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| Journal: | *Journal of Agricultural and Food Chemistry* |
| Manuscript ID: | jf-2010-001606.R2 |
| Manuscript Type: | Article |
| Date Submitted by the Author: | 26-Feb-2010 |
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# Comparison of Pasting and Gel Stability of Waxy and Normal Starches from Potato, Maize and Rice with a Novel Waxy Cassava Starch under Thermal, Chemical and Mechanical Stress.

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## ABSTRACT

Functional properties of normal and waxy starches from maize, rice, potato and cassava as well as the modified waxy maize starch COLFLO® 67 were compared. The main objective of this study is to position the recently discovered spontaneous mutation for amylose-free cassava starch in relation to the other starches with well known characteristics. Paste clarity, wavelength of maximum absorption (λ Max), pasting properties, swelling power, solubility and dispersed volume fraction measurements and gel stability (acid and alkaline resistance, shear, refrigeration and freeze/thaw stability) were evaluated in the different types and sources of starch included in this study. λ Max in the waxy cassava starch was reduced considerably in comparison with normal cassava starch (535 versus 592 nm). RVA peak viscosity of waxy cassava starch was larger than in normal cassava starch (1119 vs 937cP) and assuming a position intermediate between the waxy potato and maize starches. Acid, alkaline and shear stability of waxy cassava starch was similar to normal cassava except for alkaline pH where it showed a low effect. Gels from normal root and tubers starches after refrigeration and freeze/thaw had lower syneresis than cereal starches. Gels from waxy starches (except for potato) did not present any syneresis after 5 weeks of storage at 4 °C. Waxy cassava starch was the only one not showing any syneresis after five weeks storage at -20 °C. Natural waxy cassava starch is therefore, a promising ingredient to formulate refrigerated or frozen food.

**Key words:** syneresis; gel stability; functional properties; waxy cassava starch; storage

## INTRODUCTION

The markets for industrial starches are expanding and current demand is satisfied mostly by four crops: potato, maize, wheat and cassava or tapioca (*Manihot esculenta* Crantz) *(1, 2).* Cassava is particularly important as a source of starch in tropical and subtropical regions of the world. About 84.5% of dry root weight of cassava is starch *(3).* Compared with other root and tuber tropical crops, cassava starch and its biosynthesis have been well studied *(1-8).* The starch granules are generally round (oval), with a flat surface on one side (truncated). The size of individual granules ranges from 5 to about 40 µm, with reported averages varying from 5.4 to 17.2µm *(5, 6).* However, recently a mutation with considerably smaller starch granule size has been reported *(9).* Starches from cassava and potato share some similarities: they produce relatively bland pastes, with higher viscosity, better clarity and lower retrogradation rates than starches from cereals. They also possess lower levels of proteins and lipids *(1, 2).* On the other hand, functional properties of potato starch are influenced by the presence of phosphate monoesters groups in its amylopectin and their large granule size *(5).*

There is a widespread variation of cassava starch biochemical and functional properties reported in the literature *(5, 6, 10).* In a description of analyses made in starches from more than 4000 genotypes recently published, average amylose content was found to be 20.7% *(3).* An amylose-free (or waxy) natural mutation has also been reported *(11)* as well as the application of genetic transformation for the development of cassava with a waxy starch *(12).*

Many comparisons of the physicochemical and functional properties of starches from different crops have been published *(1, 2, 13-19).* Normal cassava and potato starches have high swelling power and dispersed volume fraction compared with starches from other tropical root and tuber crops *(19).* Comparisons between normal and transgenic waxy cassava starch demonstrated that the later increased the clarity and stability of gels, increased the granule melting temperature by almost 2 ºC, without affecting particle size or chain length distributions or phosphorous contents *(12).*

In spite of the diversity offered by native starches from different crops, they cannot satisfy the different demands by different industries (adhesives, agrochemicals, cosmetics, detergents, food, medical oil and gas, paper and board, pharmaceutical, plastics, purification, beverages, textile *(1, 2)*) and chemical or physical modifications *in vitro* are required. However, these modifications are often environment unfriendly, imply additional costs, and there is a growing preference by consumers for natural, unmodified products. Therefore, there is an increasing interest and growing opportunities (conventional breeding and genetic transformation) in achieving some of these modifications *in planta (1, 2).* The recent discovery of two starch mutations in cassava *(9, 11)* is relevant because it widens the applications and competitiveness of cassava starch, they offer the advantages of modifications *in planta*, and because this crop is a key source of starch for tropical regions of the world. There are ongoing efforts to develop commercial cassava varieties with waxy starch. Field selections will be conducted in Thailand by mid 2010.

The aim of the present study was to compare physicochemical and functional properties of normal and waxy starches from different crops, taking advantage of the recent availability of a natural mutation for waxy cassava starch in genotype AM206-5 *(11).*

## MATERIALS AND METHODS

### Raw Material

**Cassava starch isolation.** Freshly cut pieces of cassava roots were suspended in tap water and crushed in a 4 liters capacity Waring Commercial blender (New Hartford, CT, USA). The slurry was filtered through a 100 µm sieve. The starch was allowed to settle and the supernatant decanted off. Solids were washed with distilled water twice and centrifuged at 8000 rpm for ten minutes *(20).* The sample was then dried in an oven with fan-forced ventilation at 40 °C for two days (Thelco Oven Model 28, Precision Scientific Subsidiary of GCA Corporation. Chicago, USA). Starches from four non-waxy genotypes were included in this study. Mutant 7 is a starch from a genotype that has frequently shown distinctive properties. The other three genotypes have been released for commercial production and are adapted to contrasting environments in

Colombia. CM 523-7 is a variety adapted to the acid soil savannas. MPER 183 is adapted to the mid-altitude valleys environment. MTAI 8 was released in Thailand as Rayong 60 and is adapted to sub-humid conditions. The study also includes the waxy cassava starch from clone AM 206-5 *(11).* This clone was obtained from a self-pollination that allowed the expression of the recessive trait responsible for the production of amylose-free starch. It is a natural mutation similar to those observed in other crops. The starch of this clone completely lacks amylose, based on DSC analysis. All cassava starches were obtained following the same extraction protocol on root samples from plants grown in CIAT Experimental Farm in Palmira, Colombia which is approximately 1000 meters above sea level (m.a.s.l.) and harvested at 10 months after plating.

**Commercial starches.** Normal and waxy commercial starches were used in this study. Maize (*Zea mays*) starches were from Roquette, Lestrem, France and potato (*Solanum tuberosum*) starches from Avebe, The Netherlands. Normal rice (*Oryza sativa*) from Bangkok Starch Industrial CO., Ltd, Thailand and waxy rice was from Remy Industries, Belgium. In addition the modified maize starch COLFLO® 67 from National Starch (France) was also included in the study. This starch was obtained by reticulation/stabilization of native waxy maize starch.

## Starch characterization

**Moisture content.** This parameter was determined on 10 g of starch samples (iw) which were then placed at 100 °C for 24 h and weighted again (fw). Moisture expressed as percentage was determined as (fw / iw)*100. Three different quantifications per starch sample were made and mean values were then calculated.

**Amylose content.** This parameter was measured following standard colorimetric procedures *(21).* Starch granules were first dispersed with ethanol and then gelatinized with sodium hydroxide 1N. An aliquot was then acidified and treated with a 2% iodine solution, which produces blue-black stain coloration. The color intensity, which is related to amylose content, was then measured with a spectrophotometer (620 nm wavelength) and compared with standard curves (using amylose concentrations ranging from 0 to 30%). Curves were obtained using purified amylose extracted from potato tubers and amylopectin extracted from cassava roots (variety AM 206-5 *(11)*). Three different quantifications per starch sample were made and mean values were then calculated.

