Study on Variation in Crystallanity with Amylose Content in Pearl Millet Cultivars by X Ray Diffraction

Shrikrishna Nishani[1], Shalini Gaur Rudra[2], S.C. Datta[3]
[1, 2] Division of Food Science and Post Harvest Technology, Indian Agricultural Research Institute, New Delhi 110012, INDIA
[3] Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, New Delhi 110012, INDIA

Abstract- *Comparative study of pearl millet starch with different amylose content was carried out using X ray powder diffraction method. With objective to characterise the relationship between crystal type and amylose content. Varieties namely P 443, ICTP 8203, L 74, P 612, four varieties were selected to have a proportionally wide variation in the amylose contents of the flours. The four selected varieties comprised of P 443 and ICTP 8203 for higher amylose content and P 612 and L 74 to provide low amylose variants. Degree of crystallanity decreased with increasing Amylose content. These in turn affect the range of industrial applications of a starch.*

I. INTRODUCTION

Higher plants have starch as important polysaccharide reserve. It consists of two components amylose and amylopectin. Amylose is an α- (1- 4) D- glucopyranosyl polymer with linear structure and amylopectin consists of α-(1- 4) D- glucopyranosyl unit with α-(1-6) linkage at interval of 20 units and are branched depending upon plant sources. In semi crystalline polymer crystalline and amorphous regions are attributed to amylose and amylopectin respectively. The amylose content in starch affects physicochemical and functional properties such as pasting temperature, viscosity, gel stability, the water solubility. These in turn affect the range of industrial applications of a starch.

Starch alphaglucans form double helices. These form the organized array in the form of crystalline entities. Crystallanity of native semi crystalline starches form values within the range of 15-45% (Zobel et al, 1988, Imberty et al., 1991). The crystalline structure of starches has been widely studied by wide angle X-ray diffractometry. The position of the diffraction peak obtained are used to classify each starch into A,B or C an are used to identify the particular crystalline forms in the material (Singh et al.,2006). Most of cereal have the A-type X ray diffraction pattern , tuber and high amylose starches have B-type and legumes have C-type pattern. It is generally accepted that pattern C-type is combination of A and B-type polymorphs (Garnet et al.,1990). Crystal structure of A and B type of starches are based on the parallel stranded helices. Difference being the packing and water holding capacity. In A-type of starches they are closely packed, B-type starches are loosely packed and have high water holding capacity (Imberty et al., 1991).

In this study X-ray diffraction method was used to describe the crystal types and crystallinity levels in pearl millet starch granules of differing in amylose content, and to characterise the relationship between crystal type and amylose content.

II. MATERIALS AND METHODS

X-ray powder diffraction

The X-ray diffraction (XRD) pattern of the CPI powders was measured using a Philips (PW 1710, Netherlands) diffractometer with CuKa< 1 radiation. Hartley *et al.*,(1995) noted that the crystallinity of starch is also influenced by water content of starch, thus the pearlmillet flours were equilibrated to relative humidity of 0.44 using saturated potassium carbonate solution in dessicator for 90 days prior to X-ray analysis. Diffractograms were taken between 3^o and 50^o (2θ) at the rate of 1.2^o/ min with step size of 0.05^o (2θ). The starch powders were packed tightly in an rectangular aluminum cell (20 3 20 mm, thickness 0.15 cm), The samples were exposed to the X-ray beam with the X-ray generator running at 40 KV and 30 mA. Duplicate measurements were made at ambient temperature. The degree of crystallinity of samples was quantitatively estimated as shown in figure 1, following the method of Nara & Komiya (1983). The area above the smooth curve was taken to correspond to the crystalline portion, and the lower area between the smooth curve and a linear baseline. The upper diffraction peak area and total diffraction area over the diffraction angle 5–50^o 2θ were integrated manually. The ratio of upper area to total diffraction area was taken as the degree of crystallinity**.**

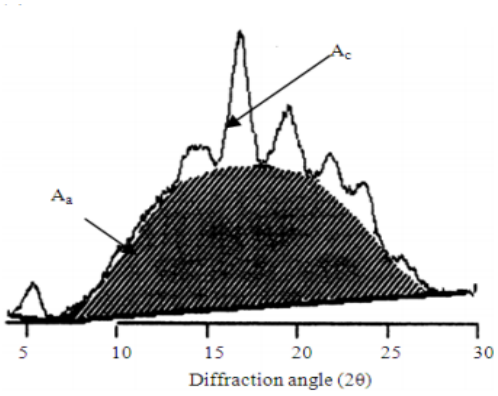

Fig 1: Calculation of relative degree of crystallization

III. RESULTS AND DISCUSSION

The amylose content in starch affects many significant physical, chemical and functional properties such as pasting temperature, birefringence end point temperature (BEPT), viscosity, gel stability, the water solubility and the degree of resistance of the starch granules to 'in vitro' digestion by amylases. These in turn affect the range of industrial applications of a starch (Cheetham& Tao, 1998)**.** The degree of crystallinity being inversely proportional to the amylose content has bearing on the starch gelatinization, pasting, cooking and digestibility properties.

