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EFFECT OF STARCH ISOLATION METHODS ON CHEMICAL, FUNCTIONAL AND STRUCTURAL PROPERTIES OF PEARL MILLET STARCH (*PENNISETUM TYPHOIDIUM*)

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Keywords:

Isolation of pearl millet starch, Chemical properties, Functional characteristics, Starch structure

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ABSTRACT: The study was carried out to determine the chemical, functional, and structural properties of starch that was isolated by various methods from pearl millet (*Pennisetum Typhoidium).* Isolation methods of starch with different chemicals influenced starch properties. Pearl millet starch was isolated by Sodium azide (alkaline) (T_1), Sodium metabisulfite & lactic acid (T_2) (neutral), and Mercuric chloride (acidic) (T_3). Chemical and functional properties were observed. T_1 yielded a higher amount of starch (56.3 /100g) (db) compared to T_2 and T_3. Proximate analyses of starch showed that the protein content was 0.21 - 0.31%, apparent amylose was 19.42 - 21.60%; however, similar values were observed in fat (0.01%) and ash (0.05%). The swelling power and solubility of the isolated starches differed significantly. The crystalline degree of pearl millet starch of T_1 T_2 T_3 was 37.91, 29.22, and 19.92%, respectively. The result showed that isolation methods brought changes in yield, chemical, and functional properties of pearl millet starch. Starch isolated with alkaline had a higher amount of yield, apparent amylose, and total starch content compared to starch isolated with neutral and acidic solutions.

INTRODUCTION: Pearl millet (*Pennisetum typhoidium*) is the sixth most important cereal worldwide and the main food source in the semiarid regions of Asia and Africa. Pearl millet is a multi-use crop widely grown for food and non-food uses, such as feed, silage, fuel, *etc*. Millet can be rich in B vitamins, minerals (calcium, iron, and phosphorus in - phytic acid form), lipids, dietary fiber, and polyphenols, depending on the specific type. It is versatile millet mainly used in traditional food preparations, such as thick/thin porridges or Indian flatbreads like chapatti/roti. Being gluten-free, pearl millet is suitable for subjects with celiac disease and has considerable potential as a novel food ingredient [1, 2].

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In order to popularize the millet, new applications must be explored. Starch, the major component of pearl millet is reported to range from 62.8 to 70.5 g/100 g in different genotypes [3, 4]. Pearl millet is a low-cost and easily available source of starch and it may be a substitute for corn starch [5].

The starch isolation methods from various studies differ greatly, especially in the steeping condition which is neutral, alkaline, or acidic. Neutral solution or at pH 6.5 with the addition of small amount of sodium azide (*e.g.*, 0.01%)/ mercury(II) chloride (*e.g.*, 0.01 M) was used for starch extraction from pearl, proso, foxtail, barnyard, kodo and little millets to inhibit bacteria growth/ amylase activity [6]. However, Acidic solution with small amounts of lactic acid (*e.g.*, 15 g/L) and/or sulfur dioxide (*e.g.*, 0.5 g/L or 0.15%) addition was used to isolate starch from proso and pearl millet [7]. This solution has profound influence on the chemical composition and the properties of the isolated starch.

The starch from different pearl millet cultivars had a low content of protein (0.32 - 0.75 g/100 g), fat (0.27 - 0.46 g/100 g), and ash content (0.22 - 0.41 g/100 g), whereas crude fiber content was not detected. The moisture content of starches from all the cultivars was around 11 g/100 g. The low ash, fat, and protein contents reflect the purity of the samples. Amylose content of starches from different cultivars ranged from 13.6 to 18.1 g/100 g [8, 9]. Starch is of commercial importance because of its numerous functional properties, particularly due to its ability to modify the texture of a product. Therefore, the objective of the present study was to characterize the pearl millet starch isolated by three different methods and analyses of chemical, functional and structural properties.

MATERIALS AND METHODS:

Materials: Pearl millet (co-10) was purchased from Tamil Nadu Agricultural University Coimbatore, India. The grains were cleaned and stored in an airtight container, and it's used for further analyses such as isolation of starch, chemical, functional and structural properties. All reagents used in the study were purchased from Sigma-Aldrich, Steinheim, Germany.

