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Crystalline and pasting properties of cassava starch are influenced by its molecular properties

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This study was undertaken to detail starch characteristics among cassava varieties and compare them to their improved progenies. Cassava starch was extracted from roots of both the parents and progenies of two popular Ugandan local varieties (Bamunanika and Nyaraboke) and three popular elite varieties (NASE 10, TME 14 and 95/SE/00036) and their properties compared. The pasting and rheological properties showed a unique pasting curve in the progenies compared to the parents with significantly low peak viscosities among the progenies. Percentage crystallinity as determined by X-ray crystallography was on average four points higher in parents compared to progenies. There were no significant differences in the average amylose contents (17 - 20%) and starch contents (about 81%) in both the progenies and their parents. Significant relationships were observed between crystalline and pasting properties of the starch among the clones and parents. The above differences suggest possible variations in the amylopectin chain structure and starch molecular properties attributable to differences in the starch branching enzyme among the progenies and their parents.

Key words: Starch, crystallinity, pasting property, amylopectin structure.

INTRODUCTION

Starch, the main plant carbohydrate is the most important plant derivative used by man. It has unlimited importance in industry and food and can be modified to suit various applications using inexpensive methods making it ideal for a number of uses (Satin, 2006). One of the major sources of starch is cassava which produces high purity and quality starch compared to other tuber and cereal crop sources. Cassava is an important root crop in sub Saharan Africa being consumed by more than 600 million people world wide. The starch produced by cassava is amenable for use in various applications both dietary and industrial. In the improvement of cassava, the importance and use of starch plays a significant role. Development of starches also occupies a central position in the quest for their commercialization hence increasing production at farmer level.

Different sources of starch exist and a lot of them include the most important crop plants which have been used by man for a long time. However, starch can also be

derived from non food plant sources (Tie et al., 2008; Jiménez-Hernández et al., 2007; Srichuwong et al., 2005; Spence and Jane, 1999). In the production of starch from plants, emphasis is put on the ease with which starch is produced after extraction and its purity. This is why cassava presents one of the most important sources of starch given its ease of extraction and high purity with less protein and other associated compounds (Ceballos et al., 2007). In particular, cassava produces high amounts of starch compared to other crops such as rice and maize. Given the increasing trends of production of this crop, (FAOSTAT, 2008) exploitation of starch from cassava is a necessary option to cater for the increased demand especially in the dietary and industrial sectors.

Pasting and crystalline characteristics which have been studied and documented (Annison and Topping, 1994; Sagilata et al., 2006; Burell, 2003; Charles et al., 2005; Zeeman et al., 2002) are important in starch applications. These studies show that cassava starches pasting and crystalline properties are typical of other tuber starches. Other functional properties of cassava starch have also been detailed in addition to cassava starches composition and other biomolecules associated with this starch

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(Nuwamanya et al., 2009). The studies reveal the apparent inefficiencies of cassava starch hence its limited use for industrial and food uses.

However, modification of starch by targeting the starch biosynthetic pathway has shown important progress into the production of novel starches. For example, modifications in the starch branching enzyme have been found to be important in influencing starch viscosity properties in waxy rice (Han et al., 2004). Other important enzyme modifications include the targeting of isoamylases to alter starch granule properties (Bustos et al., 2004; Dellate et al., 2006; Streb et al., 2008) and targeting starch synthases to determine specific amylopectin properties hence affecting starch crystallinity (Zhang et al., 2008; Morell, 2003; Zhang et al., 2004). Such modifications target enzymes important in specifying the type of starch formed after an alteration in them. This has employed both biotechnological and conventional means and starches with a number of properties have been produced.

The use of conventional means in producing plants with particular traits is a cheap and convenient way that can be employed although it is not specific. It allows the natural selection of different traits at a time hence increasing the diversity of the plant under study; in this case cassava (Ceballos et al., 2007). Thus this study employed a polycross design to produce a number of progenies with quality starch conferring traits. This was important for producing considerable variability on top of producing a maximum number of hybridisations which would hence produce a big number of progenies with different (in this case starch) characteristics (Ceballos et al., 2004). The study was aimed at producing progenies (varieties) with varied root quality traits to aid selection of starches applicable in both dietary and industrial purposes.