**Paste clarity.** The methodology suggested by Craig *et al. (22)* was used. A 1% dry base (db) aqueous dispersion of starch was boiled at 97 ºC (1000m above sea level) with shaking thoroughly every 5 min for 30 min. Transmittance was measured after cooling to room temperature at 650 nm. Two different quantifications per starch sample were made and mean values were then calculated.

**Wavelength of maximum absorption (λ Max).** The formation of iodine complexes with amylose, amylopectin and their mixture was determined after solubilization in 1M KOH for 3 days at 4 ºC under stirring. The solution was diluted to 0.1 M KOH at a final polymer concentration of 1 g $L^{-1}$. One ml of 0.1N HCl, 3 ml of distilled water and 0.2 ml of iodine solution (2% KI, 0.2% $I_2$) were added to 1 ml of glucan solution in 0.1N KOH. Absorption spectrum was recorded with a µQUANT Spectrophotometer (BioTek Instruments, Winooski, U.S.A) between 450 and 700 nm *(19).*

**Pasting properties.** Hot starch dispersion viscosity profiles were obtained with a Rapid Visco Analyzer model RVA-4 Series (Newport Scientific, Australia). Starch (1.25 g db) was dispersed in distilled water (near 23 $cm^3$) to 5% suspension. Starch concentration is critical for RVA results. The concentration used was adequate for comparing different starches falls within the range of concentrations frequently reported literature. Viscosity was recorded using the temperature profile: holding at 50 °C for 1 min, heating from 50 °C 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 $min^{-1}$. The gel was then maintained 2 min at 50 °C with continuous stirring at 160 rpm. Four parameters were measured: pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau at 90 °C (HPV) and the cool paste viscosity (CPV) at 50 °C (19.33 min analysis). With them, three additional parameters were calculated: breakdown (BD), estimated as PV-HPV; setback (SB), estimated as CPV-PV; and consistency (CS), estimated as CPV-HPV.

**Swelling power, solubility and dispersed volume fraction measurements.** Swelling power (SW) and solubility patterns (SO) *(23)* were determined using 1% db (w/w) starch dispersions (0.28 g db dispersed in 27.72 g of distilled water) at 60, 75 and 90 °C. The low concentration used in the study was chosen to obtain an optimal separation between the pellet and supernatant phases after centrifugation. Paste was prepared in RVA starting at 35 °C for 1 min, increasing temperatures at 6 °C $min^{-1}$ rate. Three different and independent analyses were made holding final temperatures at 60, 75 or 90 °C for 2.5 min. Stirring was at 960 rpm for the first minute and then maintained at 160 rpm during the entire analysis. The paste was immediately transferred to a 50 $cm^3$ centrifuge tube. The supernatant and sediment after centrifugation for 10 min at 6000 g at 25 °C were collected and weighed (Wsu and Wse, respectively) then dried at 100 °C for 24 h and 48 h respectively and weighed (Dsu and Dse, respectively). The values thus obtained were used to calculate three parameters: concentration of soluble material in the supernatant (solubility), the swelling power and the volume fraction of the dispersed phase (Φ) as follows:

$$\text{Solubility (\% db)} = 100 * \text{Dsu} / 0.28$$

$$\text{Swelling Power } (g_{water}/g_{Starch}) = (\text{Wse} - \text{Dse})/\text{Dse}$$

$$\mathbf{(\Phi)} = (27.91 - (\text{Wsu} - \text{Dsu}))/27.91$$

Factor 27.91 is calculated as total volume ($cm^3$) of the paste.

Starch specific density is 1.5 g $cm^{-3}$

27.91 = 27.72 + (0.28 / 1.5) $cm^3$

## Gels stability

**Acid and alkaline resistance.** The starch sample (5%, w/v, db) was dispersed in 25 ml of distilled water, in 25 ml potassium chloride and sodium borate solution 0.1M (pH9), or in 25 ml citrate-phosphate buffer 0.2M (pH3). A RVA was used with the same profiles mentioned above for the analysis of pasting properties. CPV: Cool paste viscosity at 50 ℃ (19.33 min of analysis) for the different pHs was reported as: CPVpH3, $CPV_{Water}$ and CPVpH9. The acidity effect was defined as the ratio between $CPVpH3/CPV_{Water}$, and the basic effect, as the ratio between $CPVpH9/CPV_{Water}$ *(14, 24).* These formulas indicate that pH-resistant gels have values around 1. Susceptible gels, on the other hand, will result in values relatively distant from 1 (either close to 0 or above 2).

**Shear stability.** The shear effect was measured using the following ratio $CPV_{shear}$ / CPV *(14).* CPV is the same parameter described above in the Pasting Properties section. $CPV_{shear}$ was recorded using the same temperature profile but there was a change in stirring: 2 min after the maximum temperature (90 ℃) had been reached stirring was increased to 960 rpm for 3 min. Then stirring and temperature profile continued as in the standard pasting properties analyses.

**Refrigeration and Freeze/thaw stability.** Paste was prepared in RVA (5% db, w/w, containing 0.1% sodium azide) from 35 ℃, increasing temperatures at 6 ℃ $min^{-1}$ rate to 93 ℃ (the water boiling temperature at Cali, Colombia is 97 ℃) and maintained during 2 min. Protocol followed common procedures *(12, 14, 18)* with slight modifications (pastes were prepared using the RVA and the starch concentration was 5% rather than 4%).

**Syneresis after refrigeration.** 10 centrifuge tubes were filled with approximately 6 g of gel (WG) and stored at 4 ºC. The study lasted for five weeks. Every 7 days, two tubes were taken out of the refrigerator and held at room temperature during 1 hour. Tubes were centrifuged at 5000 rpm for 10 min and supernatant was separated and weighted (WS). Syneresis was calculated as WS/WG*100

**Syneresis after freeze/thaw.** 10 centrifuge tubes were filled with approximately 6 g of gel (WG) and stored at -20 ºC. The study lasted for five weeks. Every 7 days, two tubes were taken out of the freezer and held during 1.5 h in a water bath (30 ºC). Samples were then centrifuged (5000 rpm for 10 min) and then supernatant was separated and weighted (WS). Syneresis was calculated as WS/WG*100.

All analyses were replicated. In the case of moisture and amylose content, paste clarity, and swelling power three independent replications were used. For wavelength of maximum absorption, pasting properties and gel stability tests results are based on two replications.

## RESULTS

The physicochemical properties of the different starches included in this study are presented in **Table 1**. Paste clarity in normal starches ranged from 10% (rice) to 88% (potato). Paste clarity of normal cassava was intermediate, around 50%. Waxy starches from each crop showed higher paste clarity than their respective normal starches. The change was particularly noticeable in the case of maize and much smaller in the case of potato and rice. The modified starch COLFLO® 67 had very low paste clarity (about 5%). As expected, amylose content in waxy starches (including COLFLO® 67) was negligible except for potato that showed values around 8% (this can be explained by the branched amylopectin molecules of potato starch *(25)*). The normal rice starch analyzed in our study had a relatively low level of amylose (around 10%). $\lambda_{\text{Max}}$ values were clearly higher in the gels from normal starches compared with waxy starches from different crops. **Figure 1** illustrates the absorbance spectrums of gels from different starches in the presence of iodine. The color of waxy gels was brown, starch containing amylose developed blue gels. The intensity of the color was related to amylose content, the highest absorbance spectrum are for the starches with highest amylose content.

