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Effect of different process parameters on starch extraction yield

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Abstract
Starch is the major composition of any cereal crops and present in the form of carbohydrates along with cellulose and other components. Paddy is one of the most commonly growing crop in different states of India such as Uttar Pradesh, West Bengal, Punjab, Telangana, Odisha, Tamil Nadu, Chhattisgarh, etc. In Chhattisgarh paddy cultivation is prime crop among the all groups of farmers and the paddy production in Chhattisgarh is continuously increases from the last decade. In 2021-2022 the overall production of paddy in Chhattisgarh was 7.90 million tonnes whereas in 2020-2021 the production was 7.16 million tonnes. It has been seen that during the milling of paddy, significant amount of broken has been generated. This broken rice are utilized in terms of extraction of starch from different rice varieties. The objective of this study was to optimize the starch extraction process from broken rice using alkaline steeping method. For the extraction of starch from the broken of different rice varieties such as Mahamaya, Swarna and MTU-1010 in different conditions of steeping time (*viz.*,18, 24 and 36 h) and chemical concentration (*viz.*, 0.2, 0.3 and 0.4%) were utilized. The average starch extraction efficiency of Mahamaya, Swarna and MTU-1010 were found to be 68.66%, 74.33%, and 70.33% respectively. The extraction of starch with 0.3% chemical concentration and 18h steeping time was optimized condition for broken of Swarna rice variety. These results indicated that the alkaline steeping method is efficient for starch extraction from broken rice.

Keywords: Starch extraction, broken rice, alkaline steeping method, rice varieties

Introduction
Rice (*Oryza sativa*) belongs to the *"Poaceae"* family and known as major cereal crop and consumed as a staple food by over half of the world's population. Production of rice is very high in developing countries and nations in Asia. Almost 95% of the rice production is done in Asian countries and about half of the world. India is one of the leading countries for rice production and stands in second position in the world next to China, accounting for 22.5% of overall world rice production (Rathna Priya *et al.*, 2019) [6].

In India, the area for rice cultivation comprises about 43,388,000 hectares of land with the production of 127.93 million tonnes in previous year (2021-2022). Literature survey shows that the rice production is continuously increasing year by year from the decades (Rathna Priya *et al.*, 2019) [6].

In Chhattisgarh, the state occupies 13.51 million hectares area with the total cultivated area of the state is 4.78 million hectare and the area under rice cultivation is about 3.7 million hectare which is mostly rain fed, covering both upland and shallow lowland areas. In Chhattisgarh, the average volume of rice production is about 6.12 million hectare from 2009 to 2021. The production of paddy in last year is about 7.90 million tonnes and an average productivity about 1.3 tonnes/ha depending upon the rainfall concentration in Chhattisgarh.

Paddy is the rich source of carbohydrates and moderate source of proteins and fats. Paddy also contains fair amount of B complex vitamins along with minerals such as calcium (Ca), magnesium (Mg), and phosphorus (P) are present along with some traces of iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn). Starch is the major dietary source of carbohydrates and is the most abundant storage of polysaccharide in paddy. Starch is major constituent of rice which comprises almost 75-85%. Starch is majorly composed of two substances i.e., amylose, which is a linear polysaccharide, and amylopectin, which is a branched polysaccharide. Usually in paddy, starch consist of 75% of amylopectin and 25% of amylose content. Generally some paddy varieties such as jasmine, basmati etc., are waxy starch consist of mainly amylopectin and 0-8% amylose whereas some paddy varieties such as Mahamaya, Shankar etc., are high amylose starch consist of 40-70% amylose.

In Chhattisgarh region, commonly paddy production of different varieties are done in two different seasons namely *kharif* in rainy season and *rabi* in summer season. The production as well as quality characteristics of paddy depends on the climatic conditions like rainfall, temperature, humidity etc. during *kharif* and *rabi* season. According to a survey usually, the paddy production is high in *kharif* season, this might be because of enough irrigation water, favourable environment or climatic conditions. The paddy crop grown in optimum environmental condition enables higher milling characteristics. In contrast to these during the summer season (*rabi* season), the rainfall is uneven and environmental conditions are different like higher temperature, lower humidity which is not much favourable for paddy production. Due to varying climatic condition in *rabi* season the quality characteristics of paddy are affected by the uneven temperature and humidity. Rice are broken during milling of paddy grains. Since, grains are passes through the high mechanical impact or shearing impact, where some whole grain rice are crushed and turns into broken rice. In *rabi* season grown paddy varieties, more broken rice are obtained due to the uneven temperature during season. The broken percentage are also depends on the rice varieties and milling conditions upto some extent. In general milling of thick rice variety more broken kernels are obtained than thin rice varieties.

In Chhattisgarh, different paddy varieties are adopted by the farmers for different uses or purposes such as preparation of *Poha* (flaked rice) or *Murra* and flour, which have a considerable value in the market. In Chhattisgarh the different paddy varieties are categorized under thick and thin varieties according to the dimensional measurements. The thick rice varieties includes Swarna, Mahamaya, Shankar, MTU 1010 etc. whereas the thin rice varieties includes Dubraj, HMT, Javaphool, Govindbhog etc. Generally, the thin varieties of rice are preferred for cooking and consumption purposes that make the thin rice varieties popular among the people and poses good market value. The higher market demand of thin rice variety (jeeraphool, dubraj and HMT) poses higher market value. Whereas thick rice varieties (Swarna, mahamaya and MTU 1010) are less preferred for human consumption and consider for animal feed and other purposes that stands for poor market value.

