INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

# ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION OF CROSSBREED AND OPEN POLLINATED MAIZE STARCHES FROM ETHIOPIA

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Article Received on: 09/05/18 Approved for publication: 08/06/18

**DOI: 10.7897/2230-8407.09696**

**ABSTRACT**

Crossbreed maize starch with code name of BH-546 and open pollinated maize starch, Gibe-3 *Zea mays* are a potential source of starch in Ethiopia. Physicochemical properties of BH-546 starch such as, densities, flow, swelling power, percentage solubility, moisture sorption, lipid and protein content, and ash values were investigated and compared to those of Gibe-3 starch. Moisture content, water absorption capacity, solubility and chemical compositions were found to be significantly different. Moisture sorption increased generally with relative humidity. Total lipid content of (BH-546) starch was found to be 7.95% which is higher than that of the (Gibe-3) Zea mays starch (7.55%). These values are higher than those reported on the maize whole kernel starches (4%) but lower than those reported for Maize germ (wet milling) starch (40%) and Maize germ (dry milling) starch (20%). In conclusion, crossbreeding affects the physicochemical properties of maize starches.

**KEYWORDS**: Crossbreed maize; BH-546; Gibe-3; Starch; Physicochemical.

## INTRODUCTION

Important heritable and desired novel properties can be expressed using different natural and artificial breading techniques. Such innovative techniques have been used to develop crops with unique features for agricultural and industrial applications. The development of traits with better stress tolerance, disease resistance, higher biomass yield, wider adaptability and improved quality have been central parts of pant breading techniques. Altering the content of specific biological molecules (proteins, sugars, lipids, vitamins, fibers, etc.) and imparting properties for easier agro-processing during harvesting, milling, baking, malting, blending is also possible through breeding.[1, 2]

Maize is the second most abundant crop in the world and has been used for different household and industrial applications. Associated with its importance and susceptibility to environmental factors, maize breeding and cross-breeding practices have been commonly practiced. Single cross, double-cross, three-way cross, top-cross and varietal-cross hybrids are the most commonly studies maize breeds. In addition to the conventional breeding techniques, advanced biotechnology techniques have been employed to further enhance the efficiency of breeding and product quality.[3]

The three-way cross hybrid is most prominent throughout Ethiopia due to its high productivity and coverage claimed to give on average seven tons per hectare. Therefore, BH-546 varieties were selected as an experimental material for the physicochemical characterization of maize starch. BH-546 Zea mays was a cross breed from CML-395×CML-202//BKL-101 maize. Half-and-half maize assortments have more noteworthy hereditary potential and should be produced and advanced. Development of mixture maize has been one of the extraordinary horticultural innovative achievements of the century.[4]

Open pollination is often triggered by natural factors such as wind, insects and birds resulting genetically diverse cultivars due to its unrestricted nature. Open pollination offers cultivars with better adaptability to the environment, stable characteristics and comparable yield to hybrid forms with relatively lower cost.[5] For maize, two types of open-pollinated cultivars have been produced. The first type is prepared by repeated selection procedures until acceptable improvement is obtained; while the second type obtained through pre-planned matting of desired genotypes and is commonly referred as a synthetic cultivar.[6]

Therefore, Gibe-3 (open pollinated Zea mays) was selected as an experimental material for the physicochemical characterization of maize starch. The utilization of starch and starch derivatives in Ethiopia is presumed to significantly increase because of the government policy to move from agricultural-led economy towards industrialization. Accordingly, the utilization of starch Industry is expected to increase by fifty percent in the coming years. In the event that starch production is to be expanded to take care of the modern demand, and convey monetary advantage to Ethiopians, there is a need to investigate different wellsprings of starch, which could supplement the current source. Maize offers this opportunity as option wellsprings of starch for the local industry.[7] The purpose of this research is to isolate and characterize the physicochemical properties of crossbreed maize starches.

## MATERIALS AND METHODS

Crossbreed Zea mays (BH-546) and open pollinated (Gibe-3) Zea mays were purchased from the Ethiopia Institute of Agricultural

Research Addis Ababa, Ethiopia. The different equipment and chemicals used were specified with in the methods section.