Methods of Isolation of Starch:

Isolation of Pearl Millet Starch using Sodium Azide (T_1): Millet grains were steeped in distilled water (1:2) containing 0.01% sodium azide to inhibit microbial growth at 4°C for 24 h. The excess water was decanted, steeped, and the washed grains were ground in a warring blender with sufficient water. The slurry was sieved on 85 mesh nylon bolting cloth. The left-overs (hulls, germ, and endosperm) were re-slurried with water to float off the germ and hulls. The grinding, sieving, and regrinding of the leftover endosperm particles had been repeated until the leftover was essentially free of starch.

The starch-protein slurry was centrifuged at 2000 rpm for 20 min. The supernatant had been discarded, and the protein layer on top of the starch was removed with a spatula. The starch was washed repeatedly by re-dispersing in distilled water and centrifuging until it appeared clean. The cleaned starch was air-dried on a glass plate for 12 h, re-dispersed in water, and wet-sieved through 100-mesh screen [10].

Isolation of Pearl Millet using Sodium Metabisulfite and Lactic Acid (T_2): Starch was isolated from pearl millet grains according to method [11] with some modifications. Cleaned pearl millet grains were steeped in 0.5% lactic acid and 0.3% sodium metabisulfite solution for 24 h at 40 °C. The steeped grains were then washed thrice for the complete removal of the steeping solution. Grains were then coarsely ground and blended in a waring blender for 5 min at 8000 rpm. The slurry thus obtained was sieved through a stack of 80 and 300 wire mesh sieves from top to bottom. The left over's from the sieves were reblended in a warning blender for 2 min followed by sieving. This step was repeated twice. The pH of the resultant slurry was adjusted to 9.0 using 0.5M NaOH solution and stirred for 2 hours in a magnetic stirrer and then allowed to sediment at 4 °C. The protein layer was scraped off using a spatula. The sediment starch was washed with water and homogenized again to remove the residual protein. The washed starch was dried at room temperature.

Isolation of Pearl Millet Starch using Mercuric Chloride (T_3): The grain was steeped in 0.2M acetate buffer (pH 6.5) containing 0.01M mercuric chloride for 30 hours at 6 °C, which was used to soften the grain and to inhibit amylases. The softened grains were then ground using a warring blender and slurried in water before being sieved successively and rapidly through 80 mesh screens. Grinding, slurring, and sieving was repeated until the material left on the sieve was free from starch. Contaminating proteinaceous material in the starch suspension was removed without causing any modification of the starch by shaking the aqueous suspension with 1/8 of its volume of toluene; the protein is denatured at water/toluene interface [12].

Chemical Properties of Starch: Quantitative estimation of moisture [13], fat, crude protein, and ash [14] were performed by the standard AOAC methods. Apparent amylose and total starch were determined by the method of [15,] and pH was determined [16].

Functional Properties of Pearl Millet Starch:

Water and Oil Absorption Capacity (WAC & OAC): Starch (2.5) on a dry weight basis (db) was mixed with 20 ml distilled water /sunflower oil and then stirred for 30 min at 25 °C. The slurry was then centrifuged at 3000 rpm for 10 min, and the supernatant was decanted. The gain in weight was expressed as water/oil absorption capacity [17].

Swelling Power (SP) and Solubility (S): The swelling power and solubility of the starches were determined by [18]. Starch (0.5g) was dispersed in 20 ml distilled water in centrifuge tubes. The suspensions were heated at 90 °C for 30 min. The gelatinized samples were then cooled to room temperature and centrifuged at 1000 × g for 20 min .the supernatant was dried at 110 °C to constant weight to quantify the soluble fraction. The solubility was expressed as the percentage of dried solid weight based on the weight of the dry sample. The swelling power was represented as the ratio of the weight of the wet sediment to the weight of the initial dry sample less the amount of soluble starch.

Bulk Density: Five g of sample was put into a 10 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume. Bulk density (g/cm) = weight of sample/volume of the sample after tapping [5].