Consumption of broken rice is very lessly preferred by people and having very less market value. For the utilization of broken rice, starch could be extracted from broken rice that could be potential source of starch for numerous industries like food, packaging, cosmetic, chemical, textile, and paper industries. Therefore, a research work has been conducted for the utilization of broken rice by extracting starch. The aim of this study was to examine the effect of different process parameter on starch extraction yield from broken rice. There are several methods for extracting starch from broken rice such as alkaline steeping, enzymatic, and alkaline-protease methods. The starch extraction method plays an important role and the mentioned methods give significantly different characteristics, functional properties and different extraction yields. On the basis of literature survey, the present study was carried out to improve the recovery of starch using alkaline steeping method from different varieties of broken rice.

On the basis of easy or local availability, high broken percentage and lower market value the three different varieties (Swarna, Mahamaya, and MTU 1010) of broken rice were obtained from the agronomy store of department of agronomy I.G.K.V. Raipur.

Materials and Methods

Procurement of materials and chemicals

The brokens of summer-grown rice varieties was procured to be used in the study of the alkaline steeping method of starch extraction. Three varieties, namely Swarna, Mahamaya and MTU 1010, were procured from the agronomy store IGKV Raipur. The chosen varieties were selected because of their easy availability and cost efficiency.

Most of the chemicals used in this investigation were procured from CDH Chemicals, HiMedia Chemicals, Sichem Biotech, Sigma Aldrich, etc. All the chemicals used during the research work were of analytical grade.

Extraction of starch

Extraction of starch from broken rice was carried out using the alkaline steeping method as suggested by Gani *et al*., (2013) [3]. The 100 g broken rice was soaked in 1000 mL (1:10 w/v) distilled water upto 4 h for softening the rice grains and the excess water was drained off using strainer. Further, the softened broken rice were soaked in NaOH solution (1:10 w/v) at different concentration *viz*. 0.2, 0.3 and 0.4% and allowed to stand for 1h to loosening of grains and for breakdown of protein present in rice grains. Subsequently, half of the NaOH solution were drained off and the loosened rice were blended for 5 min using domestic mixture grinder (model: FP 2663, USHA international ltd., 600 W) with occasional stirring and thus rice suspension was prepared. The prepared rice slurry was passed through 100 micron screens to separate the ungrounded broken rice. Further, the uniform suspension was kept for 18, 24 and 36h to precipitate the starch at ambient temperature. The precipitate was collected after draining the alkaline solution and residue was washed to eliminate traces of protein by employing a 0.2% NaOH solution until the disappearance of the yellowish layer. The residue underwent a subsequent washing process with distilled water and followed by addition of 1N HCl solution to neutralize the pH. The suspension was washed again with distilled water and the starch sediments were allowed to settle by employing centrifugation at 6000 rpm for 10 minutes (Remi elektrotechnik ltd. RSCGL). The sediment samples were subjected to a drying process at a temperature of 40°C. The samples were pulverized and stored in zip-lock high-density polyethylene (HDPE) bags until further usage.

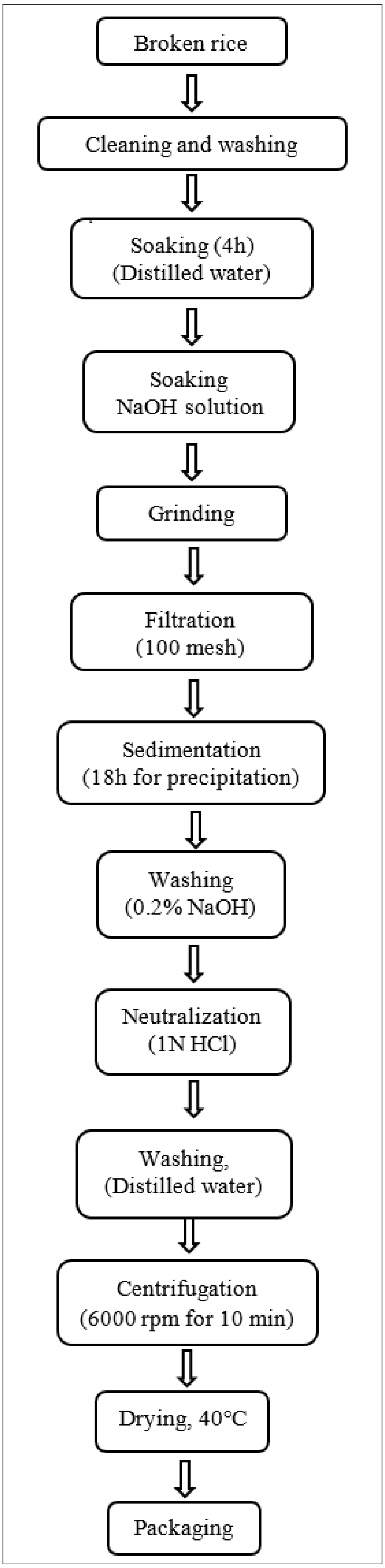


Fig 1: Extraction of starch by using alkaline steeping time

Qualitative analysis

The qualitative analysis is a process of evaluation of various quality characteristics related to product. To assure the different characteristics of extracted starch various tests were performed using standard analytical procedures. The analytical tests involves the detection of starch, absence of protein in the extract of broken rice and determination of some physico-chemical properties.

Iodine test

10 g of potassium iodide crystals were added to 100 mL distilled water and swirled until it fully dissolved. 5g iodine crystals were added to the solution and stirred until all iodine got dissolved. The solution was prevented from exposure to light to avoid the degradation of chemicals. Further, 0.2g of extracted starch from broken rice was taken in a petri dish and 5ml distilled water was added and dissolved completely. One drop of iodine solution was added on the petri dish and the occurrence of any colour indication like blue-black and yellow-orange colour that assures the presence and absence of starch in the extract (Jeyasubramanian and Balachander, 2016) [4].