Effects of pH and shearing in gels from the starches analyzed in this study are presented in **Table 2**. Acid conditions in normal starches showed a minimum effect on maize starch, increasing the viscosity of its gels (1.23), followed by rice (0.72), potato (0.58) and then the different cassava samples (from 0.39 in CM 523-7 to 0.53 in MTAI 8. Mutant 7 had the lowest resistance among non-waxy cassava samples (0.36). Differences in the performance of gels from waxy and normal starches from cassava or rice were not large (**Table 2**). On the other hand, waxy maize starch had a considerably lower resistance to acid medium than normal maize starch (0.45 versus 1.23). In the case of potato the effect on acid pH resistance was also lower in the waxy starch compared with normal starch but the difference was not as marked as in the case of maize. The modified COLFLO® 67 had better stability than standard waxy or normal maize starches with a value very close to 1.

Alkaline pH effect increased viscosity and was also highest in the case of normal maize starch (4.12) and lowest for potato (1.53). Normal cassava starches assumed intermediate values ranging from 2.77 (MPER 183) to 3.73 (MTAI 8). Waxy cassava did not show much difference compared with normal cassava starches for the effects of alkaline pH, with a slightly better resistance. The alkaline pH resistance of waxy maize gels was considerably better (2.32) than that of the normal maize gels (4.12). The effect of alkaline pH on waxy potato starch, on the other hand, increased from 1.53 to 2.26. COLFLO® 67 had the lowest effect of alkaline pH (1.33) of all the starches analyzed (**Table 2**).

Shear effect values among normal starches was lowest in maize and rice (1.13 and 0.94, respectively), highest in potato (0.52) with intermediate values for cassava (ranging from 0.55 in MTAI 8 up to 0.67 in MPER 183, **Table 2**). Waxy cassava and potato starches did not show much difference in shear effect compared with their normal counterparts. In the case of waxy maize, on the other hand, there was a drastic resistance reduction (from 1.13 down to 0.35). Shear effect of COLFLO® 67 (1.09) was similar to normal maize.

Pasting characteristics of the different starches analyzed are presented in **Table 3**, as well as in **Figures 2** and **3**. The lowest PT for normal starches was found in root and tuber starches. Cassava clones CM 523-7 and, MTAI8 had the lowest values (63.3 and 63.7 ℃, respectively), with slightly higher values in potato (65.2 ℃) and the other cassava starches (64.8 ℃ in MPER 183 and 68.0 ℃ in Mutant 7). Cereals had the highest PT: 84.0 ℃ for rice and 89.0 ℃ for maize. PT in the case of waxy potato was about the same as normal potato (65.9 vs. 65.2 ℃). Waxy cassava showed a slightly higher value (≈ 2 ℃) compared with the normal versions (except for Mutant 7, which showed the highest PT among cassava starches). Waxy maize and COLFLO® 67, on the other hand, had a drastic reduction in PT (from 89.0 down to around 70 ℃, **Table 3**).

As expected, PV of normal starches was highest in potato (2550 cP), lowest in maize (176 cP) and rice (343 cP) and intermediate in the different cassava starches (ranging from 876 up to 1006 cP). Absence of amylose in the starch increased PV in cassava (up to 111 cP) and especially in maize (up to 973 cP). Waxy rice did not show a very large increase in PV (498 compared with 343 cP).

COLFLO® 67 had a peculiar amylogram with a high heat and shear stability (**Figure 3**) continuously increasing viscosity. Therefore, it was not possible to determine PV, BD, SB or CS values. A longer analysis may have shown better changes in viscosity. The RVA profile used, however, is the standard in many laboratories and facilitates comparisons between starches.

**Table 3** also presents the results of BD, which was negative in maize (-30 cP), intermediate in normal cassava starches (ranging from 324 in Mutant 7 up to 500 in CM 523-7) and highest in potato (1204 cP). BD in waxy maize starch was much higher than in its normal counterpart (307 vs. -30 cP) and slightly higher in waxy versus normal cassava and potato starches. Potato showed the highest drop in viscosity (SB) among normal starches (-1082 cP), followed by cassava (values ranging from -157 to -364 cP) and then by maize (SB value of -15 cP). Mutant 7 showed a distinctive performance among non-waxy cassava starches, with a small SB magnitude (-157 cP). The absence of amylose accentuated the SB value of different starches: for maize from -15 to -289 cP; for cassava from an average of -323 cP (excluding Mutant 7) to –595 cP; and in potato from -1082 to -1268 cP. Normal and waxy rice starches had positive SB values and were similar to each other.

The last column in **Table 3** summarizes the values for CS. Cereal starches had lower values. Normal maize starch had actually a negative value (-45 cP) whereas rice starch had small positive one (34 cP). Normal starches from cassava and potato had more or less similar values with those from several cassava cultivars slightly higher than that of potato. Amylose-free starches had opposite effects on the CS of maize (which increased from -45 up to 31 cP) and rice (increasing from 34 to 54 cP), compared with that of cassava and potato (which were reduced to 37 and 13 cP, respectively, from values > 100 cP).

Reliability of the information provided in **Table 3** is adequate, based on the standard deviations values provided. **Figure 2** helps visualizing the main trends in viscosities of different normal starches: a very high PV for potato starch, low values for cereal starch and intermediate performance of the cassava starches. The delayed gelatinization of Mutant 7 is also apparent. **Figure 3** illustrates how the recently discovered waxy starch mutation would offer a performance intermediate between waxy maize and waxy potato starches, regarding PV.

**Figures 4a** and **4b** present the values for SO and SW, respectively, estimated at three different temperatures. **Figure 4c** illustrates the **Φ** values for the starches analyzed. The coefficient of variations *(26)* for SO and SW were ≤ 6 (data not presented) except for SO at 60 ºC (which for most cases was still too low for starch to start the absorption of water). This information is, therefore, considered to be reliable. Normal cereal starches (rice and maize) showed low SO at 60 and 75 ºC but the highest at 90 ºC. As expected, SO of waxy starches was generally lower than in their normal counterparts. However, in the case of waxy cassava

SO increased, particularly at 90 ºC. COLFLO® 67 had a very low SO which did not increased with temperature.

Normal maize starch had the lowest SW values regardless the temperature (**Figure 4b**). At 60 ºC normal potato starch had swelling values intermediate between those of maize and cassava. Differences in SW were generally not large. At 75 and 90 ºC however, SW were much higher in the waxy version of the starches of each crop, particularly in the cases of cassava and potato. SW for COLFLO® 67 was always higher than for normal maize, particularly at 75 ºC.

Φ for waxy potato starch at 90 ºC reached the value of one. There was no separation between pellet and supernatant after centrifugation of the gel (**Figure 4c**). To achieve separation it would have been necessary to reduce the concentration of the starch below 1%. This result suggests that SW of waxy potato at 90 ºC could be even higher than the value presented in **Figure 4b**.

Syneresis in gels maintained in refrigerated conditions for up to five weeks is illustrated in **Figure 5**. The highest syneresis values were observed in gels from normal maize, rice and potato starches and COLFLO® 67. Cassava Mutant 7 showed the highest syneresis for a non-waxy cassava starch, followed by MTAI 8 (but with considerably lower values). Gels from waxy potato had much lower syneresis than its normal counterpart, but it was still measurable. Gels from waxy maize, rice and cassava as well as normal rice and cassava starches (CM 523-7 and MPER 183) had negligible levels of syneresis showing a very stable behavior under storage at 4 °C.