Structural Analyses of Starch:

Scanning Electron Microscopy (SEM): A starch granule was observed using a Scanning Electron Microscope (SEM) (JEOL-Model 6390, Japan). The samples were sprinkled on a double-sided tape mounted on a SEM stub. The samples were coated with gold and placed in the SEM chamber. Photomicrographs were taken using a scanning electron microscope apparatus at an accelerating voltage of 15 kV.

X-Ray Diffraction (XRD): The isolated starch was packed in rectangular glass crucibles and exposed to an X-ray beam (8 keV) generated by an x-ray diffractometer (MiniFlex–II, desktop x-ray diffractometer, Japan) equipped with a θ-θ goniometry at 25 mA and 30 kV, with Cu kα filtered radiation. The graph were plotted between the ranges for 2θ was set to 10 – 40° and smoothed with the software powder X. The degree of crystallinity of samples was quantitatively estimated with the origin version 6.0 software.

Functional Group Analysis using FT-IR Spectroscopy: The FT-IR spectromicroscopy of the purified starch was assessed in the wavelength range of 4000-400 cm^{-1}. (make Perkin Elmer, model spectrum two specimen solids liquid).

Statistical Analysis: All experiments were performed in triplicates. The results were expressed as the mean ±S.D. One Way Analysis of Variance (ANOVA) using MS Excel 2007 and means were compared by Critical Difference (CD).

Differences at $P<0.05$ were considered to be significant. Pearson correlation (r) was also calculated using Statistical Package for Social Sciences (SPSS version 16.0) to know the relationship among the chemical and functional properties of pearl millet starch.

RESULTS AND DISCUSSION:

Chemical and Functional Properties: Starch was isolated from pearl millet using sodium azide (T_1), sodium metabisulfite & lactic acid (T_2), and Mercuric chloride (T_3). The total starch content of isolated starch was ranged from 91.96 to 97.22% (db). The chemical properties of pearl millet starches are presented in **Table 1**.

TABLE 1: CHEMICAL PROPERTIES OF PEARL MILLET STARCHES

Properties	Isolated Starch		
	T_1	T_2	T_3
Yield (%)	56.10 ± 0.57 [a]	49.01±0.57 [b]	40.00 ± 0.33 [c]
Moisture (%)	8.16 ± 0.26[a]	8. 16 ± 0.28[a]	8.66 ± 0.28[a]
Dry matter (%)	91.84 ± 0.33[a]	91.84 ± 0.92[a]	91.34 ± 1.0[a]
Protein (%)	0. 31 ± 0.03[a]	0.21 ± 0.06[a]	0.21 ± 0.05[a]
pH	3. 60 ± 0.03[a]	3.75 ± 0.09[a]	3.53 ± 0.06[a]
Fat (%)	0. 02 ± 0.05[a]	0.02 ± 0.01[a]	0.01 ± 0.03[a]
Ash (%)	0.05 ± 0.01[a]	0.05 ± 0.01[a]	0.05 ± 0.01[a]
Apparent amylose (%)	21.60 ± 1.57[a]	19.42 ± 1.33[b]	20.17 ± 0.57[c]
Total starch (%)	97.22 ± 4.14[a]	91.83 ± 1.50[b]	91.96 ± 8.59[c]

All data are mean ±S.D of triplicates. Mean values followed by the same letters within the row are not significantly different ($p>0.05$). Mean values followed by the different letters within the row are significantly different ($p< 0.05$).

Isolation with sodium azide yielded the greatest amount of starch (56 /100g)(db) followed by sodium metabisulfite & lactic acid (49 /100 g)(db) and mercuric chloride (40 / 100 g)(db). The range of starch yield of pearl millet obtained in the study was supported by [19] and also 52.1% (db) of starch yield was reported for finger millet starch [20].