**Starch isolation**

Extraction and purification of crossbreed and open pollinated maize starches were carried out according to the method by Gebre-Mariam and Schmidt with slight modification. BH-546 and Gibe-3 maize flour were sieved through a fine sieve (224µm) to remove roughages and unnecessary impurities from the flour. Sieved maize flour of 390 g was mixed with distilled water containing 15 w/v of sodium metabisulphite stirred for 2 h at room temperature, the material was allowed to settle and the supernatant was decanted. The sedimented starch was repeatedly treated with sodium metabisulphite solution until the suspension became translucent. The material was then passed through fine muslin to remove cell debris and the translucent suspension was collected, filtered through a fine sieve (224 µm) and allowed to settle. The sedimented starch was washed several times with distilled water followed by sieving after each washing until the wash water was clear and free of suspended impurities and the filtrate was centrifuged at 6000 rpm for 15 min. The supernatant was discarded, along with the tailings layered on top of the starch, which were carefully removed by scraping. The sedimented starch was collected and dried in an oven (Kottermann® 2711, Germany) at 50 °C overnight. Then it was milled to fine powder in grinding machine, sieved using 224-µm mesh size sieve and stored in plastic bags for further studies.[8]

**Determination of chemical Composition**

**Moisture Content**

Moisture content of BH-546 and Gibe-3 maize starch powders was determined by a gravimetric method. The powder sample (5 g) was spread onto pre-weighed petridishes uniformly and dried in an oven at 105 $^0$C until constant weight was obtained. The weight loss was determined by accurately weighing the samples after attainment of constant weight. The moisture content was determined from the loss of weight. The result was then expressed as a mean of three parallel determinations.

**Total Ash Determination**

Ash content was determined according to FAO manuals of food quality control.[9] The ash content was determined by weighing 2 g each of BH-546 and Gibe-3 maize starch samples into crucible that has been ignited and cooled in a desiccator and weighed soon after reaching room temperature. The samples were ignited in a furnace (Naber, D-2804, and Liliethal, Germany) at about 550 $^0$C for 1 h. The remaining mass in the crucible is the total ash, which was expressed in percentage.

**Protein Content**

Protein content was analyzed by destruction of organic matter using sulfuric acid. Nitrogen was liberated as ammonia and was distilled, collected and titrated according to Association of Official Analytical Chemists. Weighed starch sample (2 g) was placed in 500 ml digestion flask. Six ml of acid mixture (2 parts of conc. Sulfuric acid and 1 part of conc. Ortophosphoric acid) was added. The flask was placed on heater and violent reaction was observed. As soon as violent reaction was ceased, heat was increased, and the destruction was continued until the content appears light green for one hour. It was then cooled and diluted with distilled water. The digested and diluted solution was transferred in to sample compartment of the distiller. It was then distilled until a total volume of 150 ml was collected ($NH_3$ as distillate). Finally, the excess standard acid in the distillate was titrated with standard NaOH solution.[10]

**Lipid Content Determinations (Defatting)**

Defatting of BH-546 and Gibe-3 maize starches was conducted using the method described by Bligh and Dyer. Four grams of starches obtained from each maize were shaken vigorously with 100 ml of chloroform-methanol (2:1 v/v) at 25 °C for 1 h. The extract was separated into filtrate (Filtrate 1) and residue by decantation. The filtrate was used for further isolation of unbound lipids, while residue was further solvent extracted with 100 ml of n-propanol-water (3:1v/v) at 95 °C for 7 h to extract mainly bound lipids in water bath. This second extract was decanted after being cooled at room temperature. Its filtrate (Filtrate 2) was used for further isolation of starch-bound lipids. After all these, both filtrates (Filtrate 1 and Filtrate 2) were purified by extraction with chloroform-methanol-water (1:2:0.8) and chloroform-methanol-water (1:1:0.9), respectively. Then, two layers (water and chloroform) were formed and latter separated by gentle decantation. The chloroform layers were diluted with benzene and shaken slightly. Then the organic solvents were evaporated using a rotary evaporator. The weight difference was used to calculate percentage of unbound, bound and total lipids. Results are given as mean and standard deviation of three parallel determinations.[11]

**pH Determination**

The pH of BH-546 and Gibe-3 maize starch was determined using the method described by Singh and his colleagues. It was done by shaking a 1% w/v dispersion of the starch in water for 5 min and the pH was determined using a digital pH meter (Wagtech 3510, UK).[12]

