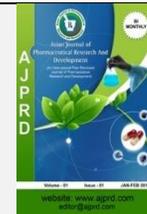


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Research Article

Characterization and Tablet Property Evaluation of Pregelatinized Starch of Teff (*Eragrostis tef*)

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ABSTRACT

Starch is a multifunctional pharmaceutical excipient in solid dosage formulations and obtained from different sources. Teff (*Eragrostis tef*) grain is a source of major staple food in Ethiopia and contains starch. Teff starch was extracted from milled teff flour using 0.075% sodium metabisulphite. The starch was pregelatinized at 60 oC, 70 oC and 80 oC temperatures. When characterized, the pregelatinized teff starches had enhanced physical attributes such as swelling capacity, water absorption capacity, granule density, Carr's index, Hausener's ratio, flow rate and angle of repose. When incorporated as disintegrant in chloroquine phosphate tablet formulation at 5% and 10% concentrations, tablets containing pregelatinized teff starches showed faster disintegration time and better compressibility compared to the native starch. Tablets containing pregelatinized teff starch at 70 oC and 10% concentration had the fastest disintegration time with 7.55 min. Pregelatinization, a non-expensive type of physical starch modification can be used to enhance excipient attributes of teff starch for its use as disintegrant in tablet formulations.

Key words: Teff starch, pregelatinized teff starch, pregelatinization, chloroquine phosphate, tablet

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INTRODUCTION

Starch is a multifunctional excipient in solid dosage forms such as tablet formulations which can be used as binder, disintegrant, or filler due to its suitable physicochemical properties and relative inertness¹. Each starch is named according to its botanical origin, e.g., potato starch, maize starch, cassava (tapioca) starch, rice starch, etc. Each starch is definitely different from the other with respect to chemical composition and physical characteristics².

Teff (*Eragrostis tef*) is cultivated as a major cereal in Ethiopia. In 2016/17 of total cereal production by individual farmers in major cereal production season, teff

production accounted 17.29% (50,204,400.47 quintals)³. There are several varieties of teff in Ethiopian markets identifiable with their color such as very white, white, light brown and dark brown⁴. The major nutritional constituents of teff grain per 100 g are protein (13.3 g), total lipid (2.38 g), carbohydrate (73.13 g), and total dietary fiber (8 g)⁵. Teff flour from its whole grain is widely used in Ethiopia for making staple foods such as *injera* (fermented, pancake-like sour bread), traditional alcoholic drinks like *tella* (opaque beer) and *katikalla* (local spirit), *kitta* (sweet dry unleavened bread), *muk* (gruel) and porridge⁴. In recent years, teff is attracting consumers around the world. Teff grain is gluten free and has great potential to make varieties

of food/beverage products to help people with celiac disease⁶.

Due to limited suitability of native starches for applications in tablet formulations, they are modified chemically or physically to improve their positive attributes or to minimize their defects⁷. One of the physical modification techniques of native starch is pregelatinization. Pregelatinized starch is also known as instant starch slurry, where native starch is simply precooked to give products that readily disperse in cold water to form moderately stable suspensions⁸. Pregelatinized starch has multiple functionalities in tablet formulations as directly compressible filler-binder, disintegrant, flow-aid and self-lubricant. In this study teff starch was extracted, pregelatinized, characterized and some tableting characteristics were evaluated.

MATERIAL AND METHODS

MATERIALS

Teff grain variety locally known as “Magna”, white in color, was purchased, sieved and the whole grain was milled in milling house. Chemicals used were sodium metabisulphite, potassium hydroxide, hydrochloric acid, potassium iodide, iodine, ethanol, sodium hydroxide, acetic acid, xylene, chloroquine phosphate, Celactose 80[®], dicalcium phosphate dehydrate, Starch 1500[®], magnesium stearate and talc.

Methods

Starch extraction

Starch was extracted according to the method of Gebremariam and Schimit⁹ with slight modifications. Teff flour was dispersed in 0.075% sodium metabisulphite solution for 2 hr stirring periodically in the first 1 hr. The supernatant containing soluble components was decanted and the suspension was repeatedly treated with the solution. Extraneous insoluble matters like fibers, sands, lipid, etc were removed from the suspension by repeatedly filtering using sieves and nylon cloth. The suspension was kept overnight to allow sedimentation of the starch and the supernatant was carefully decanted. The starch was then dried at 50 °C. The dried starch was powdered using mortar and pestle, sieved using 224 µm sieve and stored in jar.