MATERIALS AND METHODS

Field experiments were set up at the National Crops Resources Research Institute (NaCRRI), Namulonge, in Central Uganda. Parental lines included locally grown varieties namely; Bamunanika and Nyaraboke characterised by good farmer preferred characteristics but inferior in other qualities. Donor parents included two introductions from IITA (Nigeria) namely 95/SE-00036 and NASE 10 characterized by a high yield potential (20 - 35 Kg/ha) and the Nigerian landrace TME 14 that combines high dry matter content, high yielding tuber potential and resistance to cassava mosaic disease.

Due to insufficient knowledge on the specific combining abilities and flowering habits of the selected parents, a polycross mating design was used to maximize hybridization and seed production. In the following year, seeds from the polycross were pre-germinated in a high humidity chamber at 30°C. Pre-sprouting was necessary because seeds often have a dormancy period of a few months after maturity and require relatively high temperatures (30 - 35°C) for optimum germination (Ceballos et al., 2004). Adequate soil moisture and freedom from weeds was maintained to ensure high and uniform germination of the seeds. The seedlings were maintained

in the nursery for four weeks. Progeny evaluation was carried out using five half-sib families that were planted using family replication procedure (Jaramillo et al., 2005).

Harvesting and collection of root samples

Five hundred and sixty eight progenies constituting 5 progeny families and their respective parents were harvested at 12 months after planting; two roots were randomly collected per progeny and prepared for starch extraction by peeling and cleaning with distilled water.

Starch extraction

Native cassava starch extraction was carried out using a method described by Benesi (2005) and Nuwamanya et al. (2009). The starch was air-dried on aluminium pans at room temperature for 24 - 36 h and stored in plastic air tight containers at room temperature. The extracted starch from each of the progeny families for a particular parent was bulked before analysis.

Proximate analysis of cassava starch

The proximate composition of cassava starch was analyzed according to the AOAC standard methods for the determinations of ash, crude fiber, crude fat and starch content. Protein determination was carried out using the Dumas combustion method. Total amylose was determined using the amylose amylopectin kit from megazyme international.

Cassava starch pasting properties

Rheological measurements were performed using the Anton Paar GmbH rheometer. Starch and water were mixed to a ratio 2.8 : 25.2 so that the total sample size was 28 g. Starch slurries of similar concentration were loaded on the Peltier element of the rheometer, equilibrated for 5 min and heated from 50 to 95°C followed by cooling down to 50°C at 5°C/min. Viscosity parameters recorded included the peak, setback viscosity at 50°C and final viscosity. The pasting curves produced were compared across all parents and progenies.

Starch crystalline properties

A homogeneous loose starch sample powder was pressed with a glass slide into a Siemens diffractometer sample holder. The X-ray diffraction was obtained using an automated diffractometer (D-501, Siemens, München, Germany) of Cu K α (1.5418 Å) radiation, power setting of 40 kV (40 mA) in the range 3 - 70° of 2 θ at 25°C, flat plate specimen rotating at 30 rpm, 1° divergence slit/scattering slit, receiving slits 0.05°, step width 0.04° of 2 θ , time per step 1.5 s with secondary graphite monochromator and a detection by scintillation counting.

Determination of the relative crystallinity

The relative crystallinity of starch samples was quantitatively estimated as the ratio of crystalline area (A_c) to the total area drawn under the major diffraction peaks. A smooth curve of the baseline below the peaks was computer-plotted on the diffractogram. The

Table 1. Typical proximate values of cassava starch (Dry basis).

Parameter	Parents (n = 5)	Progenies (n = 568)
Moisture content	15.85 ^a ± 0.91	14.72 ^a ± 0.57
Mineral matter	0.12 ^a ± 0.04	0.23 ^b ± 0.20
Protein	0.26 ^a ± 0.02	0.28 ^a ± 0.02
Fibre	0.20 ^a ± 0.16	0.49 ^b ± 0.14
Lipid	0.21 ^a ± 0.10	0.24 ^b ± 0.04
Starch content	81.25 ^a ± 7.36	80.99 ^a ± 9.53
Amylose*	19.69 ^a ± 1.79	17.89 ^a ± 1.71

*Results of amylose content on starch content basis, n= number of parents/progenies evaluated.