Moisture content of starches ware ranged from 8.16 - 8.66% (db) similar to the result of [21, 22]. Moisture content of 8.4 - 17.3 g, was considered to be within the acceptable range and beneficial in terms of shelf life and keeping the quality of the starches. Dry matter of starches was in the range of 91.84 - 91. 34% (db), and this was comparable to the report by [23]. Protein content of pearl millet starches was in the range of 0.21 - 0.31% and fat 0.01 - 0.02%, and ash content were similar in all the isolated starches. Apparent amylose content of isolated pearl millet starches was in the range of 19.42 to 21.60%. High amylose content influences the gelatinization temperature and restricts the swelling of granule [6].

TABLE 2: FUNCTIONAL PROPERTIES OF PEARL MILLET STARCHES

Properties	T_1	T_2	T_3
WAC (ml/g)	0.63 ± 0.04^{a}	1.11 ± 0.326^{b}	1.11 ± 0.32^{c}
OAC (ml/g)	1.07 ± 0.05^{a}	1.60 ± 0.12^{b}	1.49 ± 0.12^{c}
SP (g)	2.98 ± 0.05^{a}	3.11 ± 0.10^{b}	2.96 ± 0.21^{c}
S (g/g)	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.03^{a}
BD (g/g)	0.96 ± 0.01^{a}	0.81 ± 0.01^{b}	0.85 ± 0.02^{c}

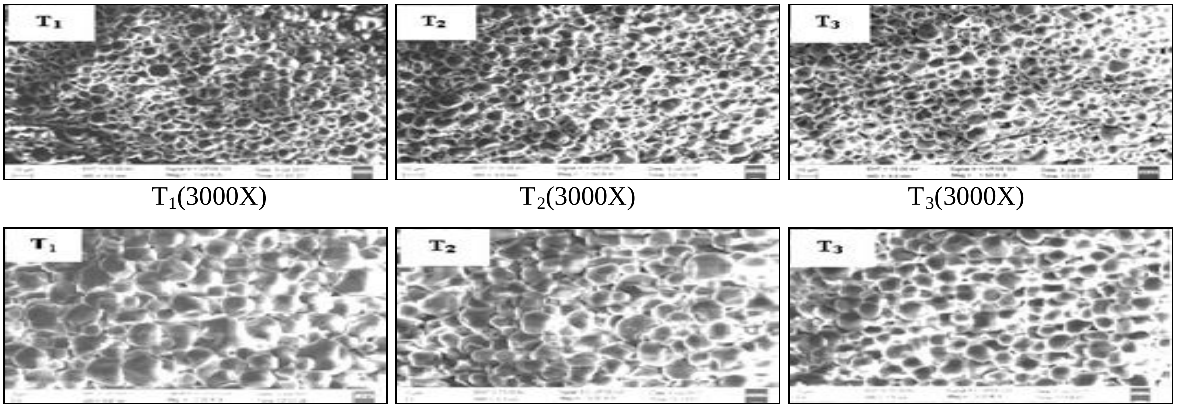
Mean values followed by the different letters in the same row indicate significant difference ($p<0.05$). WAC: Water Absorption Capacity; OAC: Oil Absorption Capacity; SP&S: Swelling power and solubility; BD: Bulk Density

Functional properties of pearl millet starches are given in **Table 2**. Water Absorption Capacity (WAC) of pearl millet starches ranged from 0.63 to 1.11 ml/g. Variations in water absorption capacity may be caused by inherent differences in the proportion of crystalline and amorphous areas in the granules. Starch containing a higher proportion of amorphous material would presumably have absorbing water [20]. The oil absorption capacity of pearl millet starches was in the range of 1.07-1.60 ml/g. Possible explanations for the increased oil uptake of starch could be due to the varietal difference and large surface area and porosity of the particles [24]. The oil absorption index was influenced by the lipophilic nature of the granular surface and interior, which influenced for functional properties of starches [25].

The swelling power of isolated starches ranged from 2.96 to 3.98 (g). A significant difference ($p<0.05$) was observed in swelling power among the starches isolated through different methods. The swelling capacity of T_1 starch increased with an increasing degree of alkalinity. However, T_1 recorded a higher value (3.98%). This result was similar [26], Solubility of pearl millet starches ranged between 0.01-.02 (g/g). The insoluble starch granules started to swell because of hydration. The amylose and amylopectin dissociated in suspension, resulting in the increase of starch solubility [27].