Table 1: Amylose content and Amylolytic activity of various Pearl millet cultivars

Variety	Percentage Amylose	Amylolytic activity **(picograms maltose equivalents/ g/ min.)**
P 612	12.15	5.76
P 443	24.42	1.15
L 74	15.73	3.55
ICTP 8203	21.71	8.78

The present study envisaged to correlate the amylose content in the pearl millet flours to crystallinity and pasting behaviour of the flours. After initial screening of various pearlmillet varieties namelyP 443, ICTP 8203, L 74, P 612, four varieties were selected to have a proportionally wide variation in the amylose contents of the flours. The four selected varieties comprised of P 443 and ICTP 8203 for higher amylose content and P 612 and L 74 to provide low amylose variants (Table 1). The pasting properties are expected to be affected by amylase fractions in pearlmillet flour. Therefore, to ascertain that mainly amylose fractions should affect the pasting behaviour of flours, the amylase activity was also determined for each of the flours.provides a description of the amylase activity of the native pearlmillet flours. It can be seen that there is not much variation in the amylase activity.

Table 2: X ray diffraction pattern of various pearl millet cultivars

Variety	**DIFFRACTION PEAKS at 2θ(ANGLE)**													**% Crystallinity**
	3°	**6°**	**12°**	**14°**	**15°**	**16°**	**17°**	**18°**	**22°**	**23°**	**29°**	**38°**	**44°**	
ICTP 8203	9.9				53.7	77.1		100	66.7					27.40
L 74					90.4	92.7		100	98.4					24.42
P 443			74			76.00	100		90.2		42.9			26.24
P 612					72.60					100				31.71

XRD has been widely accepted as the most accurate tool to ascertain the crystallinity of the starch. Numerous researchers have studied the crystallinity of starches for purposes ranging from cooking quality determination to plasticizing action of starch to manufacture bio-films. X-ray diffraction provides an elucidation of the long-range molecular order, typically termed as crystalline, which is due to ordered arrays of double helices formed by the amylopectin side chains (Perez and Bertoft 2010). Hartley *et al.*, (1995) noted that the crystallinity of starch is also influenced by water content of starch, thus the pearlmillet flours were equilibrated to relative humidity of 0.44 using saturated potassium carbonate solution in dessicator for 90 days prior to X-ray analysis. X ray diffractograms of the pearl millet varieties are shown in figures 2-5. As can be seen from XRD of ICTP 8203 (Fig. 2), high intensity peaks are evident at 3^o, 15-16^o, 18^o and 22^o which is an indication of C-type crystalline pattern (Table 2). The XRD of P 443 also showed peaks at 12^o, 16-17 ^{o}, 22 ^{o} and 29 ^{o} which an indication of transition of A-type crystalline behaviour towards C type pattern. Cheetham and Tao (1998)have reported that with an increase in amylose content, the peak at 15^o 2θ becomes progressively weaker and broader, while the peaks at 17 ^{o} and 18 ^{o} 2θ merge to a large peak, and line-widths are significantly attenuated. Similarly, the peak at 22 ^{o}2θ decreases in intensity and splits into two peaks. With continuing increase in amylose content, the peak at 2θ=22 ^{o}

undergoes further enlargement of line-width and split down. A complete transition of crystallinity types in maize starch granules from A to B via a C type has been reported to occur at approximately 40% amylose. The results of X ray diffraction at 2-50 $^{o}2\theta$ are presented in Table 2. While P 612 and L 74 show a typical A type polymorphic pattern, ICTP 8203 and P 443 seem to appear in the C type polymorph. Although lipid complexes with amylose fraction of starch also affect the crystallinity and pasting behavior (Cozzolinoet al.., 2013**),** it shall be dealt with in subsequent studies.