**Density and Related Properties**

Thirty grams of each of BH-546 and Gibe-3 maize starch were transferred into two separate 250 ml measuring cylinders; the volume occupied by each starch powder was read to the nearest 0.5 ml and bulk density was calculated as g/ml. The bulk in the cylinder was tapped for 1 min. using tapped voltmeter (ERWEKA, type SVM, Germany), which provides a fixed drop of half an inch at a rate of 250 taps/min. The volume occupied by the starch was recorded and tapped density was calculated as g/ml. The Carr's index (% compressibility) of the starches was calculated from difference between the tapped and bulk densities divided by the tapped density and the ratio expressed as percentage. The Hausner ratio was calculated by dividing the tapped density by bulk density of the starch powder.

$$\text{Carr's index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tape density}} x100 \ldots\ldots \text{(eq.1)}$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \ldots\ldots\ldots\ldots\ldots\ldots \text{(eq.2)}$$

**Angle of Repose and Flow Rate**

Flow rate and angle of repose were determined by fixed height funnel method. Thirty grams of starch powder was placed and allowed to flow through a stemless funnel having 10 mm aperture from a fixed height of 10 cm. The duration of flow was recorded and used to calculate the flow rate. Angle of repose was determined from the height and radius of powder pile according to Eq.3 below.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \ldots\ldots\ldots\ldots\ldots\ldots \text{(eq.3)}$$

Where, θ=Angle of repose, h = Height of granule, r = Radius of circle formed.

**Moisture Sorption Pattern**

Pyrex desiccators containing distilled water (100% RH), saturated solution of NaCl (75.6%), and appropriate concentrations (60% , 40% , 20%) sodium hydroxide solution were prepared to obtain different relative humidity, RH ( 20, 40, 60, 75 and 100), respectively and stored in relative humidity chamber at room temperature. BH-546 and Gibe-3 maize starch powder samples were pre-dried in an oven for 4 h at 120 $^0$C. Two grams of each maize starch powder samples were spread evenly on each petridish (dried and weighed) and transferred to a particular RH chamber. Samples were equilibrated for four weeks at room temperature. The weights were recorded and moisture uptake of each sample was calculated as the weight difference of the samples before and after equilibrium in a given RH. Water sorption capacities of the starches were expressed as percent moisture uptake.[13]

**Water Binding Capacity**

A suspension of 1g starch (dry weight) in 35 ml distilled water was agitated for 1h and centrifuged (3000 rpm) for 10 min. The free water was removed from the wet starch, which was then drained for 10 min. The wet starch was then weighed.[14]

$$\text{WBC} = \frac{B-C}{B-A} \text{x} 100 \ldots\ldots\ldots\ldots \text{(eq.4)}$$

Where: A is Weight of the empty tube; B is Weight of the tube + sample before centrifuge; C is Weight of the tube + sample after centrifuge.

**Swelling Capacity and Solubility**

Powder samples (0.5 g each) were dispersed in distilled water (10 ml) in pre-weighed test tubes. The test tubes were kept in thermostatically controlled water bath at 55, 65, 75, 85 and 95 $^0$C for 30 minutes with shaking every 5 minutes and then left to cool to room temperature. The suspensions were centrifuged for 15 minutes at 3000 rpm in order to facilitate the removal of the supernatant, which was carefully decanted and the weight of the starch paste taken. Then, the supernatant was decanted into a pre-weighed watch glass and dried in an oven (Kottermann® 2711, Germany) for 2 hr at 130 $^0$C until constant weights were obtained. The residue obtained after drying the supernatant represents the amount of starch solubilized in water. The solubility was calculated as gram per 100 g of samples on dry weight basis.[15] All determinations were done in triplicate. The relative solubility (% RS) and swelling power (SP) were determined according to eq. 5 and eq. 6.

$$\text{RS (\%)} = \left(\frac{w1}{0.5}\right) x100 \ldots\ldots\ldots\ldots\ldots \text{(eq.5)}$$

$$\text{SP} = \left(\frac{w2}{0.5x\left(100-S(\%)\right)}\right) x100 \ldots\ldots\ldots\ldots\ldots \text{(eq.6)}$$

Where w1 is the weight (g) of soluble material in the supernatant, S is solubility of starch, and w2 is the weight (g) of precipitate.