Fig 2: Iodine test for confirmation of extracted starch

Biuret test

The biuret test is a reliable way to assure the presence and absence of proteins in the extracted starch. To test the absence of protein, broken rice starch solution was prepared by dissolving 1g of extracted starch in 5 mL distilled water in a test tube and stirred frequently. Further, 0.2 mL of 1M NaOH was added to the solution and mixed, followed by addition of biuret reagent and waited for 5-10 min. The occurrence of denim blue and dark or navy blue colour indicates the absence and presence of protein in the extracted starch respectively.

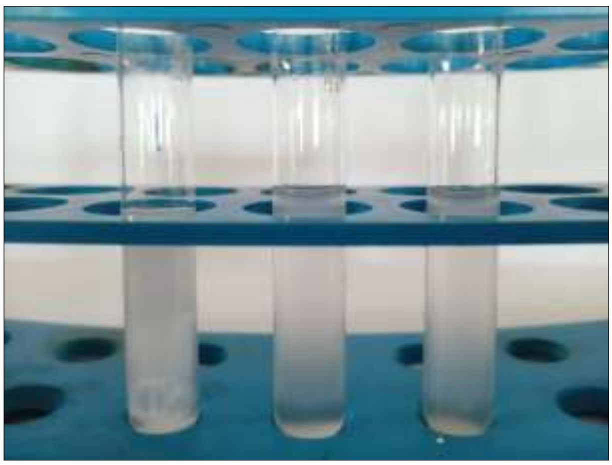


Fig 3: Test for presence and absence of protein in extracted starch

Starch yield

The yield of extracted starch from selected broken rice by alkaline steeping method was calculated by the formula given below (1) as suggested by Williams *et al.*, 2019 [23].

$$Y = \frac{M_S}{M_i} \times 100 \qquad (1)$$

Where,
Y = yield, (%)
M_s = mass of starch obtained after drying, (g)
M_i = initial mass of broken rice, (g)

Moisture content

The moisture content of extracted starch was determined by the method recommended by the AOAC (2010). Initially, 2g of starch sample was taken in a clean petri dish and samples were placed in a preheated hot air oven at 105°C for 24 h. After drying, the final weight of the sample was recorded. The following equation (2) was used to estimate the percentage of moisture content (db) in starch as suggested by the AOAC (2010).

$$M.C. = \frac{M_1 - M_2}{M_1} \times 100 \qquad (2)$$

Where,
M.C. = moisture content (%)
M_1 = weight of sample before drying (g)
M_2 = weight of sample after drying (g)

Ash content

Ash is one of the components in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents like calcium, phosphorus, iron and zinc etc.

An empty crucible was weighed (W_1) and 5g of sample was added to the crucible and weighed again (W_2). The sample was placed in a muffle furnace at 500 °C - 600 °C for 3 hrs and allowed to cool in a desiccator. The weight of the crucible and ash (W_3) was then taken again (Gani *et al.*, 2013) [3].

The ash content was calculated using the following formula.

$$\text{Ash content (\%)} = \frac{(W_3 - W_1)}{(W_3 - W_1)} \times 100 \qquad (3)$$

Where,
W_1 - Weight of empty crucible.
W_2 - Weight of crucible with sample.
W_3 - Weight of crucible with ash.

Amylose content

Starch comprises two constituents, specifically amylose and amylopectin. Amylose represents a linear or unbranched glucose polymer, where the glucose units are connected *via* α -1-4 glucosidic linkages. Amylose exists in coiled form and each coil contains six glucose residues.

Procedure

100 mg extracted starch sample was taken in a beaker and subsequently 1 mL of ethanol was added. Further, 10mL of 1N NaOH solution was added and the solutions were stirred and boiled at 100 °C for 15 minutes on a hot water bath (Digital water bath, UTS: 1.05A). The mixed samples were transferred in a clear volumetric flask and makeup with 100 ml distilled water. Then the 2.5 ml of solution was taken and 20 ml of distilled water was added with 2-3 drops of phenolphthalein indicator. The prepared solution was titrated against 0.1 N HCl until the disappearance of pink colour followed by addition of 1mL iodine reagent and volume was makeup to 50mL. The absorbance was recorded at 590 nm using spectrophotometer (model). A standard curve of amylose solution was plotted between amylose concentration and absorbance at 590nm. For preparation of blank solution, 1 mL of iodine reagent were diluted to 50 mL distilled water.

