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Study on enzymatic hydrolysis of sal (*Shorearobusta*) starch to dextrin

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ABSTRACT

Food starch processing technology has been acquiring much importance in making value-added food products by appropriate transformation reaction of starch molecules. The present study has been conducted on enzymatic transformation ofstarch isolated from Sal DOC with commercial thermostable α-amylase enzyme to form dextrin. The enzymatic process was carried out at 85°C at pH 6.8 for the production of dextrin with α-amylase. The final product i.e. dextrin was 8.13% when measured with 0.05N iodine solution at 620nm.

Keywords: Sal DOC, Starch,α amylase, Enzymatic process, Dextrin.

INTRODUCTION

In most tropical countries like India, calories are derived mostly from carbohydrate (mainly, starch) based food products. Thus, starch holds the opportunity to be utilized in increasing calorific value of food products. Isolation of starch from different food grains (such as rice, corn, millets, legumes etc.) have been performed but very few literature is available for isolation from non conventional oilseeds such as Sal seeds. Sal constitutes about 13 to 15% of the total forest area. Sal meal is the major byproduct (85to87%) in Sal seed extraction plant. It is estimated that in India the production of Sal seeds is about 5.7 million tonnes per year from which a total annual production of 5 million tones of de-oiled Sal meal could be estimated. The proximate composition shows higher percentage of carbohydrates and other constituents [1]. The information regarding isolation of starch from Sal meal is inadequate. Protocol for isolation of starch mainly follows two steps: (i) steeping and (ii) treatment with acid or alkali. But the isolation is based upon plant tissue structure and composition of different raw materials. Modification has become an integral part of food starch technology with the introduction of biocatalysis [2, 3]. Different approaches have been considered for the transformation of starch by hydrolysis process with acid and enzyme. Enzymatic hydrolysis is preferred over chemical treatment due to its substrate specificity and toxic compound production in lesser amounts. Enzymes (α- amylase) purified from bacterial sources or commercially produced are considered for transformation of starch to valuable end products [4, 5, 6].

Enzymatic hydrolysis is employed for the production of dextrin. Dextrin can also be obtained by dry heating (pyrolysis), which is called pyrodextrin and it also can be achieved through acid hydrolysis with hydrochloric acid. Dextrin are low molecular weight products occurred through partial hydrolysis of starch [7]. Due to their non-toxicity they have found widespread use as thickening agent in food processing industries. Dextrin are marketed to several food segments and offer marketing opportunities with their excellent properties of solubility, film forming, and adhesives in many foods including the new growth areas such as snacks, whole grains among others. Other than industrial purposes, it also offers many health beneficial functions. The digestion resistant property of dextrin investigated recently, provide controlled glycaemic index, weight control, effect on lipid levels and other degenerative diseases [8]. This paper deals with the transformation of starch by enzymatic hydrolysis principle with

aid of enzyme α-amylase to create low molecular weight carbohydrate i.e. dextrin from a non-traditional oilseed Sal starch.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical grade chemical i.e. Hydrochloric acid, Copper sulphate, Sodium bicarbonate, Sodium potassium tartarate, FolinCiocalteau, Anthrone, Potassium permanganate, Sodium hypochlorite, Potassium iodide, Elemental iodine and solvents were obtained from SRL, E. Merck India. Commercial α amylase is obtained from, SPEZYME® XTRA, Denmark. Sal (*Shorearobusta*) de-oiled cake is obtained from Progressive Exim Ltd., Raipur, India.

Proximate analysis of Sal De-oiled Cake

Determination of moisture Content

The moisture content was determined according to AOAC method [9].1 gm of salde-oiledcake was taken in a nickel dish and the dish was placed in a hot air oven at 110°C for 3 hours. After 3 hours, the dish was kept in desiccators and weighed.

Determination of total ash content

5 gm of salde-oiledcake was taken in a silica crucible and placed in muffle furnace at 550°C for 6hours.After 6hours, it was cooled in desiccators and weighed AOAC method [9].

Determination of crude fiber content

2 gm ofsalde-oiledcake material was subsequently treated with 0.255(N) $H_2SO_4$and 0.313(N) NaOH. After the treatment, the residue was dried, cooled in desiccators and weighed to determine the crude fibre content [10].