Determination of yield of starch

The yield of starch from the teff flour was calculated according to the following formula. The determinations were made twice.

$$\text{yield (\%)} = \frac{\text{weight of starch}}{\text{weight of flour}} \times 100$$

Preparation of pregelatinized starch

Pregelatinized teff starch was prepared according to the method of Adedokun and Itiola¹⁰. A 20% starch suspension in distilled water was heated at 60 °C, 70 °C, or 80 °C for 6 min. The resulting pregelatinized starch was placed on stainless steel tray in the form of thin film and dried in a convection oven at 60°C for 48 hr. It was then powdered

using mortar and pestle and passed through 224 µm sieve and stored in jar.

Determination of degree of gelatinization

The degree of gelatinizations of native teff starch and pregelatinized starches were determined according to the method of Baks¹¹ which relies on amylose-iodine complex formation. A sample (0.04 g) was dissolved in 50 ml of 0.15 M KOH and mixed for 15 min. The resulting solution was centrifuged. After centrifugation, 1 ml of the supernatant was removed and neutralized with 9 ml 0.017 M HCl. Subsequently, 0.1 ml iodine reagent (1 g iodine and 4 g potassium iodide in 100 ml water) was added to form a blue complex with the dissolved amylose present in the sample. The absorbance (A_1) was measured at 25 °C and 600 nm against blank. The procedure was repeated, however, in this case 0.40 M KOH was used to ensure complete solubilisation of all the amylose present in the sample. The supernatant was neutralized with 0.045 M HCl. After adding 0.1 ml iodine reagent, the absorbance (A_2) was read at 25 °C and 600 nm. The ratio A_1/A_2 was used as a measure for the degree of gelatinization. The determinations were made twice.

$$\text{gelatinization of starch (\%)} = \frac{A_1}{A_2} \times 100$$

Determination of Amylose percent

The percentages of amylose in the native teff starch and pregelatinized starches were determined according to the method of Juliano¹². About 0.1 g of the sample was weighed and transferred to 100 mL volumetric flask and heated with 1 mL 95% ethanol and 9 mL 1 N sodium hydroxide for 10 min on a boiling water bath to gelatinize the starch. The sample was then cooled and the volume was made to 100 mL using distilled water. Then 5 mL of the starch solution was taken and poured into 100 mL volumetric flask. Then 1 mL of 1N acetic acid and 2 ml of iodine solution was added and the whole volume was made to 100 ml using distilled water. Then after 20 minutes, the sample was shaken and spectrophotometric absorbance was determined at 620 nm. The determinations were made twice.

$$\% \text{ Amylose} = 3.6 \times \text{absorbance} \times 20$$

Where 3.6 is conversion factor.

Determination of swelling capacity

Swelling potentials of native teff starch and pregelatinized starches were determined according to Bowen and Vadino¹³ method. Five grams of the sample was poured into a 100-mL measuring cylinder and the bulk volume (V_1) was measured. Distilled water (90 mL) was then added and the dispersion was shaken for 5 min and then the volume was made to 100 ml with distilled water. The dispersion was made to stand for 24 hr and the sedimentation volume (V_2) was read. The swelling capacity was calculated as V_2/V_1 . The determinations were made twice.

Determination of water absorption capacity (WAC)

Water absorption capacities of native and pregelatinized starches were assessed according to the method of Solsulski

¹⁴. In this 2.5 g of each sample was placed to a weighed 50 ml centrifuge tube, and 30 ml of distilled water was added. This was then agitated for 2 minutes, centrifuged at 400 rpm for 20 minutes and the supernatant was decanted. The residue was weighed (W_1). The adsorbed drops of water was removed by drying the residue at 60 °C to constant weight (W_2) in an oven. The water absorption capacity (WAC) was then expressed as follows. The determinations were made twice.

$$\text{WAC (\%)} = \frac{W_1 - W_2}{2.5} \times 100$$

Determination of granule density

Granule densities of the samples were determined using xylene as displacement liquid. A 25 ml pycnometer was thoroughly cleaned, dried and weighed. Xylene was then poured up to 25 ml mark and the weight of xylene and pycnometer was recorded. Then 2 g of each sample was poured into empty pycnometer and the liquid was added up to the mark and the weight was recorded. The weight of displaced xylene was obtained from the recorded weights and the corresponding volume was obtained by dividing the displaced weight to specific gravity of xylene (0.855). The granule density of each starch sample was calculated by dividing weight of sample (2 g) to volume of xylene displaced. The determinations were made twice.