Fig 4: Absorbance for standard curve at 590 nm

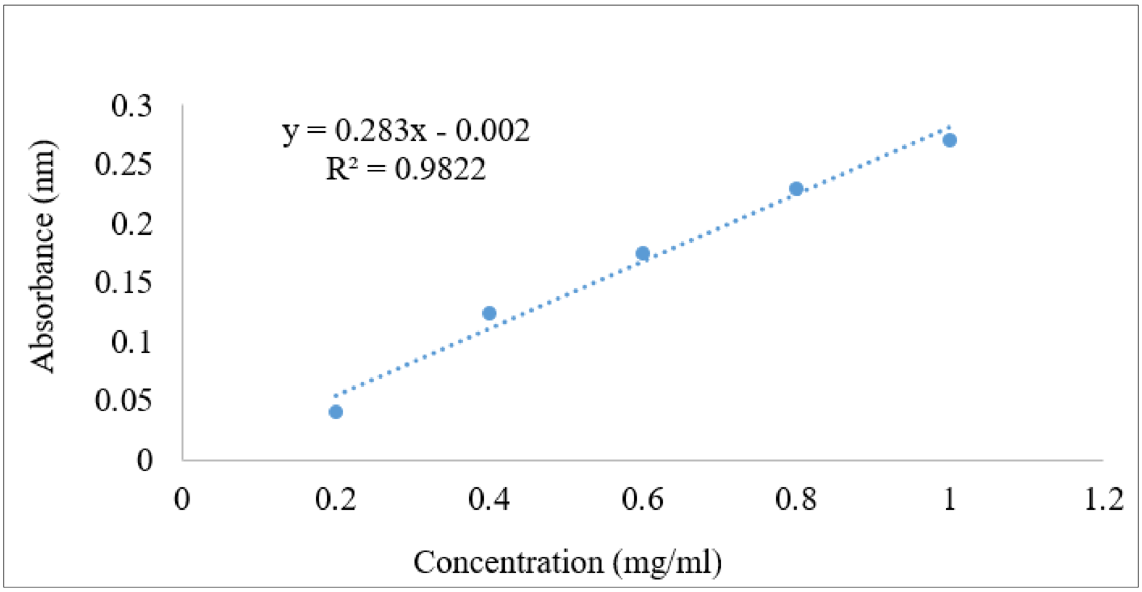


Fig 5: Standard curve for determination of amylose content

Amylopectin

The amount of amylopectin in extracted starch was calculated using the following equation as explained by Abeysundara *et al.*, (2015) [24].

$$\text{Amylopectin} = (100 - \text{Amylose}\,\%) \qquad (4)$$

Water solubility

3 g of extracted starch sample was first dispersed in 100mL distilled water (3% w/v). The solution was heated in a water bath (Digital water bath, UTS: 1.05A) and temperature was maintained at 55, 65, 75, 85 and 95 °C for 30 min. Further, suspension was centrifuged (Remi elektrotechnik ltd. RSCGL) at 3000 rpm for 10 min. The supernatant containing solubilized starch was collected and dried at 120°C to a constant weight and the residue was weighed. The solubility was determined according to the following equation (5).

$$\text{Solubility}(\%) = \frac{\text{Weight of dried supernatant}}{\text{Dry weight of initial starch}} \times 100 \qquad (5)$$

Water absorption capacity

3 g of extracted starch sample was first dispersed in 100 mL distilled water (3% w/v). The solution was heated in a water bath (Digital water bath, UTS: 1.05A) and temperature was maintained at 55, 65, 75, 85 and 95 °C for 30 min each. Further, suspension was centrifuged (Remi elektrotechnik ltd. RSCGL) at 3000 rpm for 10 min. The precipitate was collected and weight of each sample was recorded. Sedimented starch was collected and dried at 120 °C to a constant weight and the dried starch was weighed. The water absorption capacity was determined according to the equation 6.

$$\text{Water absorption} = \frac{\text{Weight of wet pellet} - \text{Weight of dry pellet}}{\text{Weight of dry sample}} \times 100 \qquad (6)$$

Result and discussion

For the utilization of broken rice obtained from different rice varieties were taken and extracted starch content using standard procedures. This section describes the results of various experiments performed to achieve the research objectives. Also, this chapter describes the results of various qualitative analysis.

Extraction of Starch

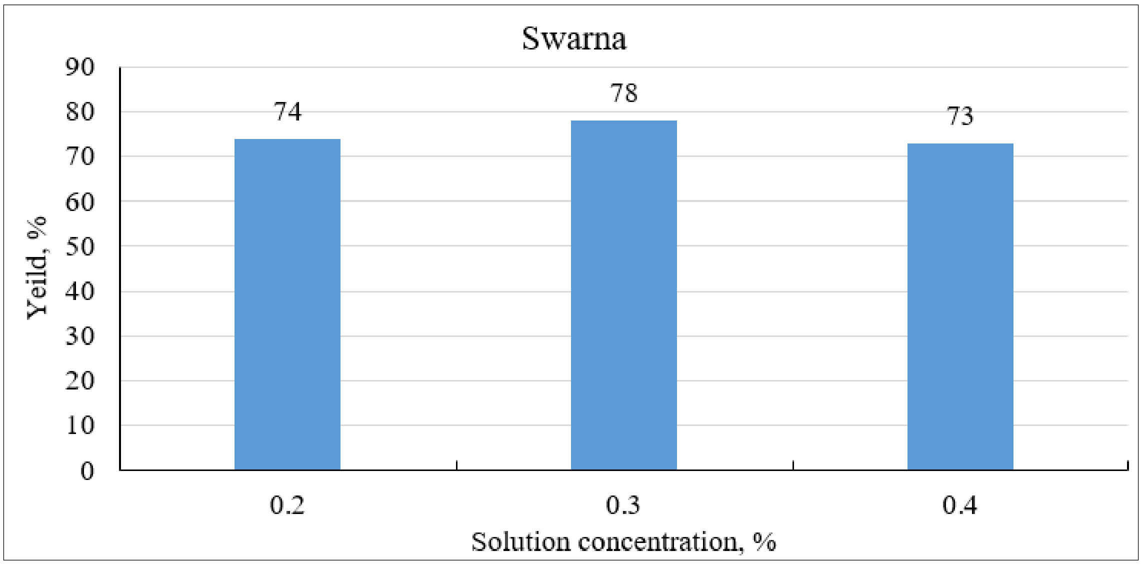
For the extraction of starch from three different varieties of broken rice *viz.* Swarna, Mahamaya and MTU 1010 was selected on the basis of local availability and their broken percentage. To extract the starch from broken rice standard alkaline steeping method was used at different steeping time (18, 24 and 36h) and solution (NaOH) concentration (0.2, 0.3 and 0.4%). From the experiment it was found that starch yield as Swarna rice variety 74, 78, and 73%, while 67, 72, and 67% was recorded for Mahamaya rice variety, similarly, the MTU 1010 rice yielded 69, 76, and 70% at 0.2, 0.3, and 0.4%, solution concentrations respectively. During the experiment, it was noticed that the yield of each rice variety majorly depends on the solution concentration, it might be noticed that by lowering an alkaline concentration, resulting the rice granules did not show significant swelling or rupturing of broken rice, while increasing the concentration may cause the gelatinization and enzymatic hydrolysis of starch molecules. Whereas, at the suitable solution concentration (0.3% NaOH) which is enough for loosen the broken rice granules to release the starch. The yield of Swarna rice variety was recorded as 75.33, 73.33 and 70.33% while, for Mahamaya rice variety, it was recorded as 68, 69.33 and 69.33% similarly, the yield of MTU 1010 rice variety, was recorded as 74, 66.66 and 68.99% at 18, 24, and 36h steeping time respectively. During the experiment, it was noticed that the steeping time has a significant impact on the yield of each rice variety, which might be due to the exposure time of broken rice under the alkaline solution was reduced, that greatly affects the loosening of grain structure resulting partial breakdown of complex molecules including protein present in rice granules. In contrast to this while increasing the steeping time for longer period leads to partial fermentation, fungal growth and enzymatic reactions that affects the quality of starch and extraction process. In addition to this, as the starch is a chain of glucose molecules, and when the breakdown of glucose molecules occurs and stays under the steeping solution for a longer period of time, some fungus and off-flavour was released. Whereas at the optimum exposure time (18 h), rice granule get the enough exposure time that enables the maximum loosening of grain structure that leads to breakdown of complex structure like protein. From the experiment it was observed that the Mahamaya broken rice had the minimum starch yield i.e., 68.66% and the Swarna rice variety has the maximum starch yield i.e., 74.33%. On the basis of the higher starch yield, starch from the Swarna rice variety was taken for further quality analysis.