Syneresis, evaluated as a parameter for freeze/thaw stability, is illustrated in **Figure 6**. The largest values were observed in normal potato, maize and rice starches and COLFLO® 67, with magnitudes increasing consistently through time. There was an interesting variation in different non-waxy starches from cassava with relatively high syneresis values for MTAI 8, followed by Mutant 7, MPER 183 and CM 523-7. Waxy potato and maize starches had a significantly lower syneresis values compared with their normal counterparts, and had a performance similar to several normal cassava starches. Waxy cassava and rice starches showed a very interesting performance with no syneresis measurable up to five weeks under freezing conditions.

## DISCUSSION

The main contribution of this study is to locate the performance of the recently discovered spontaneous mutation of amylose-free cassava starch *(11)* and to compare it with those of well known normal and waxy starches.

Paste clarity of normal cassava was intermediate between those of the cereals (maize and rice) and potato (**Table 1**). Paste clarity was low in COLFLO® 67, higher in normal and highest in waxy maize. Paste clarities of waxy starches were higher than their respective normal counterparts, as reported in the literature *(2, 27-29).* The low $\lambda_{\text{Max}}$ value for normal rice was also reported by Tetchi *et al. (19).* Acid pH resistance was lower in waxy than in normal maize. There seems to be no major change in the shear effect of waxy cassava starch compared with its normal counterpart (**Table 2**).

The general shape of the amylogram of the amylose-free cassava mutant would fall between those of waxy maize and waxy or normal potato starches (**Figures 2** and **3**). In the case of cassava, PV tends to increase but not as much as the increase observed between normal and waxy maize starch. In a comparison between transgenic waxy cassava and wild type cassava starch *(12)* it was found that the latter had a higher PV. We have found the opposite in our study. PV in normal maize and rice was lower than in the respective versions, as reported in the literature *(2, 18, 29, 30).*

Waxy and normal potato starches did not show much difference in PT. However, in the case of maize, PT was lower in waxy than in normal starch. In the case of cassava, there was a slight increase in PT of waxy compared with the non-waxy cassava samples. PT of Mutant 7 was the highest of the studied root and tubers starches. SB was smaller in waxy starches compared with their normal counterparts, as already reported in the literature *(29).*

SO at 60 ºC were very low, in agreement with the information from **Table 3** regarding pasting temperatures > 60 ºC. In general, SO in waxy starches increased compared with their normal counterparts as final temperature was increased (**Figure 4a**). Waxy cassava starch had lower SO at 60 ºC but higher SO at 90 ºC, compared with the normal cassava starches. Trends in the case of potato starch could not be clearly established because of difficulties in separating the different phases at 90 ºC. SO was measured under the shearing conditions of the RVA, which offers the advantage of a uniform paste but may result in slight overestimations.

According to the literature, swelling in normal maize increases with temperature but the rate of change was much higher in waxy maize *(2, 18).* Similarly, swelling of waxy potato starch increased much faster with higher temperatures than normal potato starch *(17).* We found similar trends in our study. Normal cassava starch showed the highest SW at 90 ºC. It had similar values than normal potato at 75 ºC, lower values at 90 ºC and higher values at 60 ºC. In this study, SW of normal cassava agree with those reported elsewhere *(31).*

Syneresis in normal cassava was low, but it was much lower in waxy cassava (**Figures 5** and **6**). This is particularly relevant in the case of freeze-thaw analyses in which waxy cassava and rice were the only starches with total absence of syneresis until the end of the experiment. This finding agrees with results on transgenic waxy cassava starch already reported *(12)* and highlights an advantage of using waxy cassava in comparison with other waxy and non-waxy starches. It has been reported that even after three freeze/thaw cycles the only starch showing no syneresis was the amylose-free transgenic cassava starch *(12).* The low syneresis value observed for normal rice in **Figure 5** may be due to the low amylose content of the particular rice starch analyzed in this study.

Pasting properties of the waxy cassava starch reported in this study have slight differences with those in the original report *(11).* This in part is due to differences in the sample arising from different environmental conditions in which AM 206-5 was grown.

As stated by BeMiller *(32),* the potential for new commercial starches can be even greater when biological and chemical modifications are combined. BeMiller, also stated that after thousand of studies, starch remains a beautifully mysterious substance. Our study provides an insight of several distinctive properties of the recently discovered spontaneous mutation of an amylose-free cassava starch. This, in turn, will define the applications where it could potentially offer advantages over normal cassava starch or as alternative to amylose-free starches from other crops.

## ACKNOWLEDGMENTS

Different starches used in this study were kindly provided by Nick de Vetten (AVEBE, The Netherlands), Agnès Sabaté (INRA, France), Klanarong Sriroth (Kasetsart University, Thailand) and National Starch Co (USA).

## ABBREVIATIONS USED

PT: pasting temperature; PV: peak viscosity; HPV: hot paste viscosity; CPV: cool paste viscosity; BD: breakdown: SB: setback; CS: consistency; SW: swelling power; SO solubility patterns

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**Table 1.** Physico-Chemical Properties of Different Types of Starch (Normal, Waxy or Modified) of Different Crops[a].

| Starch type | Amylose content (%) | Paste Clarity (%) | λ Max |
|---|---|---|---|
| **Normal starches** | | | |
| Maize | 19.9 (±0.4) | 11 (±2.7) | 590 |
| Potato | 27.7 (±0.5) | 88 (±0.8) | 591 |
| Rice | 9.7 (±0.6) | 10 (±1.2) | 570 |
| CM 523-7 (Cassava) | 19.8 (±1.3) | 50 (±3.5) | 593 |
| MPER 183 (Cassava) | 19.5 (±1.8) | 51 (±3.8) | 590 |
| MTAI 8 (Cassava) | 16.5 (±0.6) | 47 (±0.8) | 592 |
| **Waxy starches** | | | |
| Maize | 0.0 | 42 (±1.1) | 529 |
| Potato | 7.7 (±0.8) | 92 (±1.4) | 550 |
| Rice | 0.0 | 13 (±0.6) | 531 |
| Cassava (AM 206-5) | 0.0 | 61 (±0.7) | 535 |
| **Other starches** | | | |
| COLFLO® 67 | 0.0 | 5 (±0.1) | n.a. [b] |
| Mutant 7 (Cassava) | 21.3 (±1.1) | 47 (±4.1) | 592 |

[a] Standard deviations are presented within parenthesis. [b]n.a.: not available

**Table 2.** Effect of pH and Shear on Gels from Different Types of Starch (Normal, Waxy or Modified) of Different Crops [a].

| Starch type | pH Acid effect | pH Alkaline effect | Shear effect |
|---|---|---|---|
| **Normal starches** | | | |
| Maize | 1.23 (±0.06) | 4.12 (±0.41) | 1.13 (±0.02) |
| Potato | 0.58 (±0.03) | 1.53 (±0.02) | 0.52 (±0.02) |
| Rice | 0.72 (±0.05) | 3.64 (±0.26) | 0.94 (±0.04) |
| CM 523-7 | 0.39 (±0.05) | 3.22 (±0.35) | 0.59 (±0.03) |
| MPER 183 | 0.43 (±0.10) | 2.77 (±0.29) | 0.67 (±0.03) |
| MTAI 8 | 0.53 (±0.01) | 3.73 (±0.06) | 0.55 (±0.07) |
| **Waxy starches** | | | |
| Maize | 0.45 (±0.01) | 2.32 (±0.27) | 0.35 (±0.03) |
| Potato | 0.33 (±0.02) | 2.26 (±0.10) | 0.65 (±0.05) |
| Rice | 0.69 (±0.02) | 2.66 (±0.24) | 0.52 (±0.04) |
| Cassava | 0.50 (±0.04) | 2.77 (±0.19) | 0.55 (±0.05) |
| **Other starches** | | | |
| COLFLO® 67 | 1.05 (±0.03) | 1.33 (±0.05) | 1.09 (±0.12) |
| Mutant 7 | 0.36 (±0.01) | 3.15 (±0.04) | 0.60 (±0.03) |

[a] Standard deviations are presented within parenthesis.