**Crystallinity of the Starch**

X-ray powder diffraction of the starch samples were taken with X-Ray diffractometer (Bruker AXS, Bavart Zulassung, BW/508/98/Rö, Germany) operating in the 2Θ modes. A Cu target tube operated at a power setting of 40kV (15mA), receiving slit 13.0 mm, in the range of 2-90° of 2θ with single crystal graphite monochromator equipped with a microprocessor to analyze peak position and intensities was utilized. A standard polycrystalline silicon powder was used to calibrate the equipment.[16]

**RESULTS AND DISCUSSION**

**Powder Properties of Starches**

The various powder properties of the crossbreed and open pollinated maize starches obtained during the study are depicted in Table 1. Both the bulk and tapped densities of BH-546 Zea mays starch are higher than Gibe-3 Zea mays starch. The particle size and shape of the starches may be responsible for the differences in the density values. The density values were used to calculate the Hausner ratios and Carr's indices, which are measures of the flowability of a powder. Hausner ratio less than 1.25 indicate good flow, whereas greater than 1.25 indicates poor flow and Carr's index values between 5 to 10 represent excellent flow properties.[17] Hausner ratio and Carr's index values for BH-546 maize starch is significantly lower than Gibe-3 maize starch. Both BH-546 and Gibe-3 maize starches fail to flow through the funnel and as a result, angles of repose could not be determined. Similar poor flow properties of native starches have been reported by Odeku and his colleague.[18]

**Table1: Powder properties of BH-546 and Gibe-3 Zea mays starch powders**

| Powder properties | BH-546 starch ( mean ±SD) | Gibe-3 starch (mean ±SD) |
|---|---|---|
| Appearance | White powder | White powder |
| Bulk density (g/ml) | 0.34±0.005 | 0.313±0.21 |
| Tapped density (g/ml) | 0.423±0.006 | 0.42±0.0058 |
| Hausner ratio | 1.23±0.04 | 1.34±0.015 |
| Carr's index (%) | 19.04%± 0.165 | 25.5%±0.225 |
| Angle of repose (degree) | Not flow | Not flow |
| Flow rate (g/sec.) | Not flow | Not flow |

**Water binding Capacity (WBC), Moisture Content and pH**

WBC of the BH-546 Zea mays starch sample was lower than the Gibe-3 Zea mays starch sample. The WBC for BH-546 maize and Gibe-3 maize samples were 0.984% ± 0.002 and 1.2% ± 0.003, respectively. The differences in the degree of availability of water binding sites among the starches may have contributed to the variation in water holding capacity between the starches as a similar report was also reported.[19] Similarly, BH-546 maize starch had greater moisture content (3.5% ± 0.15) than the Gibe-3 maize starch (1.25% ± 0.035).

The pH of a formulation can significantly influence the stability and physiological activity of a product. Determining the potential

impact of a formulation additive on the pH of a formulation is hence an important activity during reformulation/formulation studies. In this study, the pH value of 1% w/v solutions of BH-546 and Gibe-3 maize starches were found to be 6.76 ± 0.062 and 6.46 ± 0.081, relatively in compliance with the USP recommended limit for wheat and Zea mays starches (4.0-7.0).[21] The near neutral pH suggest the potential application of the starches for the formulation of acidic, basic or neutral drugs for oral administration as the possibility of mucosal irritation will be very minimal. [20]

**Protein Content, Lipid Content and Ash Value**

The chemical composition of crossbreed Zea mays and open pollinated Zea mays is indicated in Table 2. The exact quantity of protein and lipid associated with the starch depends on both the botanical origin of the starch and its degree of purification during extraction.[22] The total lipid content of crossbreed (BH-546) Zea mays starch was found to be 7.95% indicating that the lipid content of BH-546 starch is higher than that of open pollinated (Gibe-3) Zea mays starch (7.55%). This value is higher than that reported on the maize whole kernel, starches (4%) but lower than those reported for Maize germ (wet milling) starch (40%) and Maize germ (dry milling (20%).[23] Presence of higher lipid content can significantly affect some physicochemical properties of starch such as delaying/decreasing of starch granules swelling, reducing the solubility, reducing rate of gelatinization and reduction of water uptake especially if the lipid is medium and long chain compound through interaction with linear amylose chains. It has also the potential to affect the size and shape of the starch granules at higher temperatures.[24-26]

Protein content of BH-546 Zea mays starch (6.71%) was higher than that found in Gibe-3 Zea mays (6.58%). It was reported elsewhere that maize starch has 8.3% protein content. The value obtained in this study is within the limit specified in maize starch which states not more than 8.3% protein content were allowed.[27]

**Table 2: The chemical composition of BH-546 and Gibe-3 Zea mays starch**

| Composition | BH-546 starch | Gibe-3 starch |
|---|---|---|
| Protein content | 6.71% | 6.58% |
| Lipid content | 7.95% | 7.55% |
| Ash value | 1.78% | 0.90% |