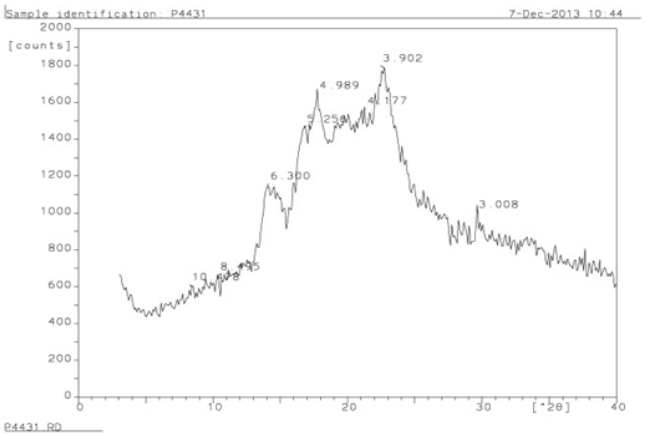

Fig 2: x ray diffractogram of P 443

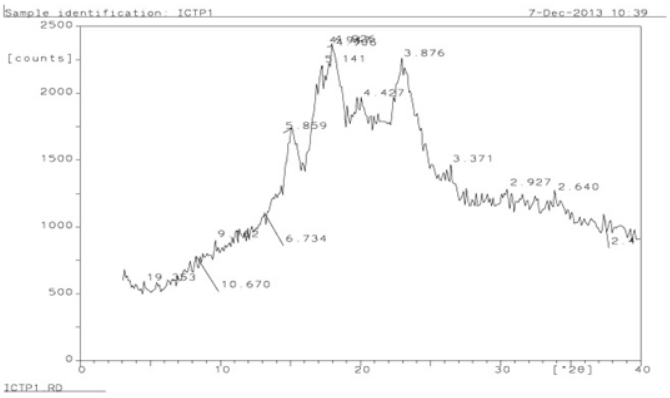

Fig 3: X ray diffractogram of ICTP 8203

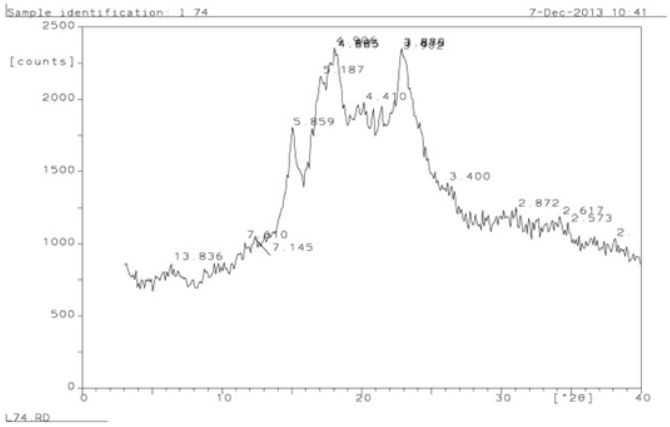

Fig 4: X ray diffractogram of L74

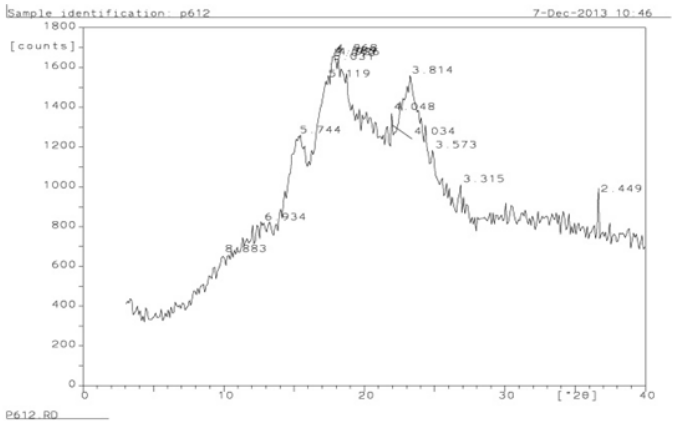

Fig 5: X ray diffractogram of P 612

IV. CONCLUSION

Study showed the difference in Amylose content has the effect on crystal pattern. Crystaltype changes from A to B with increase in amylose content . Degree of starch crystallinity decreases with increase in amylose content. This shows that crystal types are inherent characteristic of the starches and direct result of the amylose content. X ray diffraction method used in this study is widely used method provide structural finger prints directly related to material properties.

REFERENCES

[1] Cheetham, N.W.H., Leping, T. (1998). Variation in crystalline type with amylose contentin maize starch granules: an X-ray powder diffraction study. Carbohydrate Polymers, 36: 277–284.

[2] Cozzolino D, Roumeliotis S, Eglinton J. (2013) Relationships between starch pasting properties, free fatty acids and amylose content in barley Food Research International 51: 444–449

[3] Gernat C, Radosta S, Damaschun G and Schierbaum F (1990).Supramolecular structure of polymer of legume starches revealed by X ray scattering Starch/ starke, 42: 175-178.

[4] Hartley L, Chevance F, Hill SE, Mitcjell JR and Blanshard JMV (1995). Partitioning of water in binary biopolymer mixtures at low water con- tent. Carbohydrate Polymers 28(2): 83-89.

[5] ImbertyA ,Bulcon A, Tran V, and Perez S (1991). Recent advances in knowledge of starch structures.Starch/starke, 43:375-384.

[6] Nara, S. and Komiya, T. (1983). Studies on the relationship between water saturated state and crystallinity by the diffraction method for moistened potato starch. *Starch*, 35: 407–410.

[7] Perez S and Bertoft E (2010) The molecular structures of starch components and their con- tribution to the architecture of starch granules: A comprehensive review. Starch/Starke 62: 389– 420.

[8] Singh V, Ali , S.Z, Somshekar R and Mukharjee P.S (2006).Nature of crystallanity in native and acid modified starches. International journal of food properties, 9:845-854.

[9] Zobel H.F (1988). Starch crystal transformations and their industrial importance starch/ starke, 40: 1-7.

[10] Zobel H.F, Young S.N and Rocca, L.A (1988). Starch gelatinization :an x ray diffraction. Cereal chemistry, 65: 443-446.