**Table 3**. Pasting Characteristics from Different Types of Starch (Normal, Waxy or Modified) of Different Crops [a].

| Starch type | PT (°C) | PV (cP) | BD (cP) | SB(cP) | Consistency (cP) |
|---|---|---|---|---|---|
| **Native starches** | | | | | |
| Maize | 89.0 (±0.85) | 176 (±4) | -30 (±4) | -15 (±3) | -45 (±6) |
| Potato | 65.2 (±0.06) | 2550(±15) | 1204 (±29) | -1082 (±2) | 108 (±5) |
| Rice | 84.0 (±0.40) | 343(±7) | 22 (±2) | 12 (±0) | 34 (±2) |
| CM 523-7 | 63.3 (±0.12) | 1006 (±14) | 500 (±22) | -364 (±8) | 137 (±14) |
| MPER 183 | 64.8 (±0.12) | 979 (±12) | 482 (±15) | -267 (±10) | 215 (±8) |
| MTAI 8 | 63.7 (±0.00) | 876 (±13) | 455 (±0) | -338 (±4) | 117 (±4) |
| **Waxy starches** | | | | | |
| Maize | 70.9 (±0.00) | 973(±22) | 307 (±25) | -289 (±4) | 31 (±2) |
| Potato | 65.9 (±0.12) | 2491 (±49) | 1287 (±30) | -1268 (±35) | 13(±3) |
| Rice | 67.0 (±0.71) | 498 (±16) | 39 (±1) | 15 (±6) | 54 (±4) |
| Cassava | 67.4 (±0.00) | 1119 (±11) | 631 (±8) | -595 (±12) | 37 (±4) |
| **Other starches** | | | | | |
| COLFLO® 67 | 69.0 (±0.12) | -.- | -.- | -.- | -.- |
| Mutant 7 | 68.0 (±0.17) | 887 (±20) | 324 (±4) | -157 (±16) | 167 (±12) |

[a] Standard deviations are presented within parenthesis.

## FIGURE LEGENDS

**Figure 1**. Absorption spectra of different starches analyzed in this study. The rings group together the three commercial cassava clones.

**Figure 2**. RVA amylograms (5% suspensions) of normal starches from different crops. Amylograms going through the rings in the plot belong to the three commercial cassava clones included in the study (CM523-7, MTAI 8 and MPER 183).

**Figure 3**. RVA amylograms (5% suspensions) of waxy starches from different crops. COLFLO® 67 is a modified commercial waxy maize starch.

**Figure 4a**. Solubility at 60, 75 and 90 ºC of the different types of starches analyzed in this study. The circles group the performance of the four non-waxy cassava starches.

**Figure 4b**. Swelling power at 60, 75 and 90 ºC of the different types of starches analyzed in this study.

**Figure 4c**. Volume fraction of the dispersed phase (Φ) at 60, 75 and 90 ºC of the different types of starches analyzed in this study. The circles group non-waxy cassava starches (except Mutant 7), non-waxy potato and waxy maize starches.

**Figure 5**. Refrigeration stability for up to five weeks of gels from different types (normal, waxy or modified) of starches from different crops (maize, potato, rice and cassava).

**Figure 6**. Freeze/thaw stability for up to five weeks of gels from different types (normal, waxy or modified) of starches from different crops (maize, potato, rice and cassava).

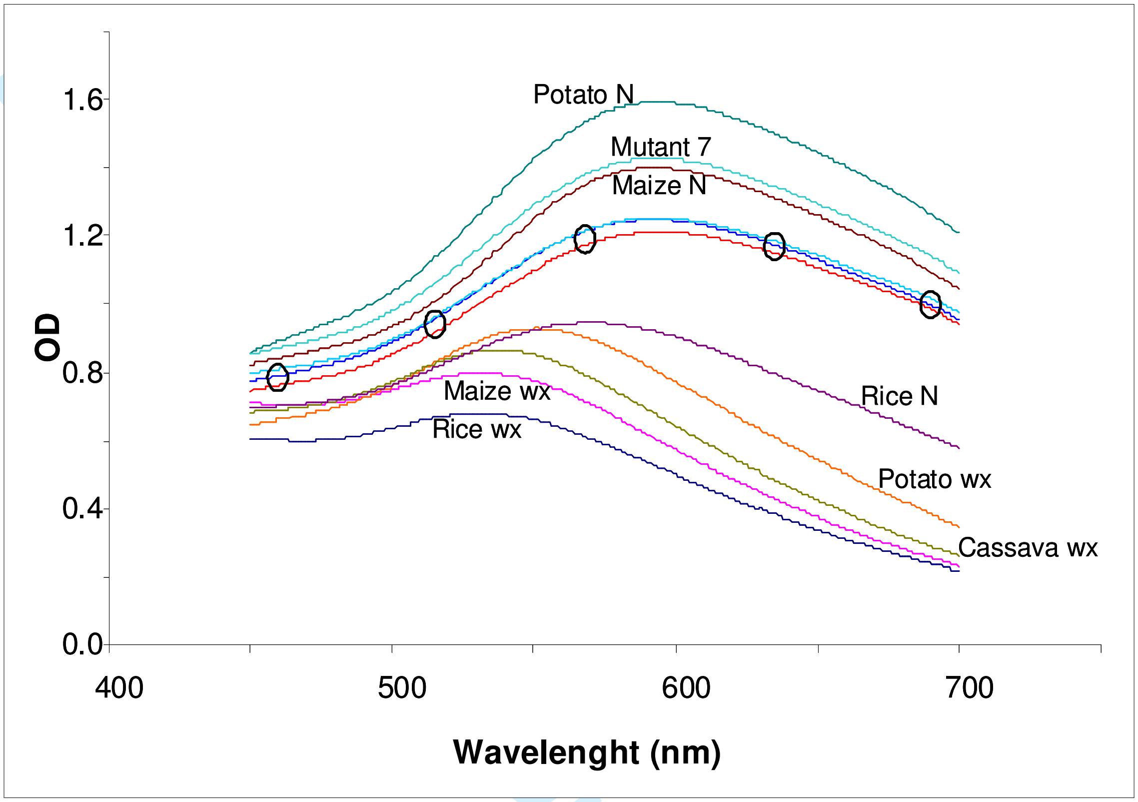
OD
1.6
1.2
0.8
0.4
0.0
400
500
600
700
Wavelenght (nm)
Potato N
Mutant 7
Maize N
Rice N
Maize wx
Rice wx
Potato wx
Cassava wx