Determination of bulk density, tapped density, Carr's index, and Hausner's ratio

Both bulk and tapped densities of the samples were measured according to USP 30-NF 25 ¹⁵ method. Accordingly, 30 g of powder was introduced into a 250 mL measuring cylinder. And the initial reading was taken. Then the cylinder was tapped using a tap densitometer that provides a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute, until no further change in the volume was noted (500 times) and the tapped volume was read. Finally, the bulk and tapped densities were calculated using equations below. The determinations were made twice.

$$\text{Bulk density} = \frac{\text{Weight of the powder}}{\text{Volume of the powder}}$$

$$\text{Tapped density} = \frac{\text{weight of the powder}}{\text{Tapped volume of the powder}}$$

Carr's Index and Hausner's ratio of the samples were calculated using equations below.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Determination of flow rate and angle of repose

Flow rate and angle of repose of each powder sample was determined using the funnel method. A powder (30 g) was made to flow through a stemless funnel having a 18 mm opening from a height of 10 cm. Time in second for the duration of flow was recorded. The average diameter and height of the powder piles formed were also recorded. The determinations were made twice.

$$\text{Flow rate (g/sec)} = \frac{\text{Weight of powder passed (g)}}{\text{Time elapsed (sec)}}$$

$$\text{Angle of repose (degree)} = \tan^{-1} \left(\frac{h}{r} \right)$$

Where h and r are the height and radius of the starch powder pile, respectively.

Determination of tableting characteristics

Tableting characteristics such as disintegration time, hardness and friability of native and pregelatinized teff starches were studied by preparing chloroquine phosphate tablets of 250 mg strength. The native and pregelatinized teff starches were used as disintegrants and incorporated at 5 % or 10% concentrations (Table 1).

Table: 1 The quantities of ingredients used in chloroquine phosphate tablets

Ingredient	Quantity
Chloroquine phosphate	250 mg
Celactose 80 [®]	250 mg
Dicalcium phosphate dihydrate	50 mg
Starch paste (10%)	165 mg
Disintegrant	5% or 10%
Magnesium stearate	0.25%
Talc	0.75%

The tablets were prepared by wet granulation method. Chloroquine phosphate, Celactose 80[®], dicalcium phosphate dehydrate and half of the disintegrant were mixed for 5 min using mixer (TURBULA[®] mixer, Switzerland). The powder mixture was wet massed using starch paste in mortar and pestle. The wet mass was granulated through 1.6 mm sieve and dried at 50 °C. The dried granules were milled and passed through 710 μm sieve and mixed for 5 min with half of the disintegrant, magnesium stearate and talc. Compression was performed using single press machine feeding the granules manually into the die cavity.

Hardness test. Hardness of three tablets was measured using hardness tester (CALEVA THT-2, Germany). Each tablet was placed between two anvils and the force that caused each tablet to break was recorded. The mean hardness and standard deviation were calculated.

Friability test: Friability of three tablets was tested using friability tester (ERWEKA TAR20, Germany). The tablets were rotated at motor speed of 25 rpm for 4 min. The tablets were dedusted and reweighed.

Disintegration test (DT). DT of the six tablets were evaluated using disintegration apparatus (ERWEKA, Germany). Each tablet was placed in tubes of basket-rack which immerses in a beaker containing distilled water maintained at 37 ± 2 °C. The time taken for each tablet to disintegrate was recorded. The mean and standard deviation were computed.

RESULTS

Starch Yield

The yield of starch from teff grain flour on dry weight basis was $8.36\% \pm 0.04$ (two trials).

Gelatinization degree

The percentage of starch gelatinized at 60 °C, 70 °C, and 80 °C is shown in Table 2. As the temperature was increased, the degree of gelatinization was increased except gelatinization at 80 °C (41.1%) which is slightly lower than that at 70 °C (44.5%). On the other hand the degree of gelatinization of Starch 1500® was significantly higher than pregelatinized teff starches (i.e., 87.7%).

Amylose Content

In this study the percent amylose content of the teff native starch, pregelatinized teff starch at 60 °C, 70 °C, 80 °C, and Starch 1500® were 46.9, 36.8, 37.7, 34.6 and 35.3, respectively (Table 2).

