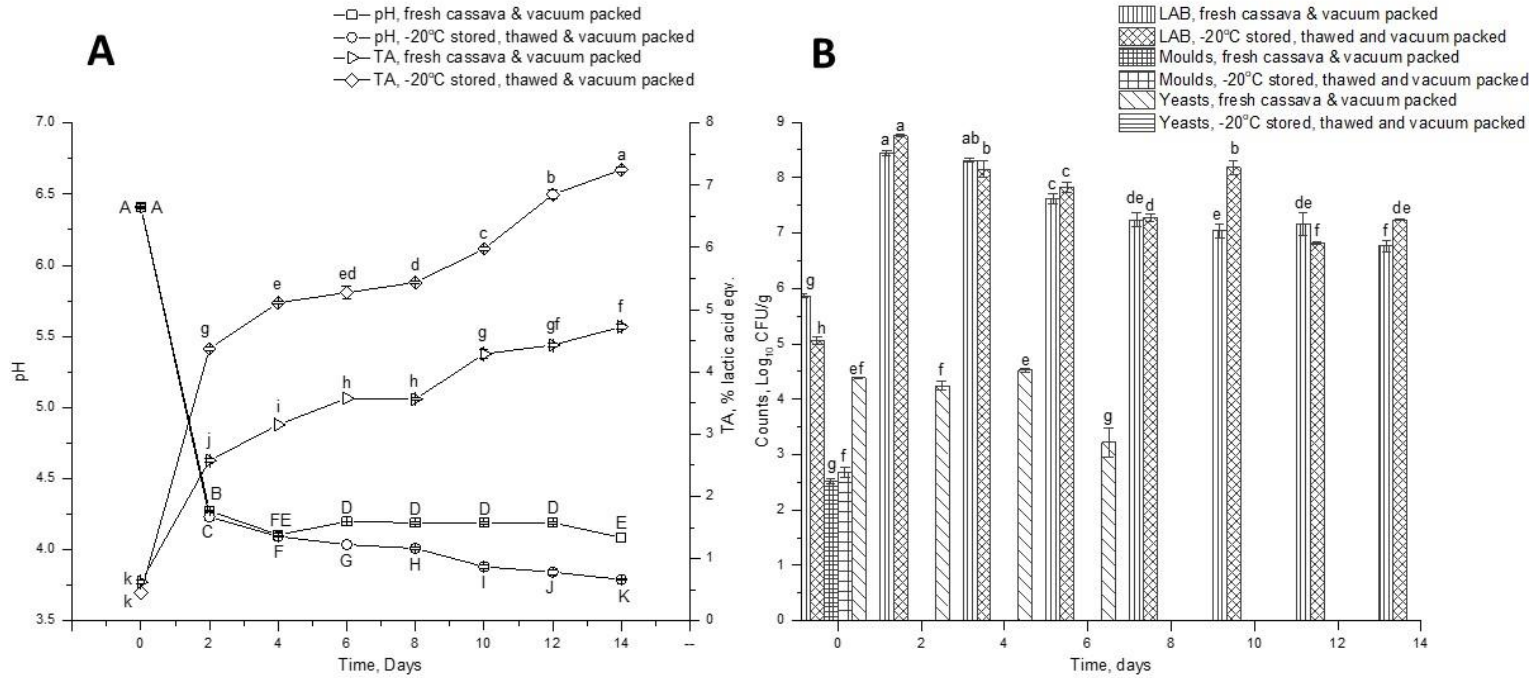

Figure 3 Vacuum packaged storage at 25ºC for 14 days of fresh and -20ºC stored then thawed cassava pieces. (A) Effects on pH and titratable acidity, (B) Effects on populations of presumptive lactic acid bacteria (LAB), moulds and yeasts.
In graphs (A) and (B) at the same time interval and between time intervals, data points with different letters for the same parameter are significantly ($p<0.05$) different. Error bars indicate standard deviation, n = 3