**Figure 1**.

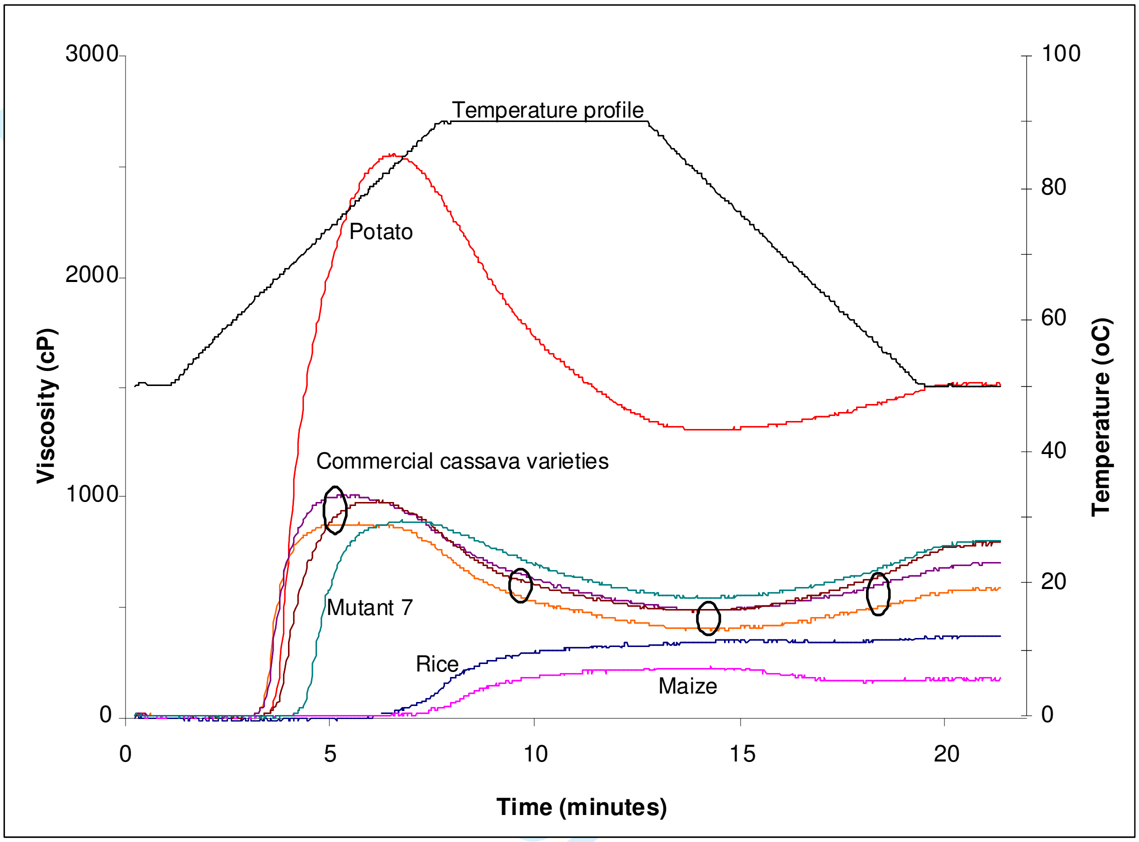
Viscosity (cP)
3000
2000
1000
0
Temperature (oC)
100
80
60
40
20
0
Time (minutes)
0
5
10
15
20
Temperature profile
Potato
Commercial cassava varieties
Mutant 7
Rice
Maize

**Figure 2**.

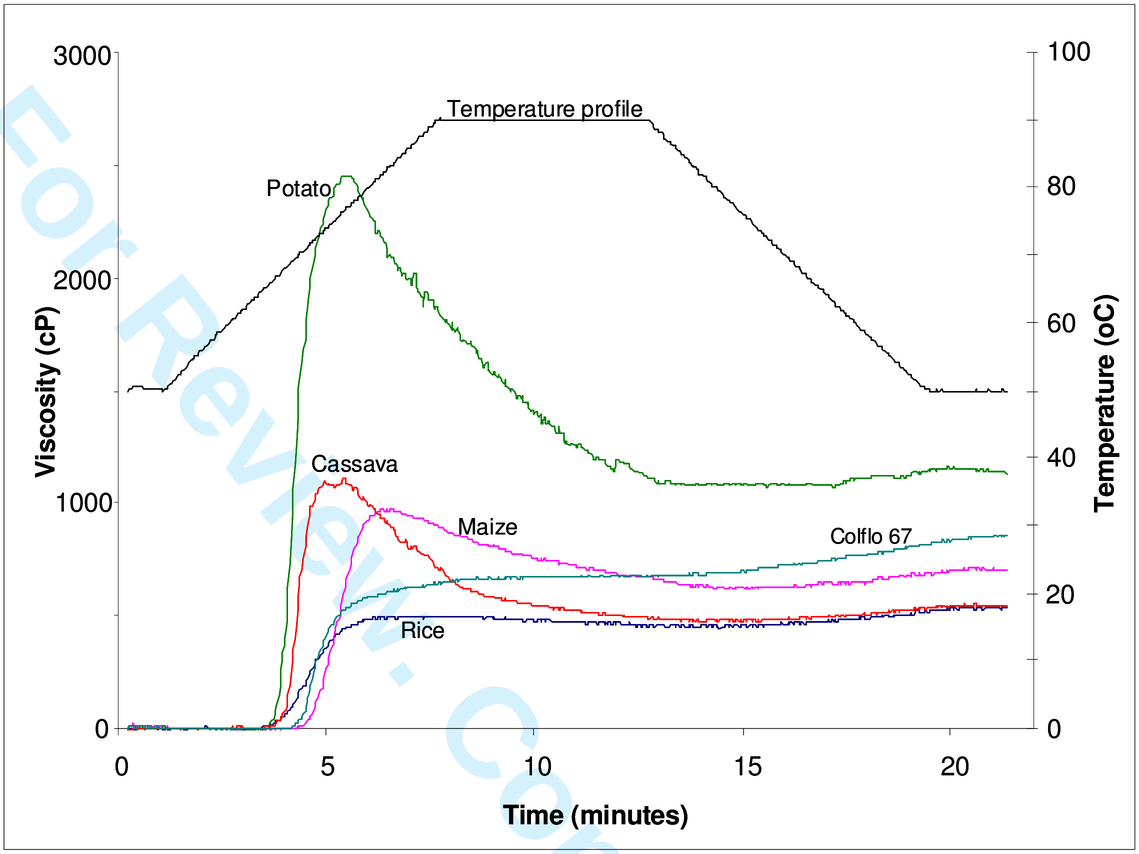
3000
2000
1000
0
100
80
60
40
20
0
0
5
10
15
20
Viscosity (cP)
Temperature (oC)
Time (minutes)
Temperature profile
Potato
Cassava
Maize
Colflo 67
Rice