The pearl millet starch had a moisture content of about 10 g/100 g and exhibited a bulk density of 0.96 g, 0.81 g, and 0.85 g/10 g for T_1, T_2, and T_3, respectively. Bulk Density (BD) was influenced by the moisture content of the starch. T_1 had a significantly ($P < 0.05$) higher bulk density (0.96 g/mm³) as compared to other samples.

Starch Granule Morphology: The scanning electron micrographs of the three types of isolated starch (Magnifications *viz*. 1500× and 3000×) are presented in **Fig. 1**.



T_1(3000X) T_2(3000X) T_3(3000X)

T_1(1500X) T_2(1500X) T_3(1500X)

FIG. 1: STARCH GRANULE MORPHOLOGY

These micrographs showed more polygonal, few small spherical and round granules, and porous. These pores had normal structures related to the genetic make- up of the source plant and were not artifacts of isolation, preparation or observation techniques [19]. The granule sizes of the pearl millet starches were similar and it's ranged from 2 to 5 µm in diameter.

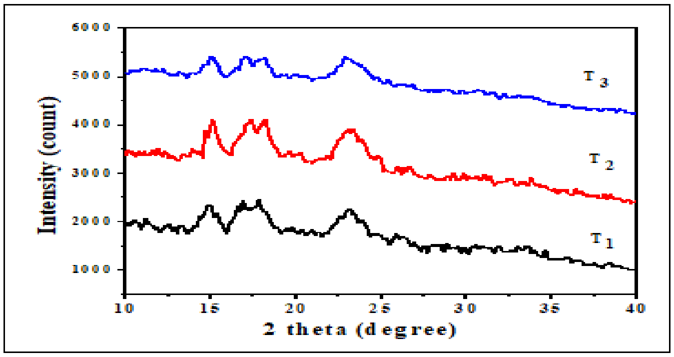
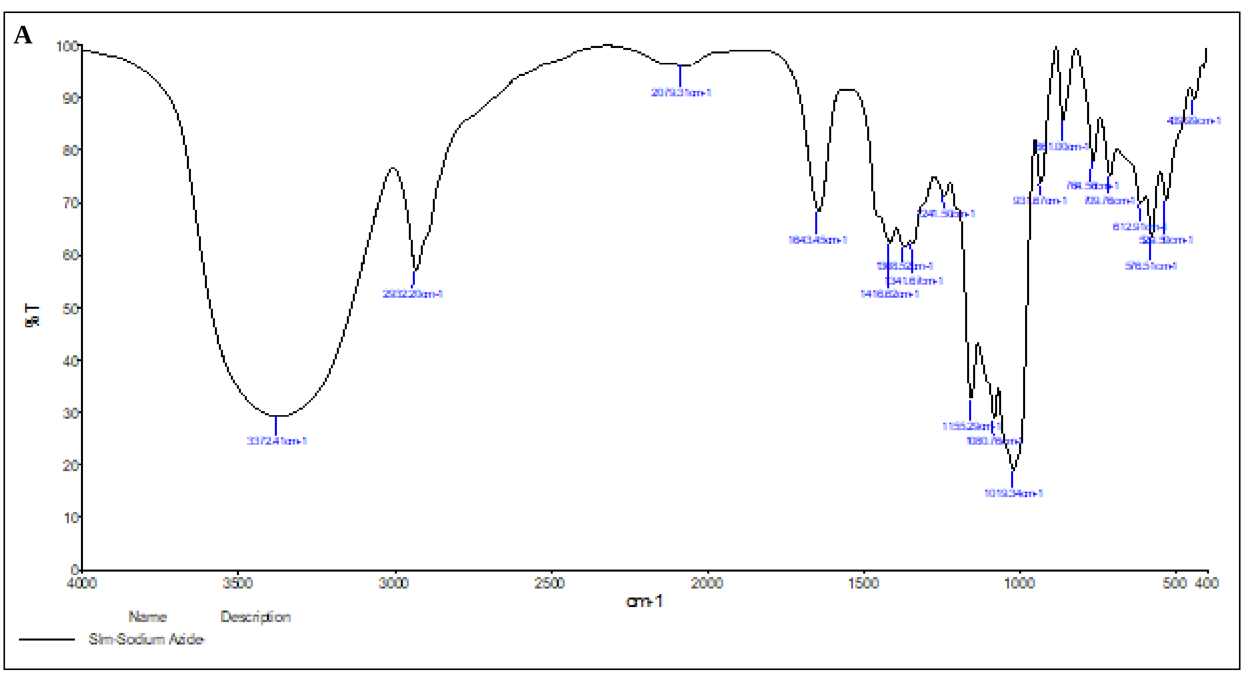


