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SEPARATION OF STARCH FROM *ZINGIBER OFFICINALE* (GINGER) AND STUDY IT'S CHARACTERIZATION

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ABSTRACT: *Zingiber officinalis* belonging to family Zingiberaceae is a plant of ancient cultivation that is traditionally used in several disorders. The principal components of ginger reported are gingerols, shogaols, and high content of starch (40 to 60 %). Not much work has been done on the separation of starch from ginger and its characterization. The main objective of this study was to investigate the potential of ginger starch to be used in Food, Pharma, as well as other industries. A simple, easy, convenient, and inexpensive method was designed to separate starch from ginger powder. The isolated ginger starch was then subjected to various physical and chemical characterization tests. The morphology of the starch granules (size and shape) was studied with scanning electron microscopy (SEM), which revealed oval and round forms of starch granules. Dynamic rheological properties of ginger starch were measured using Viscolead viscometer. The Molecular weight of starch was determined by Gel Permeation Chromatography (GPC) (1289858 g/mol). Ginger starch showed a gradual increase in swelling power and solubility index with an increase in temperature. Other Parameters that were evaluated include Gelatinization temperature (80 ± 2° C), pH (5.8), Flow properties, Iron content (Less than 10 PPM), specific surface area (0.69 m^2/g), moisture content (0.17 %).

INTRODUCTION: Starch, the main reserve carbohydrate of various crops, is plentiful in nature and can be easily isolated with high purity and low cost [1]. Starch is formed in the amyloplasts of seeds, grain, roots, and tubers of many plants and provides energy to the plants derived from the sun. Each botanical species has its own characteristic storage pattern of starch granules in each plant tissue, shape, size, structure, and composition [2].

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It is a carbohydrate comprising of a large number of glucose units joined together by glycosidic bonds. Starch is the most commonly consumed carbohydrate, which is present in large quantities in such staple foods as potatoes, wheat, maize (corn), rice, and cassava.

It comprises two types of molecules, as shown in **Fig. 1** amylose which is a linear helical molecule of α-1, 4 glucans with limited branching points at the α-1, 6 positions and the branched amylopectin which comprises of linear chains of glucose units linked by α-1, 4 glycosidic bonds and is highly branched at the α-1, 6 positions by small glucose chains. Starch mainly contains 20 to 30% amylose and 70 to 80% amylopectin by weight.

Starch is an important ingredient that is widely employed in food, textile, cosmetics, plastic, adhesives, paper, and pharmaceutical industries. The various industrial applications of starch are distinctly described by its physicochemical properties [3, 4, 5, 6].

Ginger belonging to the family Zingiberaceae, a perennial herbaceous monocotyledon, usually grown annually and is known as a medicinal and spice crop [7]. Ginger is widely cultivated in India and is a popular spice with significant nutraceutical attributes [8]. Gingerols and the related dehydration products shogaols are the principles of pungent components. Ginger rhizomes have been reported to contain starch between 40 to 60% [9]. Studies have been carried out on the separation of Ginger starch for its use as pharmaceutical excipients. However, not many studies have been done on its characterization. Therefore, the Physical, Chemical, and Functional properties of Ginger starch needs to be studied, which may find suitable Industrial applications.