Table 2. Pasting characteristics of cassava starch from the progenies and their parents.

Cultivar	PV (cP)	HPV (cP)	Final (cP)	CPV (cP)
Bamunanika	2289.5 ^a ± 6.36	671.2 ^a ± 0.14	1168 ^a ± 2.12	9.03 ^a ± 0.03
Bamunanika ¹	1666.5 ^b ± 0.71	1069.5 ^b ± 3.53	1397 ^b ± 1.41	10.39 ^b ± 0.02
Nyaraboke	2304.5 ^a ± 4.95	728.1 ^c ± 0.28	1361.5 ^c ± 0.71	8.83 ^c ± 0.26
Nyaraboke ¹	1911 ^c ± 0.00	883.84 ^d ± 0.64	1302.5 ^d ± 6.36	8.87 ^c ± 0.26
95/SE/00036	2854 ^d ± 12.02	794.6 ^e ± 0.85	1417 ^e ± 0.28	8.89 ^c ± 0.08
95/SE/00036 ¹	1881.0 ^e ± 5.65	717.6 ^f ± 0.99	1051 ^f ± 1.41	9.05 ^d ± 0.05
NASE 10	2293.5 ^a ± 3.54	730.2 ^g ± 0.42	901.0 ^g ± 4.95	9.61 ^e ± 0.13
NASE 10 ¹	1932 ^f ± 2.83	841.4 ^h ± 0.28	947.5 ^h ± 5.44	9.59 ^e ± 0.01
TME 14	2797.5 ^g ± 0.71	849.7 ⁱ ± 0.28	1593 ⁱ ± 5.65	9.12 ^d ± 0.08
TME 14 ¹	1754.5 ^h ± 0.71	975.7 ^j ± 1.77	1301.5 ^d ± 0.71	9.27 ^f ± 0.03

^aValues with the same superscript in a column are not significantly different at P < 0.05. ¹Progenies of the respective parents or cultivar.

crystalline portion was assigned to the area above the smooth curve (upper diffraction peak area), while the lower area situated between the smooth curve and a linear baseline, which connected the peaks from 5 to 35 q was taken as the amorphous region. The upper diffraction peak area (Ac) and total diffraction area over the diffraction angle (2 q) from 5 to 35 q was measured using the image tool software (UTHSCSA, 2002). The ratio of upper area to total diffraction was calculated as the relative crystallinity.

RESULTS AND DISCUSSION

Proximate analysis of cassava starch

The starch showed characteristics typical of cassava starch as shown in Table 1 with high moisture contents ranging from 14 - 16% compared to other starches where moisture contents ranges from 10 - 12% (Moorthy, 2002). Typical cassava starches have high moisture contents which are dependent on processing and storage methods and hence vary among starches. The starch content averaged at 81% showing the relatively high purity of cassava starch compared to other starches (Nuwamanya et al., 2009). This also shows the relatively low average percentages of biomolecules associated with the cassava starch granule such as protein which averaged at 0.27%,

mineral matter (0.12 - 0.23%), dietary fibre with averages between 0.2 - 0.5% and lipids with an average of 0.22% (Table 1). In particular, the amylose content ranged between 17.9 - 19.7% and was typical of cassava starch. Differences occurred in the above parameters when the progenies were compared to the parents with the moisture content being averagely lower in the progenies. Other parameters such as the protein, dietary fibre, mineral matter and lipid contents were higher in the progenies than in the parents (Table 1). However, the starch contents were similar across the parents and the progenies with no significant differences between them. Minor differences were observed in the amylose content among the parents and progenies.