**Solubility and Swelling Power**

The solubility and swelling power of the starches are depicted in Figure 1 and Figure 2, respectively. The solubility and swelling power of the starches were generally low at low temperature (55 °C) but increased significantly at higher temperature (95 °C). Waliszewski *et al.,* reported that the solubility and swelling power of both native maize starches increased with temperature. Odeku and his colleagues also reported comparable results. These could be due to the degree of macromolecular disorganization and to variations in the degradation of starch during thermal treatment.[17, 28]

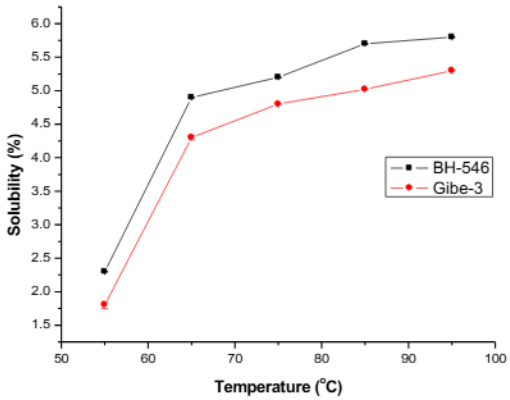


**Figure 1: Solubility of BH-546 Zea mays starch and Gibe-3 Zea mays starch at different temperatures.**

Swelling power and solubility can be used to assess the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule.[29] In this study, swelling power of BH-546 Zea mays starch sample was observed to be slightly higher than that of Gibe-3 Zea mays starch sample; however these differences were almost similar as shown in **Figure 2** Singh and his colleagues reported that starch swelling occurs concomitantly with loss of birefringence and precedes solubilization.[30]

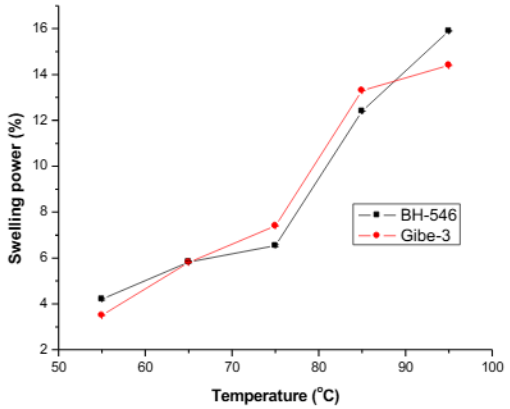


**Figure 2: Swelling power of BH-546 starches and Gibe-3 Zea mays at different temperatures.**

**Moisture Sorption Pattern**

Moisture sorption profiles of the starches are depicted in Figure 3. Moisture sorption increased generally with relative humidity. Percent moisture sorbed ranged between 4.4% at low (20 %) relative humidity to over 75.6 % at high (100 %) relative humidity. Gibe-3 had the highest moisture sorption at 100 % and 20% relative humidity (54.25 % ± 0.02, 11.6±.132), while BH-546 starch had the highest at 75.6% relative humidity (31.08% ± 0.012). At 75.6% relative humidity, Gibe-3 starch had the lowest moisture sorption (30.326±0.03); while at 20% relative humidity, BH-546 had the lowest (4.4% ±0.163). The hydrophilic nature of the starch molecule is probably responsible for the observed high moisture sorption by the starches and reinforces the necessity for moisture preclusion during storage.[31]

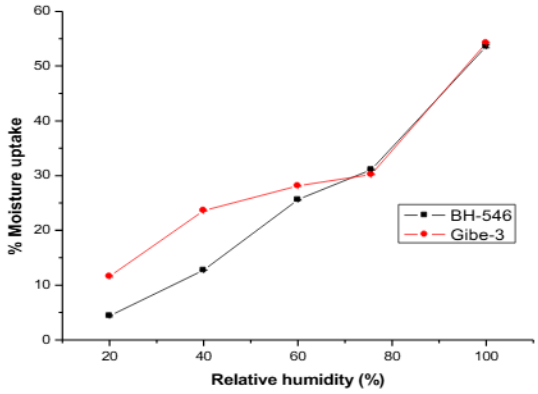


**Figure 3: Moisture sorption patterns of HB-546 and Gibe-3 Zea mays Starch.**

Higher water content in starch can affect its physicochemical and microbial stability, and also some of the important technical parameters like flowability.[32] Even though water can be bound in the starch molecular structure through hydrogen bonding with amylose and amylopectin fractions, greater amylopectin proportion may result in physical entrapment of water in the structure. It has been reported that starch granules with greater amylopectin content have greater moisture sorption capacity.[33]