Starch extraction yield

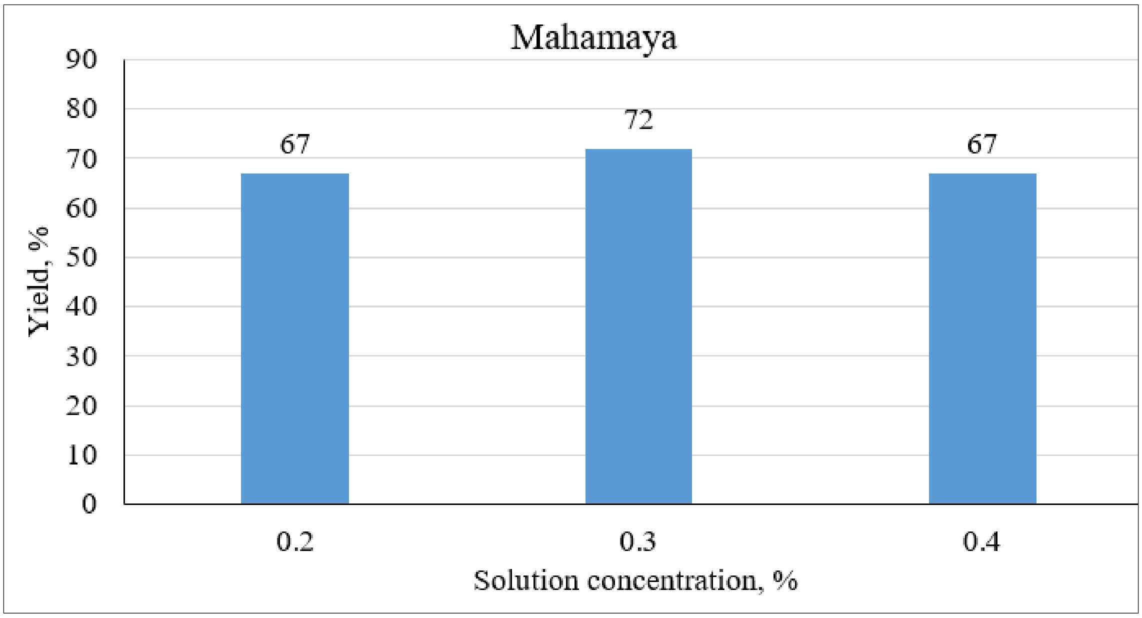
The yield of extracted starch from broken rice by the alkaline steeping method was recorded at different steeping time and solution (NaOH) concentration.

Table 1: Starch extraction yield at different alkaline concentration

S. N.	Rice Variety	Solution concentration (%)	Yield (%)
1.	Swarna	0.2	74
2.	Mahamaya		67
3.	MTU-1010		69
4.	Swarna		78
5.	Mahamaya	0.3	72
6.	MTU-1010		76
7.	Swarna		73
8.	Mahamaya	0.4	67
9.	MTU-1010		70



a)