Determination of total carbohydrate content

0.1 gmsalde-oiledcakewas acid hydrolyzed and the total carbohydrate content wasobserved by using Anthrone reagent by measuring absorption at 630nm[11].

Determination of total protein content

Total protein content of de-oiled Sal Doc was estimated by FolinCiocalteau process [12].

Determination of tannin content

The tannin content of Sal De-oiled Cake was determined by Lowerthal's method [13].

Isolation of starch from Sal De-oiled Cake

Sal (*Shorearobusta*) De-oiled Cake was obtained from PROGRESSIVE EXIM LTD. Raipur, India. The process of isolation of starch from de-oiled Sal meal was carried out according to Bhattacharya et al [1]. Properly sized Sal meal was subsequently treated with 5% NaOH and 0.1N HCl and the refining were done with sodium hypochlorite. The obtained refined starch was dried in vacuum oven for further use.

Analysis of starch isolated from sal de-oiled cake

The composition of isolated starch namely, amylose, starch, was done by using methods recommended by AOAC [9].All the observations were done by using UV-VIS spectrophotometer(JASCO V-630 UV-Vis Spectrophotometer, India).

Determination of total amylose content

Known amount of sample was taken, to which distilled water and phenolphthalein was added dropwise. 0.1N HClwas added drop wise and the absorption was measured by adding iodine reagent at 590nm [14].

Determination of total starch content

About 1 gram of starch was taken and 10 ml of distilled water was added, it was boiled for 3 minutes in hot plate. The boiled solution was diluted to 100 ml. The solution was then centrifuged at 6000 x *g* and the supernatant was taken for analysis. The OD value of starch was measured at 680 nm. The concentration of the starch was determined from the standard curve [11].

Enzymatic hydrolysis of isolated starch from Sal De-oiled Cake

The hydrolysis of Sal (*Shorearobusta*) starch was carried out by using commercial amylase enzyme. The endo-amylase obtained from SPEZYME® XTRA, Denmark randomly hydrolyzes α-1, 4-glucosidic bonds to quickly reduce the viscosity of gelatinized starch, producing soluble dextrin and oligosaccharides under a variety of process

conditions [7, 15]. One gram of sample i.e. Sal (*Shorearobusta*) starch was weighed and to it 9ml of distilled water was added. The suspension was heated for 3 minutes to gelatinize the starch. After the boiling was completed, the volume was made upto 100 ml. The pH of the gelatinized starch slurry was adjusted to 6.8 with the addition of phosphate buffer. Thermostable*α- amylase* from genetically modified strain of *Bacillus licheniformis*was added to 0.4 ml to the starch slurry. The temperature of the slurry was adjusted to 85°C with continuous mild agitation until dextrin was formed. The incubation period was maintained for 10 mins. After the completion of hydrolysis, the hydrolyzed starch was centrifuged at 6000 x *g* for 15 min (R-24 Microprocessor Research Centrifuge, REMI, India). The dextrin production was measured by using 0.05 (N) iodine in UV-VIS spectrophotometer at 620 nm.

Statistical analyses

The data evaluated for each sample is done in triplicate. The analysis data is done with ANOVA one-way analysis with probability of 0.05($p \leq 0.05$). The mean of the sample are done with standard deviation of ± 0.01. Microsoft Excel software is used for evaluation of results. For the differences reported significant, a confidence level of 95% was considered.

RESULTS AND DISCUSSION

Raw material composition

The raw material composition of Sal De-oiled Cake was determined and from the results obtained it was observed that Sal De-oiled Cake contains high amounts of total carbohydrate, which is near about 52%. It also contains appreciable amounts of tannin i.e.14.3%, which is the marker of antioxidant properties of Sal De-oiled cake but the presence of tannin also caused obstacle in isolation of starch. There is 8.5 % protein in Sal De-oiled cake and thus it can be used as a protein rich food. The ash content was very low around 4.6% whichindicates low mineral content. Sal De-oiled Cake also contains crude fiber in moderate quantity i.e. 6.5%.The original composition of Sal De-oiled Cakewas moisture 9.6%, protein 9.2%, ash 3.1%, crude fiber 9.9%, and total carbohydrate 58.4%[16].The obtained result was as per to the original composition and is represented graphically