Starch pasting properties

Results for pasting characteristics of starch from both the progenies and the different varieties (parents) used are presented in Table 2. The properties of the parents were distinctively different from those of the progenies showing the effect of different hybridisations on cassava starch and the quality of the roots where this starch was obtained. The peak viscosity was consistently high among

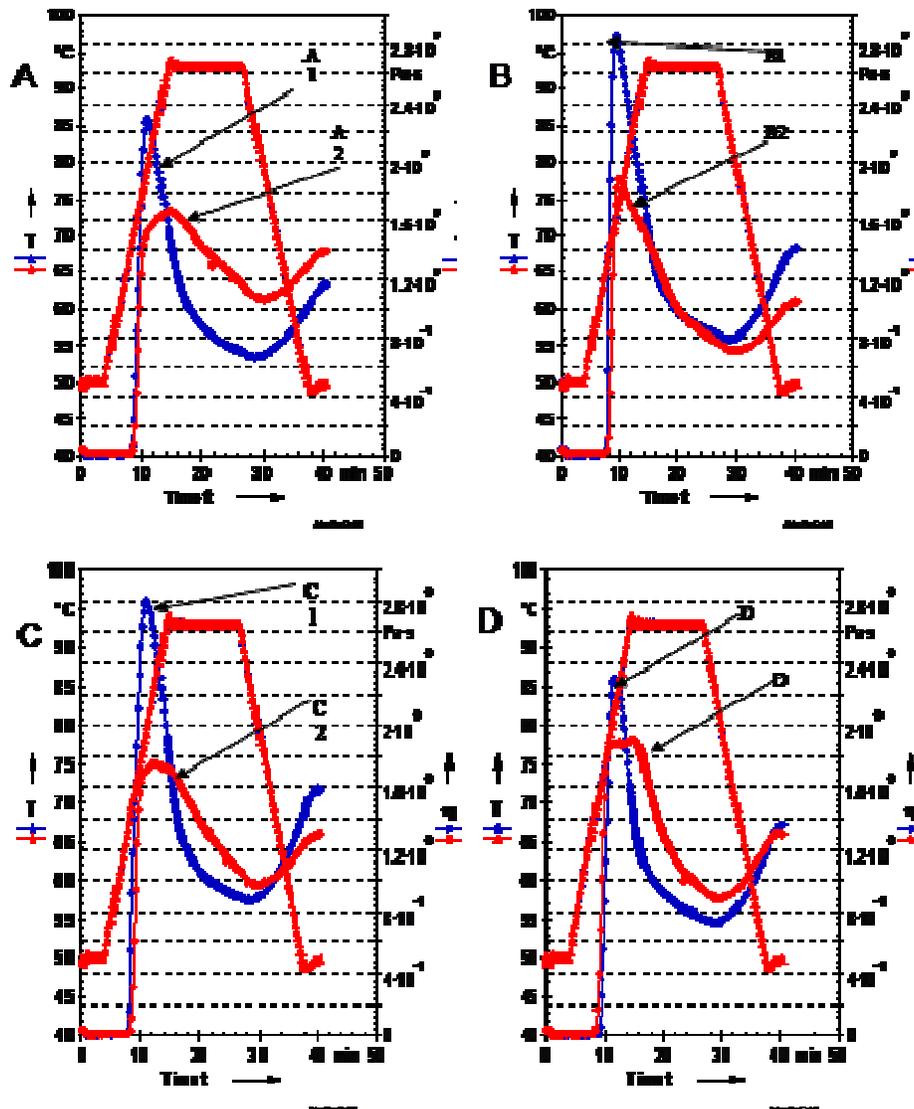


Figure 1. Pasting curves showing the different pasting properties of the parents used in comparison with the derived progenies A= Bamunanika, B= 95/SE/00036, C= TME14, D= Nyaraboke.

the parents compared to their respective progenies. Significant differences in peak viscosity were observed among the different varieties used in the poly cross. Varieties 95/SE/00036 and TME 14 showed higher peak viscosities in the range of 2.8×10^3 cP compared to other varieties considered with viscosities in the range of 2.3×10^3 cP. The low viscosities among these parents clearly show the better culinary properties presented by these varieties compared to their counterparts 95/SE/00036 and TME 14. In particular, the low peak viscosities observed among the progenies (1.6×10^3 - 2.2×10^3 cP) show a major improvement in their culinary properties. The high peak viscosity among the parents could be attributed to the minor differences in the amylose content observed among them compared to the progenies but it

is largely due to the differences in amylopectin structure as shown by differences in relative crystallinity for both the parents and progenies. Other viscosity parameters followed the same trend showing significant differences among the progenies and the parents. The hot paste viscosity was consistently low among the parents compared to the progenies while the final viscosity was low in the parents compared to progenies among Bamunanika and NASE 10. In the other parents and their progenies, the final viscosity was higher in the parents. The cold paste viscosity (CPV) was also lower in the parents compared to their progenies except for NASE 10. As seen from Figure 1, the pasting curves of the different progenies and parents were significantly different. While a flat topped curve (plateau) was observed in Nyaraboke,

Table 3. Relative crystallinity of cassava starch from both the parents and the progenies.