FIG. 2: X-RAY DIFFRACTOGRAMS OF PEARL MILLET STARCHES (T_1), (T_2), (T_3)

X-Ray Diffraction Pattern: X-ray diffraction patterns of pearl millet starches are given in **Fig. 2**. Pearl millet starches passed a typical A-type crystallinity with strong reflection at 2θ about 16, 18, 20, and 23° and weaker peak at 2θ around 14°. The ratio of the crystallinity area to the total diffraction area was calculated as the degree of crystallinity (%). The crystalline degree of pearl millet (T_1), (T_2), (T_3) isolated starch 37.91, 29.22, and 19.92%, respectively. The differences in relative crystallinity among starches can be attributed to crystal size, the orientation of the double helices within the crystalline domains, extent of interaction between double helices and amount of crystalline regions (influenced by amylose and amylopectin chain length).

FTIR Spectroscopic Analyses of Starch: Fig. 3 shows the FTIR spectrum of the T_1, T_2, and T_3 samples in the range of 4,000 - 400 cm^{-1}. The IR spectrum of starch samples was described by six main modes, with maximum absorbance peaks near 4,000, 3,000, 2,500, 1,500, 1,000, 800, and 400 cm^{-1}. The band absorbance in starch has been assigned and matched with the vibration modes of the chemical bonds and the structures of starch molecules. The broadband observed at 3,372 cm^{-1} corresponds to O–H stretching vibrations arising mainly from water and protein. Furthermore, bands at 2,932 cm^{-1} (C–H stretching of neutral lipids and proteins). All the samples showed that similar characteristics peaks at 3,372 and 2,932 cm^{-1} could be attributed to O–H and C–H bond stretching, respectively, while the peaks at 1,648 and 1,416 cm^{-1} were attributable to the bending modes of H–C–H, C–H and O–H. The peaks at 1,300~1,000 cm^{-1} were attributed to C–O–H stretching; however, 1080 and 1019 cm^{-1} were associated with the ordered and amorphous structure of starch, respectively. The bands at 930 ~860 cm^{-1} were attributed to D-glucopyranosyl ring vibration modes.