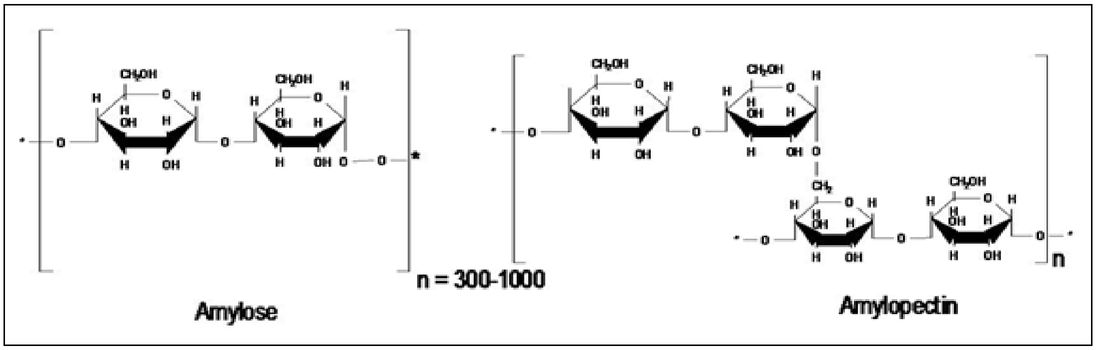

FIG. 1: REPRESENTATION OF AMYLOSE AND AMYLOPECTIN MOLECULE

MATERIALS AND METHODS: Ginger powder was purchased from the local market in Mumbai. Water used was distilled, and other reagents used were of analytical grade.

Methodology:

Separation of Ginger Starch from Ginger Powder: The Ginger powder (250 g) was soaked in water for 15 min. It was then passed through 120# sieve. The filtrate was allowed to settle for 20 min. Starch settles down. The supernatant layer was decanted, and 1 litre fresh water was added to wash the starch grains. This was then further allowed to settle for 10-15 min. Furthermore, water was removed by decanting the upper layer. The sedimented starch was stirred with sufficient distilled water and was centrifuged at 3000 rpm for 3 min to facilitate the removal of impurities. Additionally, to facilitate drying and removal of dirt, starch was washed with methanol two times by centrifugation at 3000 rpm for 3 min. The alcohol layer was removed, and settled starch was subjected to drying in a hot air oven at 60 °C.

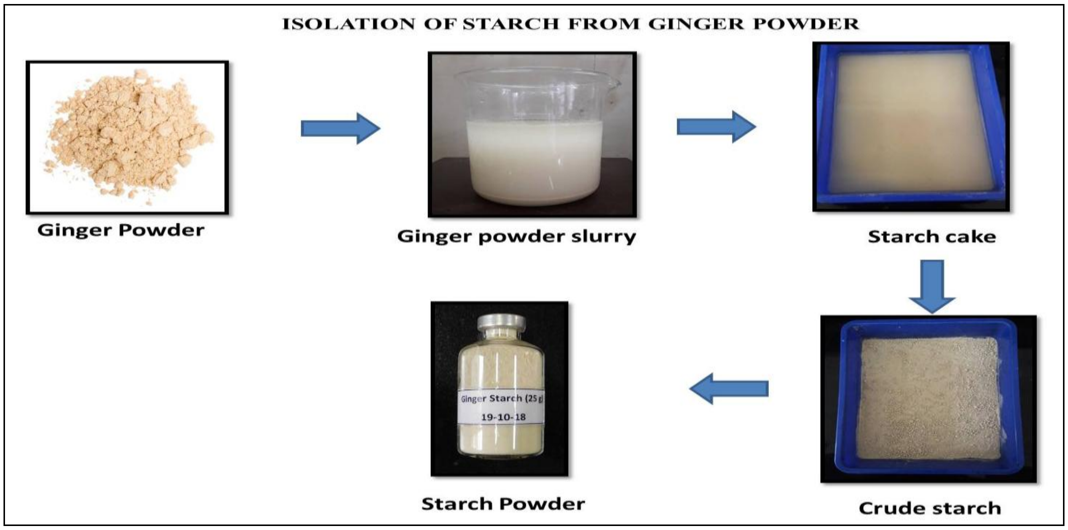

FIG. 2: SCHEMATIC REPRESENTATION OF ISOLATION OF STARCH FROM GINGER

The dried starch was pulverized, weighed, and stored in a sample bottle for further analysis [10]. The schematic representation of ginger starch separation is given in **Fig. 2**.

Physicochemical Characterization of Ginger Starch:

The following Physicochemical Parameters were Studied:

Gelatinization Temperature: The Ginger starch (5g) was put in a 100 ml beaker, and 50 ml water was added with a thermometer suspended in it. The dispersion was heated on a water bath. The gelatinization temperature was recorded by a thermometer suspended in starch slurry [10, 11].