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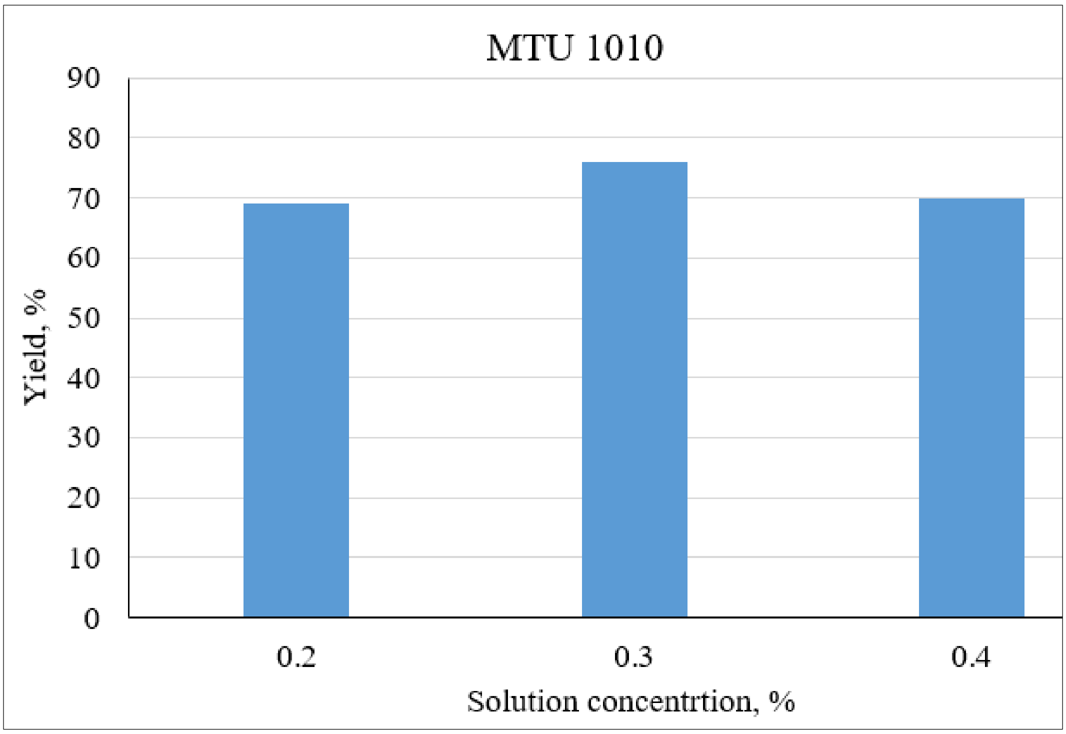
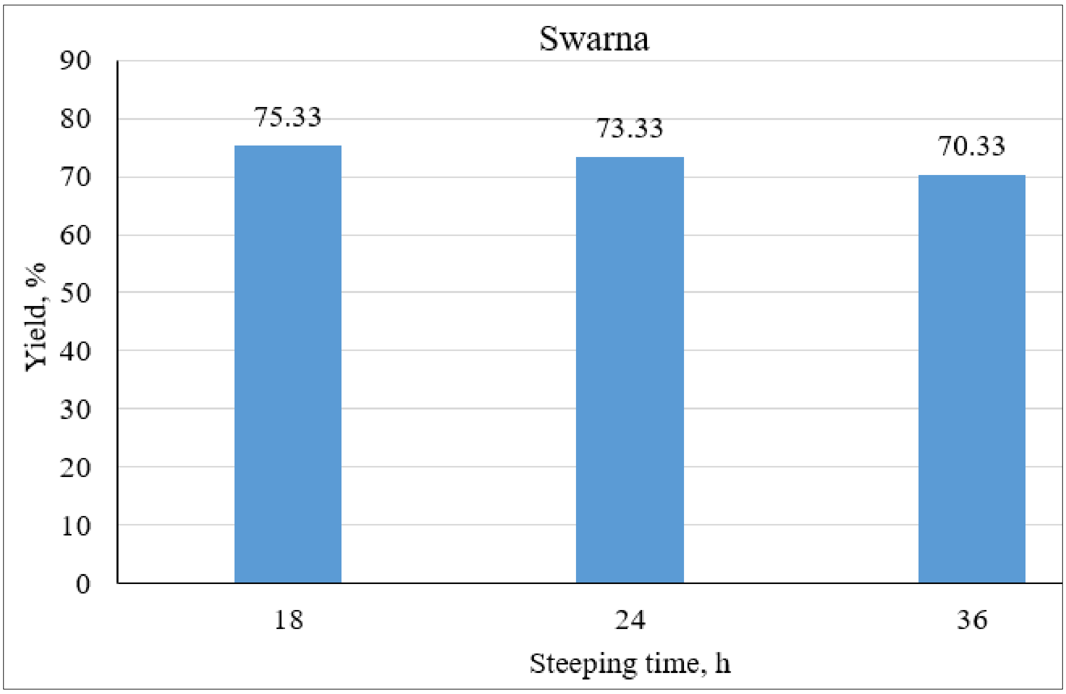


c)

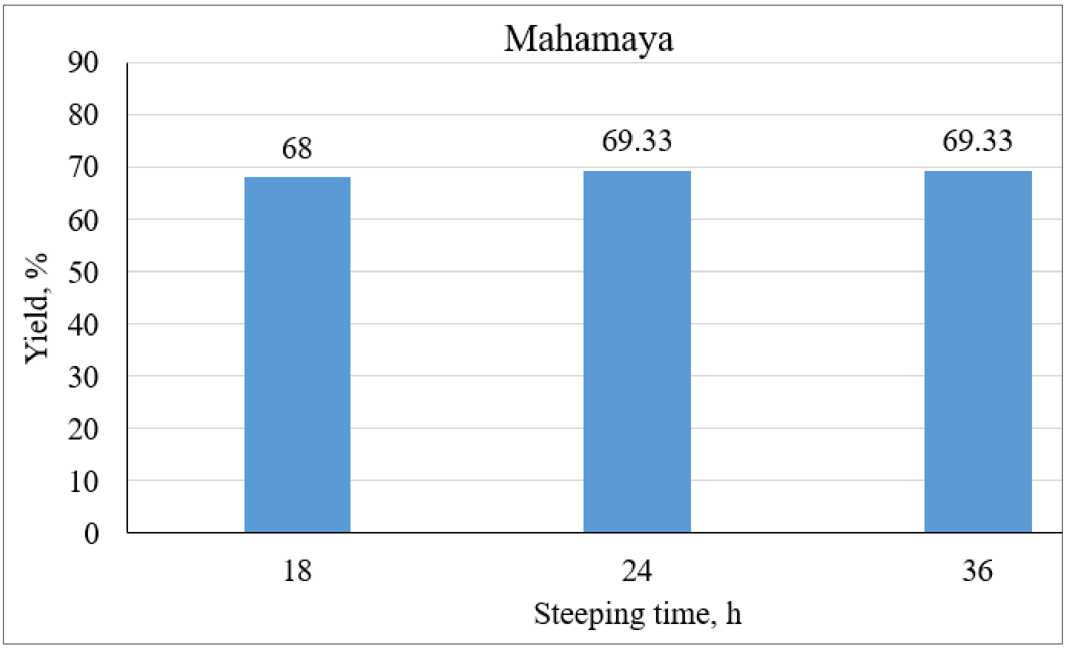
Fig 6: Graph A, B and C shows the yield of each variety of broken rice i.e., Swarna, Mahamaya and MTU 1010 respectively, at different solution concentration