Variety	Parents	Progenies
Bamunanika	39.23 ^a ± 0.62	37.35 ^a ± 0.68
Nyaraboke	39.13 ^a ± 2.22	37.59 ^a ± 2.34
95/SE/00036	40.36 ^a ± 1.82	36.72 ^a ± 0.25
NASE 10	38.01 ^a ± 0.44	36.86 ^a ± 0.89
TME 14	40.27 ^a ± 0.67	28.89 ^b ± 1.08

^aValues with the same superscript in a column are not significantly different at $p = 0.05$.

the curve in Bamunanika and TME 14 had a 'shoulder' with a delayed peak during holding while in 95/SE/00036 it resembled that of the parents. Such differences may define the inherent differences in the structure of starch from the different progenies and may be also due to interactions between starch and its associated compounds during pasting (Zhang and Hamaker, 2008). These differences are also due to differences in amylopectin structure and branching patterns. Differences among parents were observed in the peak and final viscosities much as the shape of the pasting curve followed the same pattern (Figure 1).

The differences observed above could not be completely attributed to differences in the amylose content which was almost the same throughout all the varieties and the respective progeny families or the lipid content of the starch. However the differences are due to variations in the molecular structure of the starch since the ratio of the crystalline area to the amorphous area was lower in the clones compared to their respective parental lines. According to Juliano et al. (1987), varietal differences in pasting characteristics of starch can be attributed to the differences in amylopectin molecular structure rather than amylose. In rice it was reported that differences in the amylopectin chain length and the degree of polymerization (DP)_n resulted in significant differences in the gel consistency, gel viscosity and strength (Simi and Abraham, 2008; Katayama et al., 2006). Therefore, the differences in the properties of cassava starch obtained from progenies and their respective parents are likely due to their respective molecular structures. Differences in the amylopectin chain structure and molecular properties are mainly due to differences in the starch branching enzyme (SBE). Han et al. (2003) reported that differences in the polymorphic forms of the SBE (Sbe 2 and Sbe 3) in waxy rice had significant effects on starch viscosity. Though this has not been verified in cassava, such differences are to be held responsible for the variations observed. Since the progenies were derived from the different parents with practically different characteristics, it is worth noting that such differences could have resulted into the significant differences observed. However, there is need to confirm this by detailing the differences in enzyme profiles among these progenies. This will explain properly the differences observed in progenies compared to their

parents.

Crystallinity of cassava starch

The relative crystallinity of cassava starch from both the progenies and their parents is given in Table 3. A typical A-crystalline pattern that is characteristic of cassava (Defloor et al., 1998a; Defloor et al., 1998b) was observed among the parents with relative crystallinity above 35%. Significant differences were observed when the parents were compared to the progenies with the progenies (28.9 - 37.4%) showing consistently lower crystallinity than their respective parents (38.0 - 40.4%). As much as there were no significant differences in the relative crystallinity of the parents, TME 14 and 95/SE/00036 showed higher values above 40%. Differences were observed among the progenies with those from TME 14 showing the lowest crystallinity percentage below 30% producing an almost completely different crystalline pattern (B-type) that approximates that of corn and rice rather than normal cassava (Srichuwong et al., 2005). The observed differences are in line with the pasting properties where progeny families/parents with high crystallinity showed high peak temperatures. Such differences could most likely be attributed to the amylopectin molecular properties which affect starch crystallinity. The X-ray diffraction curves obtained are shown in Figures 2, 3, and 4. The curves show significant differences among the progenies and the parents with the progenies showing lower crystallinity. Three major peaks were observed with differences on attainment of these peaks between the progenies and the parents. These differences are well presented in TME 14 and Nyaraboke where differences between the progenies and the parents can be observed with the lower curve distinctively different from the upper curve. The crystalline region is an ordered arrangement of double helical amylopectin structures (Tukomane et al., 2007; Rodriguez-Sandoval et al., 2005) which reduces with increase in the amylose and is increased by hydrolysis of starch using amylases which target amylose (Charles et al., 2005). It is affected by presence of other compounds that occur with starch since it is low in flour (17.5% for cassava) (Rodriguez-Sandoval et al., 2005) than in starch (>35.0% for cassa-