Scanning Electron Microscopy: Scanning electron microscopy was performed, and the photomicrographs of starch granules were obtained at different magnifications (500 X, 1.00 K X, 2.00 K X, 5.00 K X).

Specific Surface Area: Specific surface area of ginger starch was determined by using BET analysis. BET surface area analysis makes use of nitrogen due to its availability in high purity and its strong interaction with most solids. Known amounts of nitrogen gas were released stepwise into the sample cell. The sample was removed from the nitrogen atmosphere after the formation of adsorption layers. The data was presented in the form of a BET isotherm, which plots the amount of gas adsorbed as a function of the relative pressure [12].

Determination of Viscosity: The viscosity of the starch sample was determined by using Viscolead Viscometer (Fungi Lab). Five grams of the Ginger starch sample was added to a beaker with 100 ml distilled water followed by continuous stirring on a water bath. The viscosity was measured at room temperature (30 °C) at 100 rpm for 1 min using spindle no. 6. The viscosity readings were recorded in centipoises (cP) [10].

Swelling Power and Solubility (%): Swelling power was determined at temperatures 55 °C, 65 °C, 75 °C and 85 °C. The starch sample was added in a weighed centrifuge tube containing 50 ml of water. The sample in the centrifuge tube was heated at 55 °C, 65 °C, 75 °C and 85 °C in a thermostatically controlled water bath for 30 minutes with constant stirring. The tube was then cooled at room temperature, followed by centrifugation for 15 min at 3000 rpm. The solubility was determined by evaporating the supernatant and weighing the residue. The swelling power was calculated as follows: [2, 10, 13].

Swelling power = Weight of starch paste / Weight of dry sample

% Solubility = Weight of the soluble starch (g) × 2 × 100 % / Weight of the sample (dry basis) (g)

Water Absorption Capacity: The starch sample (5% w/v) was taken in a pre-weighed centrifuge tube. The tube was shaken in a vortex mixer for 2 min. The supernatant was then decanted, and the weight of the tube and a hydrated sample was noted. The water absorption capacity (WAC) is calculated using the following equation:

$$\text{WAC (g/g)} = m_2\text{-}m_1 / m_1$$

Where m_2 is the weight of sample with the absorbed water, whereas m_1 is the initial weight of the dried sample taken [14].

Determination of Iron Content:

Standard Iron Stock Solution A: This was prepared by dissolving 863.4 mg of ferric ammonium sulfate in water, 10 ml of 2 N H_2SO_4 was added and was diluted with water to 100 ml.10 ml of this solution was pipetted into a 1000 ml volumetric flask, 10 ml of 2 N H_2SO_4 was added, and finally the volume was made with water.

Standard Stock Solution B: 1 µg/ml of iron from standard iron stock solution A in water was taken to prepare Stock solution B.

Standard Iron Solution: 10 ml of Standard iron stock solution B was transferred to a test tube, 2 ml citric acid was added, followed by addition of 0.1 ml thioglycolic acid. 10 N NH_4OH was added until the solution was alkaline to litmus. It was then diluted with water to 20 ml.

Sample Preparation: 1.5 g of starch sample was dissolved in 15 ml of 2 N HCl followed by filtration.10 ml of this filtrate was transferred to a test tube, 2 ml of citric acid, and 0.1 ml of thioglycolic acid were added. This was followed by the addition of 10 N ammonium hydroxide until the solution was alkaline to litmus. Finally, the volume was made with water to 20 ml. [15]

Molecular Weight Determination by GPC (Gel Permeation Chromatography): Molecular weight of the starch sample was determined by Gel Permeation Chromatography.

Equipment used	:	Agilent 1260 Multidetector System
Column	:	PL aquagel-OH 40
Guard Column	:	PL aquagel- OH
Eluent	:	Buffer
Flow rate	:	1 ml/min
Detector	:	Refractive Index detector
Sample concentration	:	2 mg/ml
Sample injected	:	100 µl
Temperature	:	35°C

Moisture Content: 2 g Ginger starch sample was weighed in a tarred crucible and was kept in hot air oven at 105 °C until a constant weight was achieved [16]. The moisture content was calculated by the formula given below.

$$MC = W_0 – W_1 / W_0$$

Where MC is the moisture content and w_0, w_1 are initial and final weights of the starch, respectively.