**Figure 3**.

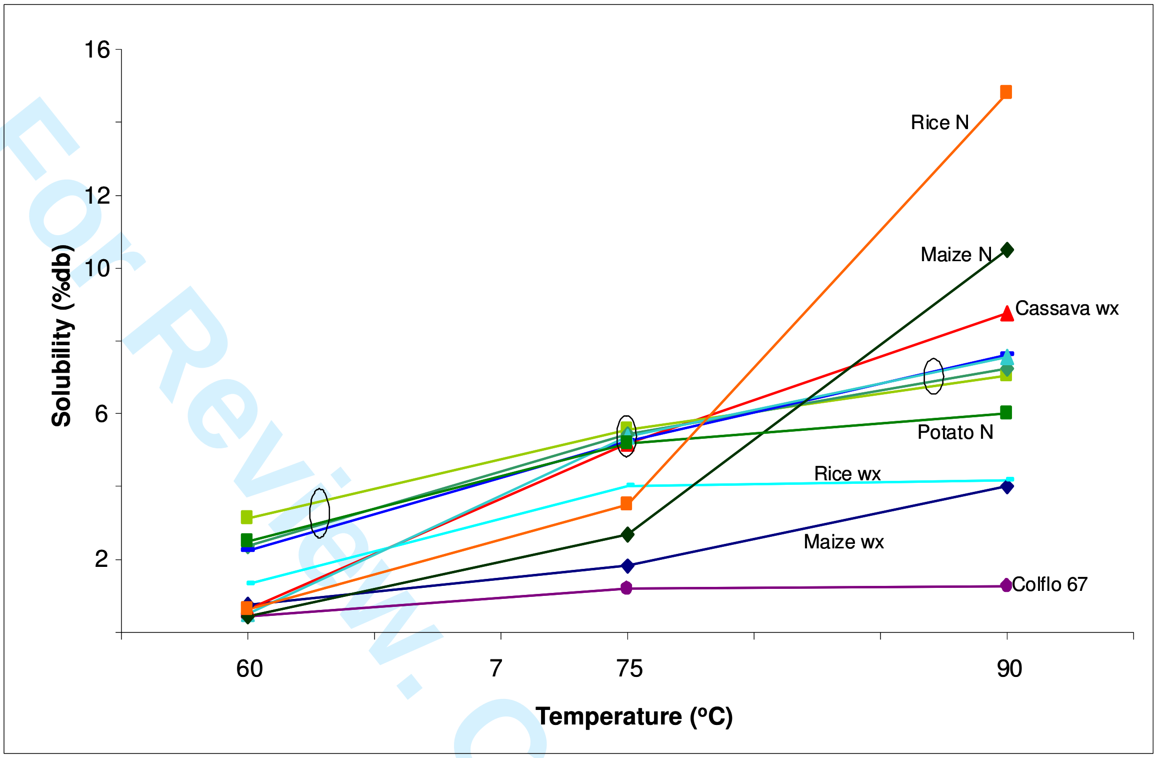
Solubility (%db)
16
12
10
6
2
60
7
75
90
Temperature (ºC)
Rice N
Maize N
Cassava wx
Potato N
Rice wx
Maize wx
Colflo 67

**Figure 4a**.

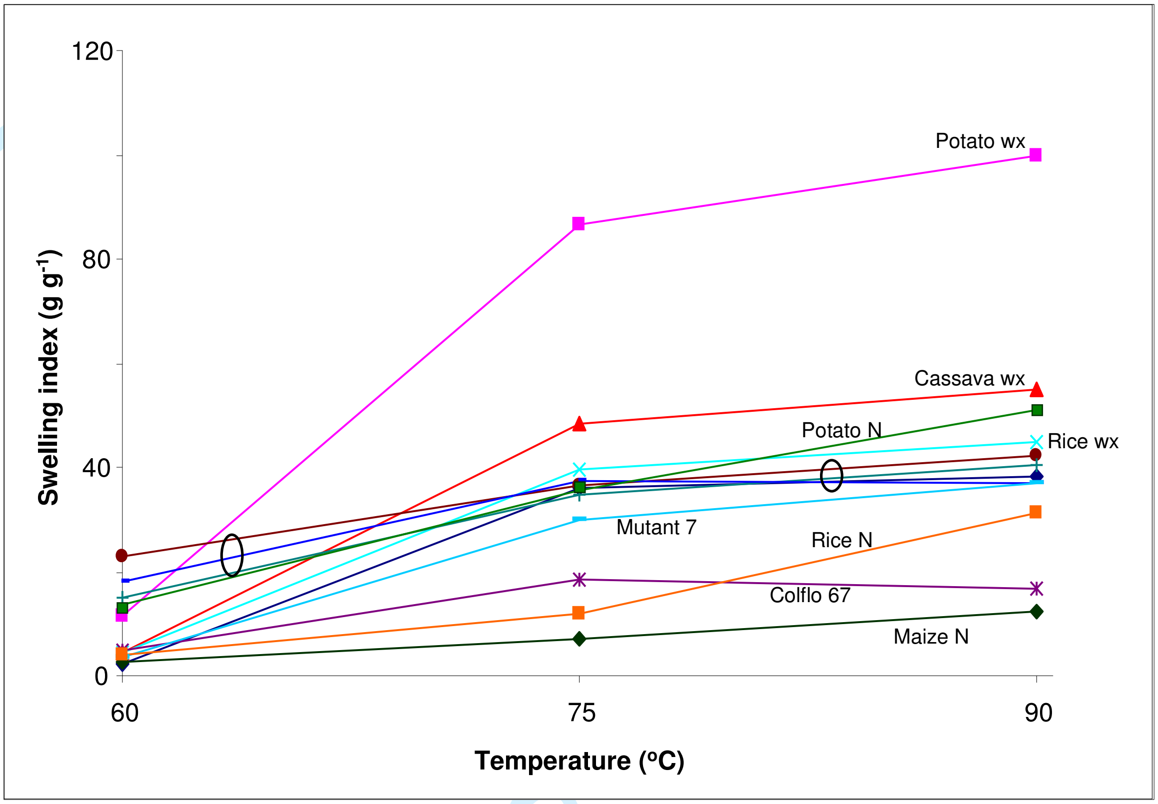
Swelling index (g g$^{-1}$)
120
80
40
0
60
75
90
Temperature (°C)
Potato wx
Cassava wx
Potato N
Rice wx
Mutant 7
Rice N
Colflo 67
Maize N

**Figure 4b**.

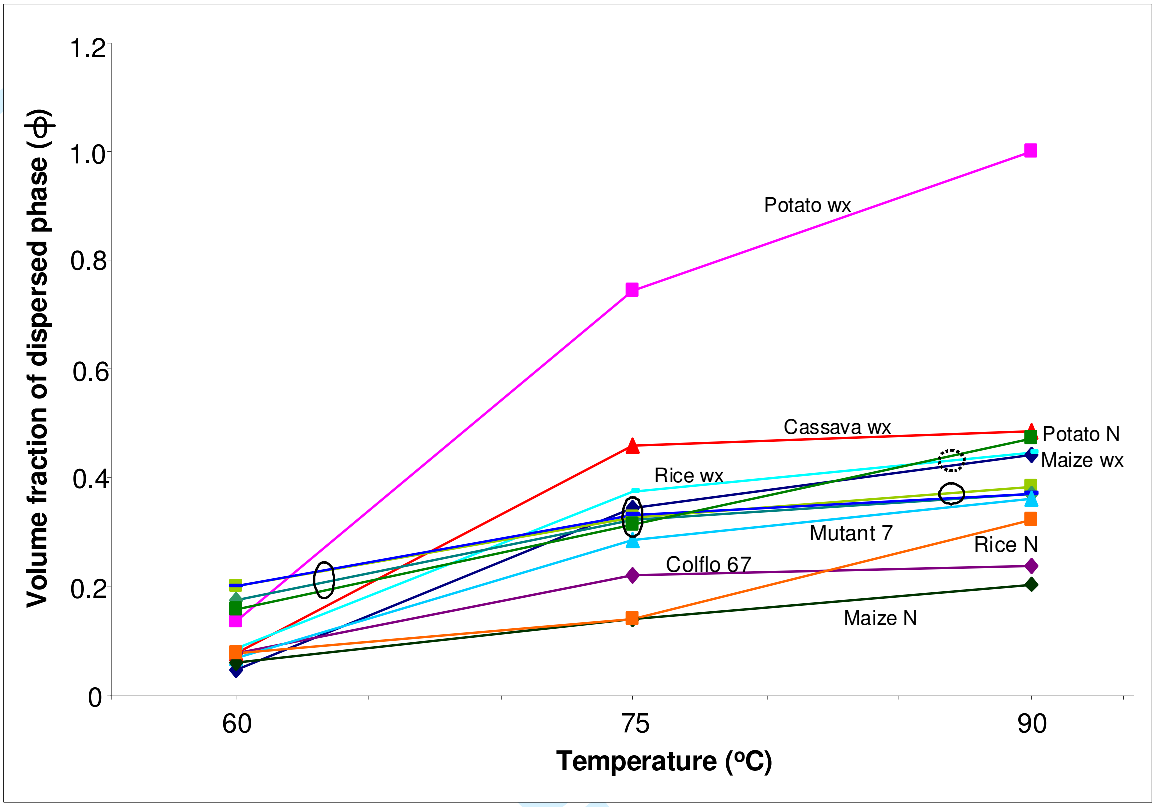
Volume fraction of dispersed phase (ϕ)
1.2
1.0
0.8
0.6
0.4
0.2
0
60
75
90
Temperature (°C)
Potato wx
Cassava wx
Potato N
Maize wx
Rice wx
Mutant 7
Rice N
Colflo 67
Maize N