In this study; however, open pollinated maize starch with higher amylopectin content displayed lower moisture uptake than the crossbreed maize starch. The difference in moisture sorption pattern, contrary to accepted hypothesis, may be accounted for differences in the degree of crystallinity between the two forms. Polymers with greater crystallinity are characterized by higher degree of secondary intermolecular bonding which results in interaction of hydroxyl groups on adjacent glucose units thereby decreasing free sites for water adsorption.[34] Accordingly, Gibe-3 at 75.6% relatively humidity with lower moisture contents was found to be stable upon storage.

### Crystallinity of the Starches

The definite crystalline nature of starch is attributed to the well-ordered amylopectin arrangement in its granular structure. Based on the length of amylopectin lattice and the density of packing, the crystalline structure of starch can be classified s A, B or C types. A pattern is common in native cereals, while B types are obtained from tuber starches. The C Type is a mixture of the two forms or can be found from smooth-seeded peas and beans. Type A adopts a close-packed arrangement with water molecules between each double helical structure while the B type is more open accommodating more water molecules within its central cavity surrounded by six double helices explaining the basic differences between the two crystalline forms. [16]

The X-ray powder diffractogram of crossbred maize starch is presented in Figure 4, which shows similar peaks with corn starch but different from those of potato. Furthermore, the characteristic peak around 5° 2θ of B-type crystallinity of most tuber starch is not observed for crossbred maize starch. Both the BH-546 Zea mays and Gibe-3 Zea mays starches exhibited maximum peaks at 18.2° and 23.06$^0$ 2θ angles. The other significant peaks were around at 17.2°, 18.3°, and 23.3° 2θ angles. These peaks are characteristics of cereal starch. Therefore, like other cereal starches, BH-546 Zea mays and Gibe-3 Zea mays starch have A-type crystallinity which shows a close packed arrangement of amylopectin within the granule.

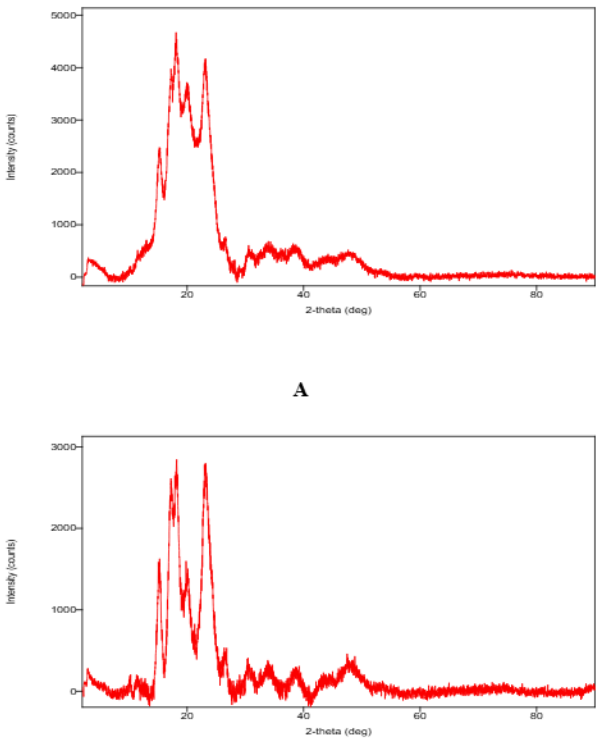


**Figure 4: X-ray powder diffractogram of starch of (A) BH-546 Zea mays (B) Gibe-3 Zea mays.**

## CONCLUSION

The study results showed variation in absorption capacity, solubility and swelling power of the starches isolated from crossbreed Zea mays (BH-546) and open pollinated Zea mays (Gibe-3). In addition, there are some differences in the result of protein and fat contents and ash values of crossbreed Zea mays (BH-546) and open pollinated Zea mays (Gibe-3). In conclusion, crossbreeding affects the physicochemical properties of the starches isolated from Zea mays.

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**Cite this article as:**

Yeabsira Lake *et al*. Isolation and physicochemical characterization of crossbreed and open pollinated maize starches from Ethiopia. Int. Res. J. Pharm. 2018;9(6):94-99 http://dx.doi.org/10.7897/2230-8407.09696

Source of support: Nil, Conflict of interest: None Declared


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