Sulfated Ash: Crucible was heated to redness and was cooled in a desiccator and weighed. 2 g of starch sample was accurately weighed into a crucible and was heated until the sample was thoroughly charred and was allowed to cool at room temperature. This residue was further moistened with 1 ml sulphuric acid and heated at 80 ± 25 °C until the white fumes of sulphuric acid were no longer evolved, and all-black particles got disappeared. The crucible was cooled, and few drops of sulphuric acid were added and heated. It was ignited as earlier, allowed to cool, and weight was recorded. The process was repeated until two weighing does not differ by 0.5 mg. [16]

TABLE 1: SOME OTHER PHYSICOCHEMICAL PARAMETERS STUDIED

S. no.	Physicochemical Parameters
1	pH
2	Bulk density [4, 15]
3	Tapped density [4, 15]
4	Compressibility index [4, 15]
5	Flowability [15]
6	Moisture Content [16]
7	Microscopic examination of Starch granules [6]
8	Particle size distribution [6]
9	Sulfated Ash [16]

RESULTS AND DISCUSSION:

Granule Shape and Particle Size Distribution: The starch granules of ginger displayed round and oval shapes, as shown in **Fig. 3**. Also, these granules presented an average particle size ranging between 9.9 µm and 56.1 µm.

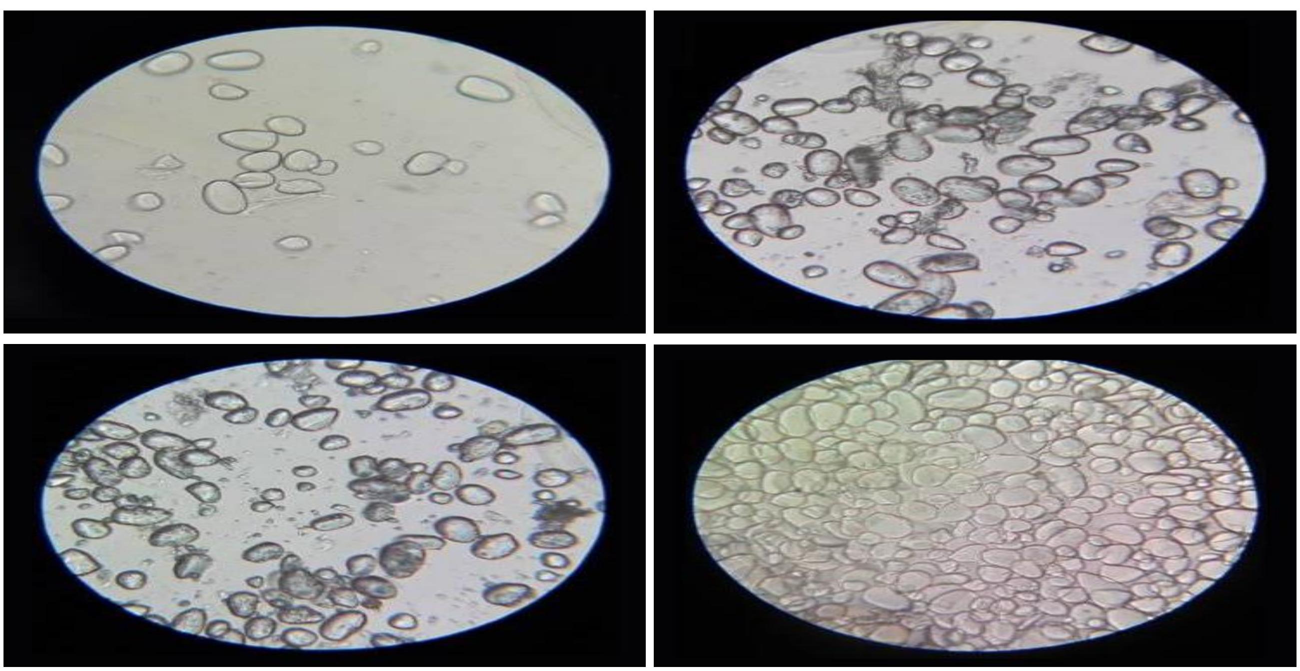

FIG. 3: PHOTOMICROGRAPHS OF STARCH GRANULES UNDER NORMAL LIGHT AT 45X MAGNIFICATION

Scanning Electron Microscopy: SEM of the sample displayed the morphological characteristics of isolated starch granule as shown in **Fig. 4**.