Table 2: Starch extraction yield at different steeping time

S. N.	Rice Variety	Steeping Time (h)	Yield (%)
1.	Swarna	18	75.33
2.	Mahamaya		68
3.	MTU-1010		74
4.	Swarna		73.33
5.	Mahamaya	24	69.33
6.	MTU-1010		66.66
7.	Swarna		70.33
8.	Mahamaya	36	69.33
9.	MTU-1010		68.99



a)



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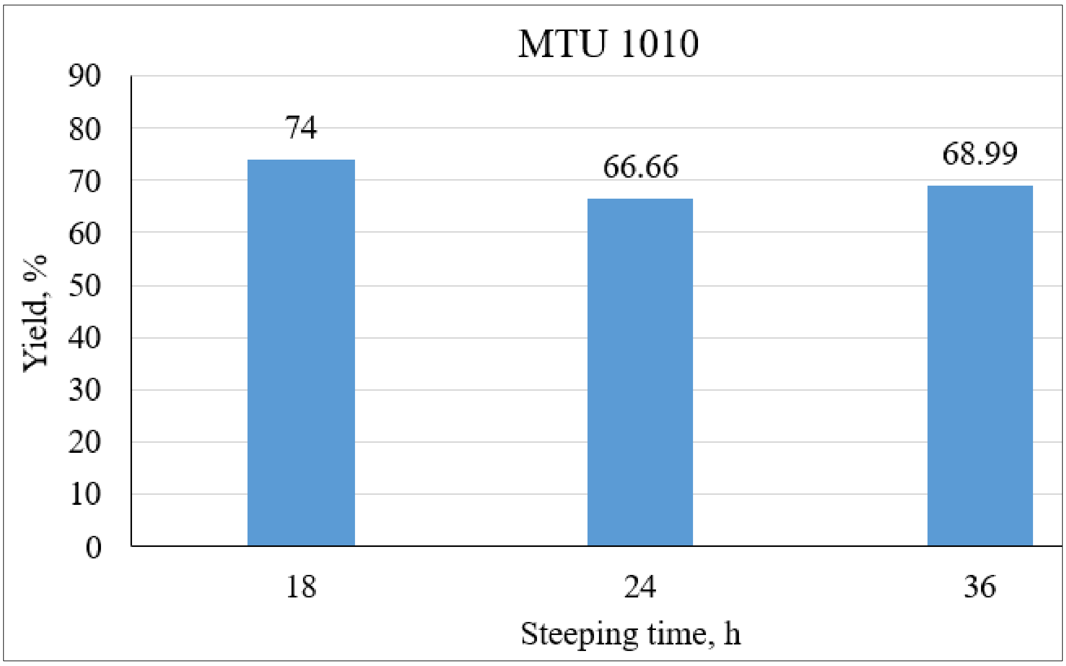

c)

Fig 7: Graph a, b and c shows the yield of each variety of broken rice i.e., Swarna, Mahamaya and MTU 1010 respectively, at different steeping time

Starch analysis

The starch test was done by iodine reagent to check the obtained starch was positive or negative in extracted starch from broken rice. During the experiment, the occurrence of Blue-black colour was detected during the analysis that indicates the presence of starch in extracted broken rice starch.

Moisture content

The physicochemical properties of starch are affected by its high water content. The water content of the extracted rice starch was recorded as 7.67±0.3% on a dry basis. Similarly, 7.95±0.24% moisture content of extracted starch on a dry basis was reported by Souza *et al.*, (2016) [15].

Ash content

The ash content indicates the presence of inorganic elements in salt form. The presence of ash in broken rice starch was 0.841±0.097%. Souza *et al.*, (2016) [15] studied the rice starch obtained from different method and their ash content was lower than the range from 1.81±0.05 to 3.25±0.20% and Lawal *et al*., (2011) [9] also reported as the ash content of rice starch was high and ranging from 1.47±0.0 to 1.81±0.0%.

Amylose content

The amylose content was investigated of extracted broken rice starch. The average percentage of amylose in extracted broken rice starch from Swarna rice variety was found to be 22.84%. The selected rice had intermediate amylose content

(20 to 25%). A similar result was found by Lawal *et al.*, (2011) [9] which estimated the amylose content varied from 22.88 to 24.48%.

Amylopectin content

The percentage of amylopectin found in extracted broken rice starch was 77.16%.

Water Solubility: The temperature affected the solubility of starch granules in water. The solubility of extracted rice starch in water was calculated at various temperatures from 55, 65, 75, 85 and 95 °C and their mean was recorded as 2.56, 3.39, 6.12, 13.74 and 20.17%, respectively. The solubility of starch is temperature dependent, as an increase in solution temperature leads to an increase in starch solubility, as shown in Fig. 8, and it might be possible due to an increase in solution temperature, which weakens the inter-granular structure of starch.