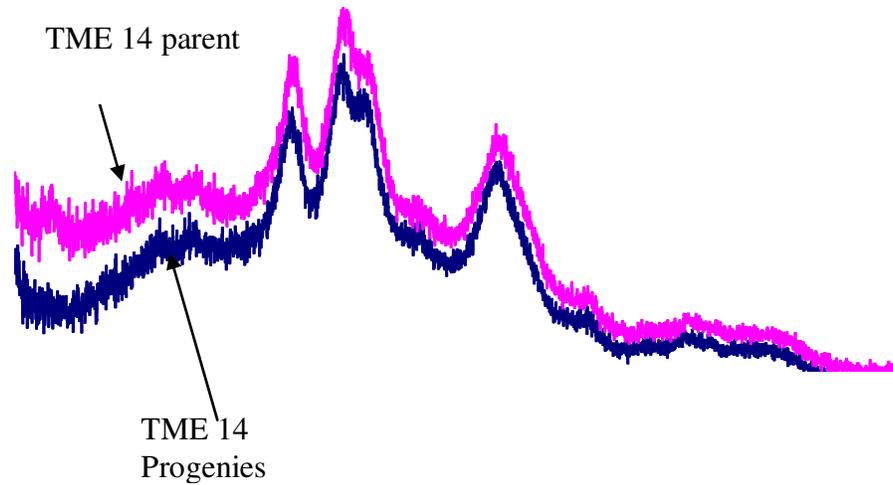


Figure 2. Crystalline curves showing the difference in relative crystallinity patterns of the parents and the progenies.