Swelling capacity

Pregelatinization of teff starch increased swelling potential of teff starch up to four times (Table 2). As temperature of gelatinization increased, swelling potential of teff starch increased. Swelling potential of Starch 1500® > pregelatinized starch at 80 °C > pregelatinized starch at 70 °C > pregelatinized starch at 60 °C.

Water Absorption Capacity

Water absorption capacities of native and pregelatinized teff starches are shown in Table 2. The ranking for water

absorption capacity is Starch 1500® > pregelatinized starch at 80 °C > pregelatinized starch at 70 °C > pregelatinized starch at 60 °C.

Granule Density

The granule density of native teff starch was 1.39 g/ml where as the granule density of pregelatinized teff starch at 60 °C, 70 °C, and 80 °C were 1.48 g/ml, 1.51 g/ml and 1.49 g/ml, respectively (Table 2).

Bulk Density, Tapped Density, Carr's index and Hausner's ratio

The bulk and tap densities of pregelatinized teff starches are higher than the native starch. The Carr's index and Hausner's ratio of the pregelatinized teff starches were lower than the native starch (Table 2).

Flow rate and angle of repose

Native teff starch did not flow during flow rate and angle of repose determinations. The angle of repose of pregelatinized teff starches at 60 °C, 70 °C, and 80 °C were 24.9°, 23.2°, and 22.7° respectively. The angle of repose of Starch 1500® was 12.2° which is almost half of the angle of repose of pregelatinized teff starches (Table 2). The flow rate of pregelatinized teff starch at 80 °C was the highest with 70.6 g/sec and Starch 1500® had the lowest flow rate with 40.0 g/sec.

Table: 2 Physicochemical properties of native and pregelatinized starches, mean (SD), n=2

Property	NTS	PTS 60	PTS 70	PTS 80	Starch 1500®
Gelatinization degree (%)	19.2(0)	34.9(0)	44.5(0)	41.1(0)	87.7(0)
Amylose content (%)	46.9(0)	36.8(0)	37.7(0)	34.6(0)	35.3(0)
Swelling capacity	0.59(0.000)	2.31(0.056)	2.56(0.000)	2.76(0.025)	3.11(0.16)
Water absorption capacity(%)	226.3(0.000)	393.0(0.001)	417.3(0.022)	422.0(0.112)	498.3(0.321)
Granule density (g/ml)	1.39(0.080)	1.48(0.005)	1.51(0.040)	1.49(0.005)	1.42(0.055)
Bulk density (g/ml)	0.30(0.003)	0.59(0.012)	0.61(0.013)	0.64(0.002)	0.62(0.014)
Tapped density (g/ml)	0.38(0.002)	0.71(0.008)	0.70(0.016)	0.72(0.008)	0.71(0.00)
Carr's index (%)	21	18	12	12	13
Hausner's ratio	1.26	1.21	1.14	1.13	1.16
Angle of repose(degree)	-	24.9(1.14)	23.2(0.99)	22.7(0.22)	12.2(1.85)
Flow rate(g/sec)	-	51.7(6.084)	50.0(6.787)	70.6(0.830)	40.0(2.678)
NTS= Native teff starch; PTS 60 = Pregelatinized teff starch at 60 °C ; PTS 70= pregelatinized teff starch at 70 °C ; PTS 80= pregelatinized teff starch at 80 °C; “ - ” indicates did not flow					

Tablet characteristics

Hardness: The lowest hardness of tablets was 112.7 N which was from pregelatinized teff starch at 70 °C and 10% usage (Table 3). The highest hardness was 255 N which was recorded from pregelatinized teff starch at 80 °C and 5% usage. In all disintegrants the harnesses of the tablets were higher at 5% than at 10% concentration.

Friability: In the present work tablets containing native teff starch had higher friability than the pregelatinized teff starches (Table 3). The friability of tablets containing native teff starch was greater than 1%. Tablets incorporated

with the pregelatinized forms of starches had friabilites of less than 1%.

Disintegration time: tablets of native teff starch and pregelatinized starches containing 10% disintegrant concentration had faster disintegration times compared to tablets containing 5% disintegrant concentration (Table 3). At 5 % disintegrant concentration, the tablets which disintegrated fastest were those containing Starch 1500® with disintegration time of 16.86 min. At disintegrant concentration of 10%, tablets containing pregelatinized teff starch at 70 °C disintegrated fastest with disintegration time of 7.55 min.