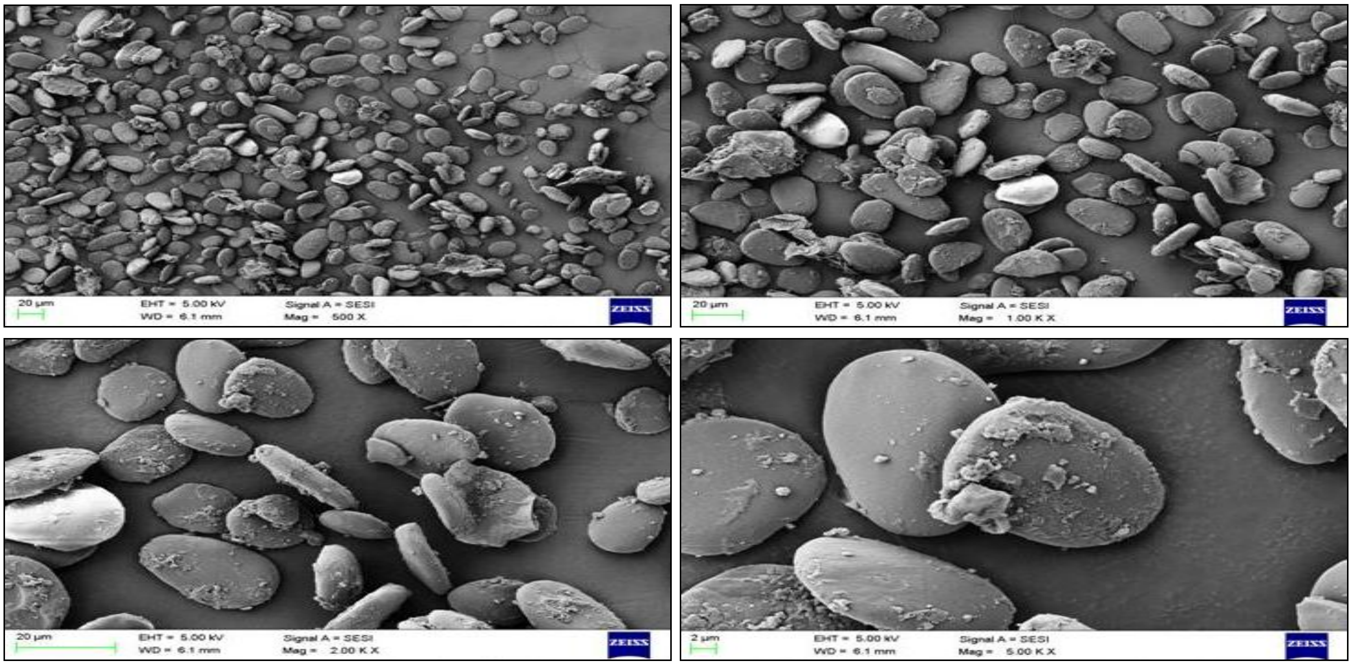

FIG. 4: SCANNING ELECTRON MICROGRAPHS OF STARCH SAMPLE A: AT 500 X; B: AT 1.00 K X; C: AT 2.00 K X; D: 5.00 K X

Iron Content: The pink color produced in the sample solution was less intense than that produced in the standard solution. Therefore, the sample passes the test (Less than 10 PPM of Iron).