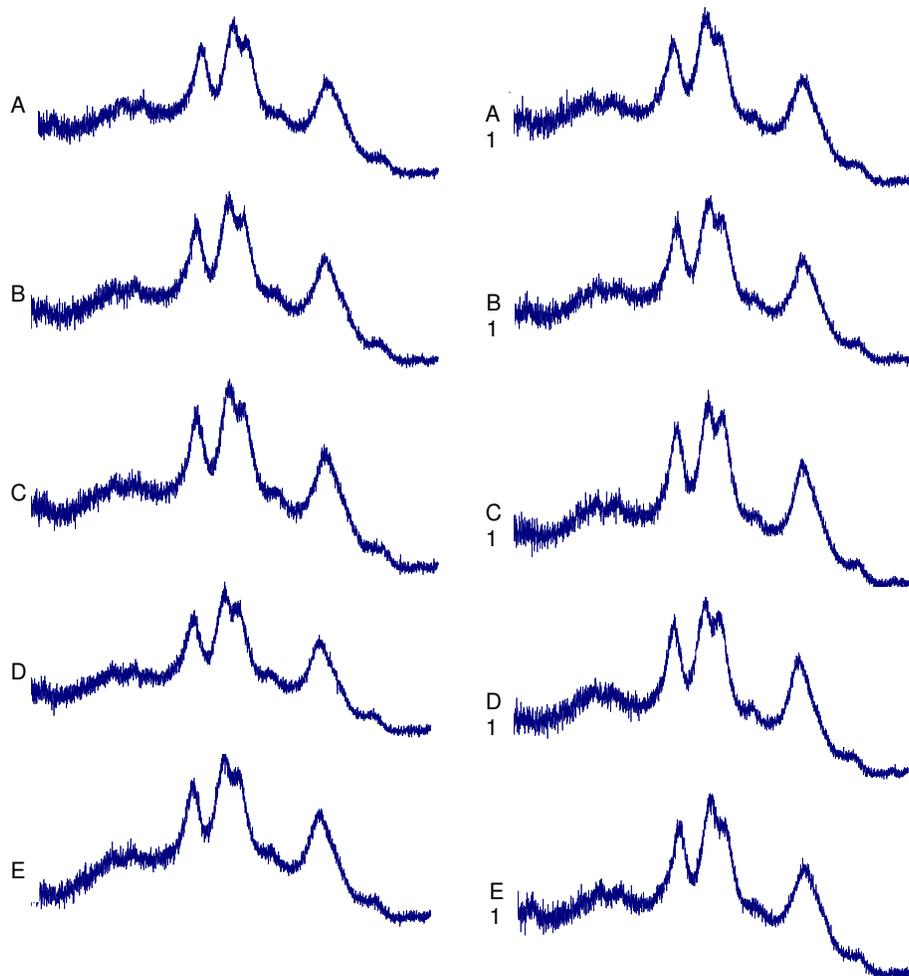


Figure 3. XRD curves comparing the progenies and the parents A= Parents, A₁= Progenies, A= 95/SE/00036, A₁= 95/SE/00036 progenies, B=Bamunanika, B₁= Bamunanika Progenies, C= NASE 10 C₁= NASE 10 Progenies, D= Nyaraboke parent D₁= Nyaraboke Progenies E= TME 14, C₁= TME.

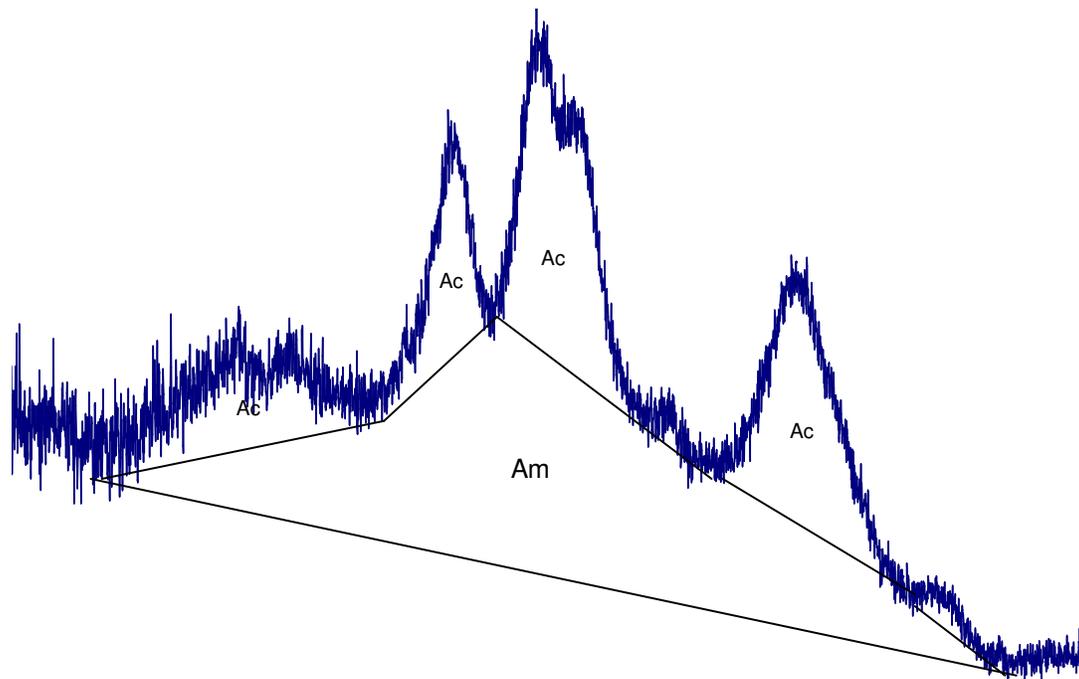


Figure 4. Measurement of relative crystallinity using image tool software UTHSCSA (2002). Ac = Crystallinity region; Am = Amorphous region.

va). Higher crystallinity results in higher gelatinisation temperature (Defloor et al., 1998b) and hence higher peak viscosities.

Conclusions

Results produced showed significant variations among progeny families and their parents which are attributed to the differences in the genetics of the parents. Different gene combinations resulted into progenies that were significantly different from their progenitors hence increasing their diversity and range of functions. A significant relationship was observed among the crystalline and pasting properties with low crystallinity and viscosity on heating among the progenies compared to their parents. Such differences are attributed to relative differences in amylopectin structure which are important in influencing starch crystalline structures.

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