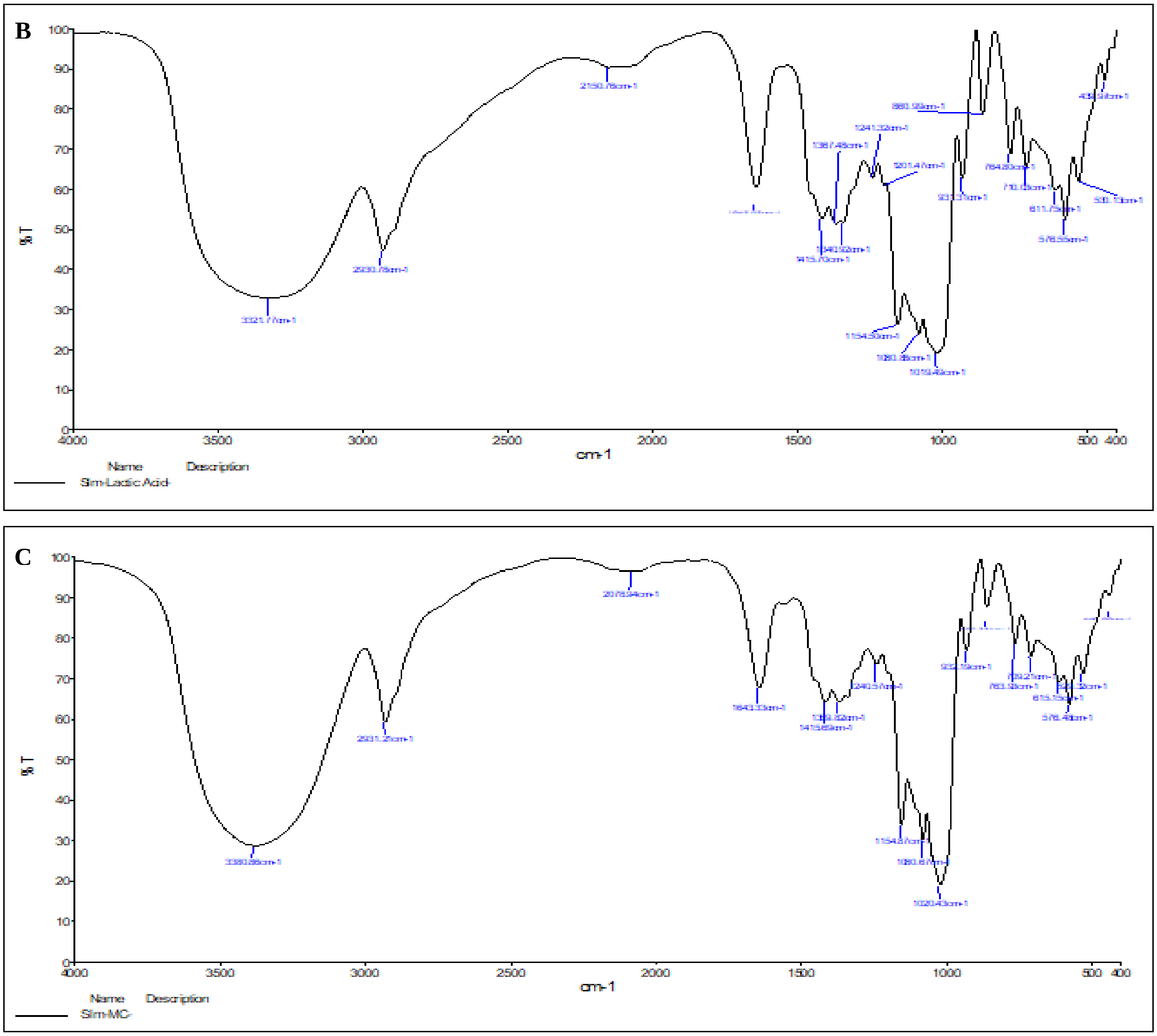


FIG. 3: SHOWS (A) (T_1) ISOLATION OF STARCH USING SODIUM AZIDE, (B) (T_2) ISOLATION OF STARCH USING SODIUM METABISULFITE AND LACTIC ACID, (C) (T_3) ISOLATION OF STARCH USING MERCURIC CHLORIDE

Correlation among the Chemical and Functional Properties of Pearl Millet Starches: Functional properties of starch depend on the chemical composition of starch, and it is given in **Table 3, 4, 5**.

TABLE 3: CORRELATION COEFFICIENTS OF FUNCTIONAL PROPERTIES AND CHEMICAL COMPOSITION OF PEARL MILLET STARCH (T_1)

	Ash	M	DM	CF	AA	SP	S	WAC	OAC	pH	CP
Ash	1										
M	.866	1									
DM	.000	-.500	1								
CF	-1.00	-.866	.000	1							
AA	.723	-.975	.682	.732	1						
SP	.723	.281	.691	-.723	-.059	1					
S	-.866	-.500	-.500	.866	.293	-.972	1				
WAC	-.115	.397	-.993	.115	-.593	-.769	.596	1			
OAC	-.980	-.949	.201	.980	.854	-.570	.748	-.087	1		
pH	.189	.655	-.982	-.189	-.808	-.542	.327	.954	-.382	1	
CP	-.500	. 000	-.866	.500	-.224	-.542	.866	.918	.316	.756	1