TABLE 2: MOLECULAR WEIGHT AVERAGES

Peak	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Mz+1 (g/mol)	Mv (g/mol)	PD
Peak 1	2471463	64590	1289858	2467877	2983272	2377876	19.97

Molecular Weight Determination by Gel Permeation Chromatography (GPC): The Molecular weight (Mw) of Ginger starch determined by Gel Permeation Chromatography was found to be 12,89,858 g/mol. Molecular weight distributions of starch components are presented in **Fig. 5**.

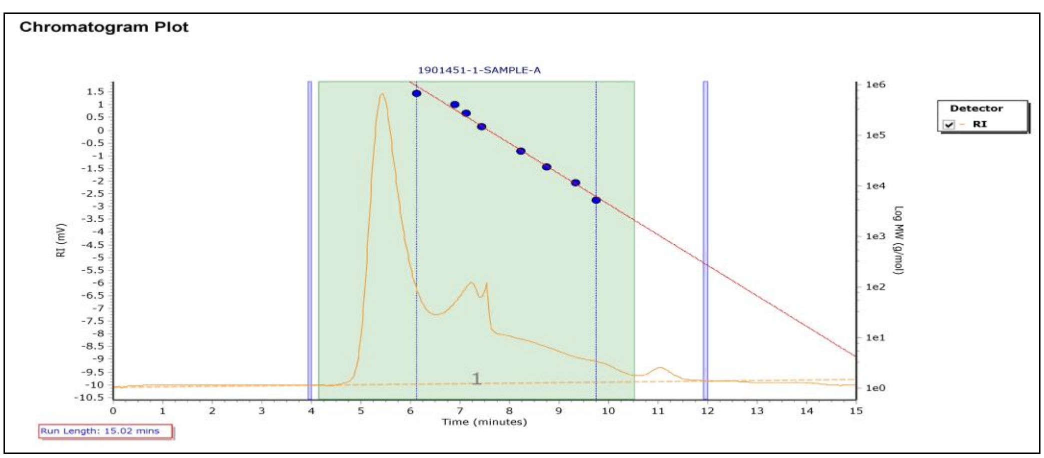

FIG. 5: ELUTION PROFILE OF GINGER STARCH SAMPLE ON PL AQUAGEL- OH 40 COLUMN.

Swelling Power and Solubility Index: The swelling power (SP) and solubility (S) of starches are directly proportional to temperature. Ginger starch exhibited a gradual increase in swelling power and solubility with an increase in temperature, indicating that Ginger starch had weaker internal associative forces, thereby maintaining the granule structure. Swelling power and solubility in starch granules reveal the magnitude of the interaction between starch chains within the amorphous and crystalline domains.

TABLE 3: SWELLING POWER AND SOLUBILITY INDEX

Temperature	Swelling Power	Solubility Index (%)
55 °C	1.24	4
65 °C	2.77	8
75 °C	3.05	10
85 °C	3.46	12

TABLE 4: PHYSICOCHEMICAL CHARACTERIZATION

S. no.	Parameters	Observation
1	pH	5.28
2	Bulk density	0.53 g/ml
3	Tapped density	0.63 g/ml
4	Compressibility (%)	15.87%
5	Flowability	6.5 ± 0.1g/s
6	Gelatinization temperature	80 ± 2°C
7	Moisture content	0.17%
8	Specific surface area	0.69 m^2/g
9	Viscosity	
	Hot Paste Viscosity	1666 cP
	Cold Paste Viscosity	2912 cP
10	Water absorption capacity	1.9 g/g
11	Sulphated Ash	0.5%

CONCLUSION: Starch isolated from ginger has been characterized for various physical, chemical parameters. The shapes of the ginger starch granules by microscopy, SEM analysis were found to be round as well as oval with average granule size in the range of 9.9 µm and 56.1 µm. The physicochemical and pasting properties of Ginger starch revealed that it can be used in food industries as viscosifiers and texturizers in soups, sauces, gravies, bakery and dairy products.

The potential long term applications of ginger starch include as a binder, disintegrant in Pharma industries, as a glue in paper industries, as an absorbent and thickener in Cosmetic industries. This study could provide valuable information pertaining to applications of ginger starch in various other industries.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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