**Figure 4c**.

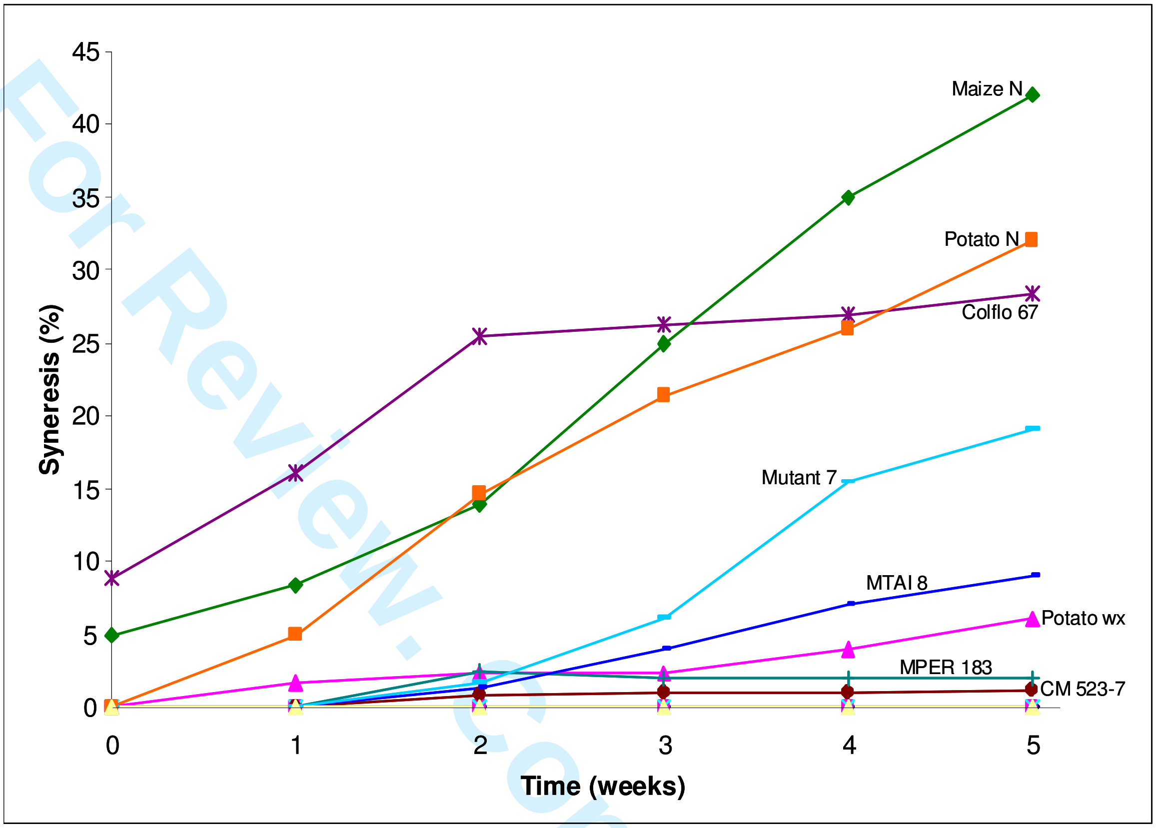
Syneresis (%)
0
5
10
15
20
25
30
35
40
45
Time (weeks)
0
1
2
3
4
5
Maize N
Potato N
Colflo 67
Mutant 7
MTAI 8
Potato wx
MPER 183
CM 523-7

**Figure 5**.

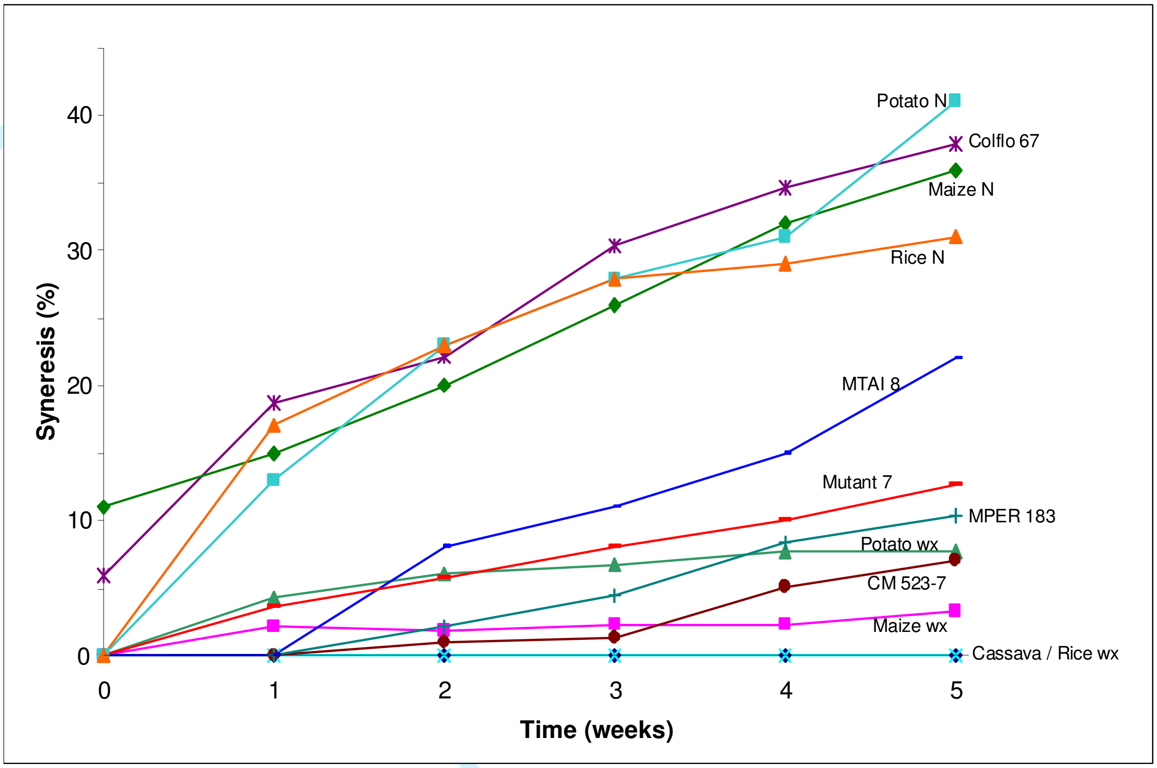
Syneresis (%)
40
30
20
10
0
0
1
2
3
4
5
Time (weeks)
Potato N
Colflo 67
Maize N
Rice N
MTAI 8
Mutant 7
MPER 183
Potato wx
CM 523-7
Maize wx
Cassava / Rice wx

**Figure 6**.