Table 3: Water solubility and water absorption capacity of extracted starch from Swarna rice variety

S. No.	Temperature (°C)	Water solubility (%)	Water absorption capacity (%)
1.	55	2.56	75.33
2.	65	3.39	80.67
3.	75	6.12	105.00
4.	85	13.74	139.33
5.	95	20.17	194.67

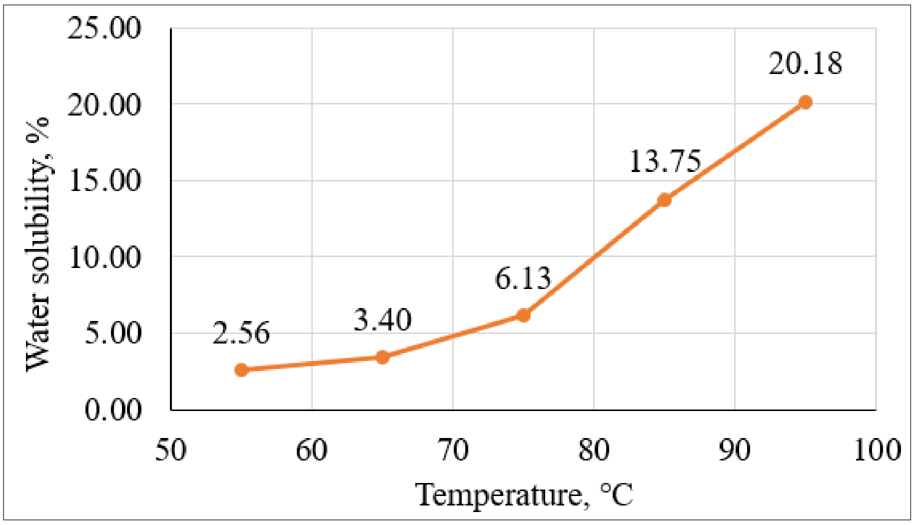

Fig 8: Effect of temperature in water solubility

Water absorption capacity

The water absorption capacity of starch is a crucial property that impacts its behaviour and functionality in various usage, especially in the food industry. This property refers to the ability of starch to absorb and hold water under specific conditions. The water absorption capacity of extracted rice starch in water was calculated at various temperatures from 55, 65, 75, 85 and 95 °C and their mean was recorded as 75.33, 80.67, 105.00, 139.33 and 194.67% respectively. The water absorption capacity (%) of the extracted starch is temperature dependent as the water absorption capacity increases with increase in solution temperature. It might be due to the increase in temperature, resulting gelatinization of starch.

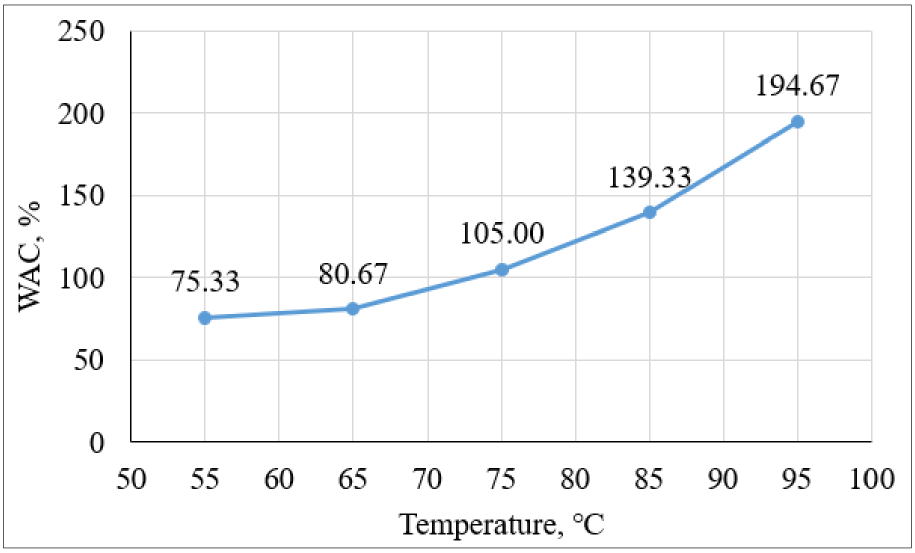

Fig 9: Water absorption capacity

Conclusion

The study was focused on the effects of different process parameters on starch extraction yield. The starch extraction was carried out using the alkaline steeping method at different NaOH concentrations of 0.2, 0.3, and 0.4% and different steeping times of 18, 24, and 36h. The result showed that the Swarna rice variety yielded the maximum starch of 74.33%, compared to Mahamaya and MTU 1010 which is 68.66 and 70.33% respectively.

The moisture content of the extracted rice starch was recorded as 7.67% while it was dry. The average percentage of amylose in the extracted broken rice starch from the Swarna rice variety was found to be 22.84%. Starches with a higher amylose content tend to form more rigid gels compared to those with lower amylose content. The Swarna rice variety has a good amylose content, making it a good source of thickening agents in sauces. The percentage of amylopectin found in extracted broken rice starch was 77.16%. A good amount of amylopectin in Swarna is helpful in the textural, thickening, and stabilising characteristics of its starch, making it valuable in a wide range of applications in the food industry. The solubility of extracted rice starch in water was calculated at various temperatures from 55-95 °C in the interval of 10 °C and their mean was observed as 2.56, 3.39, 6.12, 13.74 and 20.17%, respectively. The water absorption capacity increase with respect to the temperature

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