Table: 3 Tablet properties of native and pregelatinized starches, mean (SD)

Starch form	Hardness(N), n=3	Friability(%), n=3	Disintegration time (min), n=6
NTS-5%	233.7(134.3)	1.96	20.14(6.1)
NTS-10%	217.3(51.1)	1.01	11.84(7.2)
PTS 60-5%	207.5(149.2)	0.21	33.75(10.3)
PTS 60-10%	181.3(55.6)	0.75	10.0(4.4)
PTS 70-5%	251.0(5.7)	0.50	33.23(8.6)
PTS 70-10%	112.7(18.0)	0.23	7.55(4.0)
PTS 80-5%	255.0(60.5)	0.17	26.51(7.1)
PTS 80-10%	243.3(73.2)	0.27	11.58(8.0)
Starch 1500 [®] -5%	249.0(66.7)	0.21	16.86(11)
Starch 1500 [®] -10%	180.3(86.9)	0.17	16.20(6.5)

NTS= Native teff starch; PTS 60 = Pregelatinized teff starch at 60 °C ; PTS 70= pregelatinized teff starch at 70 °C ; PTS 80= pregelatinized teff starch at 80 °C;

DISCUSSION

In the present study the yield of starch from teff grain flour on dry weight basis was 8.36%. Due to the smaller particle size of teff starch and high quantity of associated fiber and lipidic components, there was unavoidably some starch loss during extraction. Other studies reported that the starch content of teff grain may be over 70% on dry weight basis⁵.

The amylose content of the native starch was found to be 46.9% which is higher than the amylose content obtained by Bultosa et al⁴ for five varieties of teff (24.9% – 31.7%). The possible reason could be the use of different determination techniques and starch extraction methods. Moreover, the amylose content of native teff starch was higher than the pregelatinized forms. Similarly, amylose contents of native starches of Trifloate yam, rice and corn were found to be higher than their pregelatinized forms¹⁰.

Pregelatinization of teff starch increased swelling potential of teff starch up to four times (Table 2). Starch 1500[®] had the highest swelling capacity compared to pregelatinized teff starches and this could be due to its higher degree of gelatinization. Pregelatinized starches of Trifloate yam, rice and corn showed higher swelling ability, percentage solubility and water absorption capacity than the natural starches¹⁰. Similar to swelling potential of the pregelatinized teff starches, water absorption capacity increased as temperature of gelatinization increased. Water absorption capacity of native teff starch obtained in this study was higher than that of Bultosa et al⁴ which was (103-114%) for five varieties of teff. The possible reasons for the observed differences could be species of teff employed or method of starch extraction used.

In the present study the Carr's index and Hausner's ratio of the pregelatinized teff starches were lower than the native starch. Similarly, Carr's index and Hausner's ratio of pregelatinized starches of sorgum and maize were found to be lower than unmodified starches¹⁶. Also, pregelatinized starches of Trifloate yam, rice and corn had lower Hausner's ratio than natural starches¹⁰. The angles of repose of pregelatinized teff starches were less than 25° indicating that pregelatinized teff starches had excellent flow characteristics. Similarly, the angle of repose of pregelatinized sorgum and maize starches were lower than the unmodified starches¹⁶.

In the present work tablets containing pregelatinized forms of teff starch as disintegrant had lower friability than the

native starch. Contrarily tablets containing pregelatinized starches of white trifoliate yam, yellow trifoliate yam, rice and corn as disintegrants had higher friability than natural starches¹⁷.

Pregelatinized teff starches at 70 °C and 80 °C have nearly similar results in physicochemical tests (Table 2) except disintegration time particularly at 10% disintegrant concentration (Table 3). It could be said that if the aim is to use pregelatinized teff starch as a disintegrant, pregelatinizing teff starch at 70 °C would be recommended.

CONCLUSION

Pregelatinization of native teff starch has improved important physicochemical attributes of the native starch such as swelling capacity, water absorption capacity and flowability (as reflected by angle of repose and flow rate). Moreover, chloroquine phosphate tablets containing pregelatinized teff starches had faster disintegration time (at 10% conc.) and better compressibility (as reflected by lower friability). Pregelatinized teff starches prepared at 70 °C and 80 °C had nearly similar results in physicochemical tests but superior results compared to pregelatinized teff starch prepared at 60 °C. Pregelatinizing native teff starch at 70 °C could be better if the intention is to use as tablet disintegrant.

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