M = Moisture, DM = Dry Matter, CF = Crude Fat, AA = Apparent Amylose, SP = Swelling Power, S = Solubility, WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity, CP = Crude Protein

Ash content of starch isolated with sodium azide had high degree positive correlation with moisture (r = 0.86) and amylose content (r = 0.723), whereas low degree positive correlation was found with WAC, OAC and pH. Crude fat was positively correlated with oil absorption capacity (r = 0.980). Apparent amylose was positively correlated with solubility, and OAC had a negative correlation with swelling power (r = − 0.059) and pH (r = −0.808). A high level of amylose and lipid strongly inhibited the swelling property of starch granules [28].

TABLE 4: CORRELATIONS COEFFICIENTS OF FUNCTIONAL PROPERTIES WITH CHEMICAL COMPOSITION OF PEARL MILLET STARCH (T_2)

	Ash	M	DM	CF	AA	SP	S	WAC	OAC	pH	CP
Ash	1										
M	.000	1									
DM	.778	-.629	1								
CF	000	-1.000	.629	1							
AA	-.442	-.897	.220	.897	1						
SP	-.064	-.998	.578	.998	.924	1					
S	-.500	.866	-.933	-.866	-.556	-.832	1				
WAC	-.682	-.731	-.071	.731	.957	.773	-.292	1			
OAC	.819	.189	.276	-.574	-.877	-.625	.088	-.978	1		
pH	.397	.918	-.268	-.918	-.999	-.941	.596	-.942	.852	1	
CP	-.982	.189	-.882	-.189	.264	-.126	.655	.532	-.696	-.217	1

M = Moisture, DM = Dry Matter, CF = Crude Fat, AA = Apparent Amylose, SP = Swelling Power, S = Solubility, WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity, CP = Crude Protein

Ash content had a high degree of positive correlation with dry matter and pH. Apparent amylose content was positively correlated with swelling power (r = 0.924) and water absorption capacity (r = 0. 957), (P<0.05). This might be due to the leaching of amylose. It was reported that solubility and leached amylose had been shown to be positively correlated with swelling power [27].

TABLE 5: CORRELATIONS COEFFICIENTS OF FUNCTIONAL PROPERTIES WITH CHEMICAL COMPOSITION OF PEARL MILLET (T_3)

	Ash	M	DM	CF	AA	SP	S	WAC	OAC	pH	CP
Ash	1										
M	.866	1									
DM	-.602	-.920	1								
CF	.000	-.500	.799	1							
AA	.944	.652	-.303	.331	1						
SP	.673	.953	-.996	-.740	.390	1					
S	.000	.500	-.799	-1.000	-.331	.740	1				
WAC	-.062	-.553	.835	.998	.272	-.780	-.998	1			
OAC	-.300	-.737	.943	.954	.032	-.908	-.954	.971	1		
pH	.500	.000	.391	.866	.758	-.304	-.866	.833	.676	1	
CP	.082	-.427	.747	.997	.407	-.682	-.997	.990	.926	.904	1

M = Moisture, DM = Dry Matter, CF = Crude Fat, AA = Apparent Amylose, SP = Swelling Power, S = Solubility, WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity, CP = Crude Protein

Ash content had high degree positive correlation with moisture (r= 0.866) and amylose content (r = 0.944) of the starch. Moisture content had a high degree positive correlation with swelling power (r = 0.953) of the starch. Swelling power can be influenced by strongly amylopectin molecular structure [28]. WAC was positively correlated with dry matter (r = 0.835) and negatively correlated with crude fat (r = - 0.998). OAC is positively correlated with crude fat (r = 0.954) [29].

CONCLUSION: The result showed that **s**tarch was isolated from pearl millet with different chemicals. The starches showed a significant difference in composition and physical properties. The highest starch yield (56%) was observed while using sodium azide (T_1). It had a higher starch yield, apparent amylose, and total starch content. SEM analysis showed that small granules and XRD pattern and high degree crystalline structure was observed. Hence the study concluded that alkali like sodium azide could be used to isolate starch with desirable properties.

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