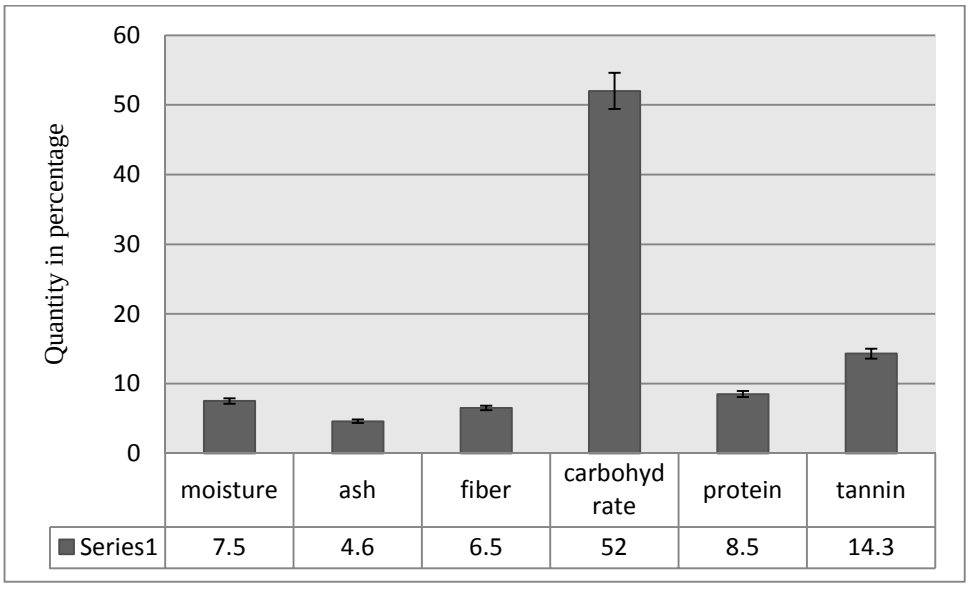

Figure 1: Proximate analysis of de-oiled Sal DOC

Analysis of starch isolated from Sal De-oiled Cake:

Fig 2 described that Sal De-oiled Cake contained 79.66% i.e. very high amount of starch. This indicates that starch was isolated in purified form with moderate to negligible amounts of other constituents

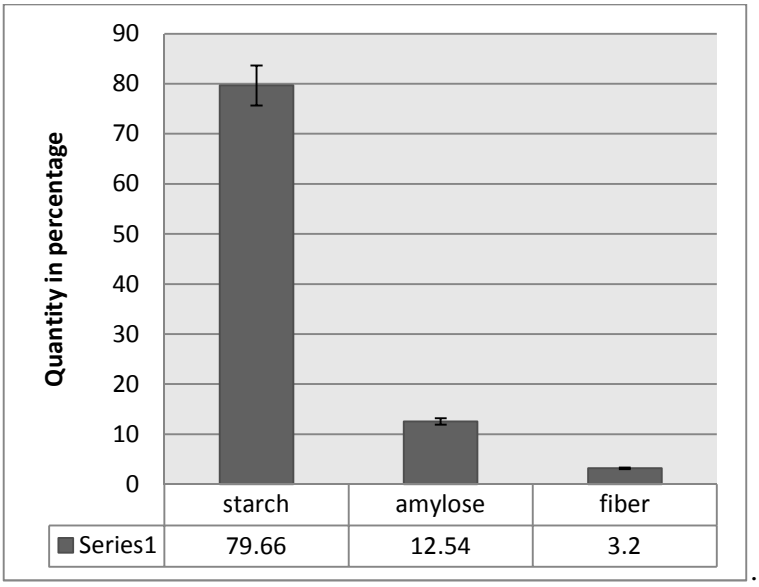

Figure 2: Composition of starch isolated from de-oiled Sal meal

(amylose i.e. 12.54%, 3.2% of fiber and in negligible amount tannin and protein). The isolated starch should be devoid of fiber content but to increase the food value of Sal starch, and the many other beneficial effects of fiber, it was not isolated from the starch [2, 3].

Enzymatic hydrolysis of isolated starch from Sal De-oiled Cake:

Partial hydrolysis of starch forms low molecular weight carbohydrate i.e. dextrin, which offers opportunity in enhancing functional and nutritional importance of food products. The starch obtained after treatment of Sal De-oiled Cake as per the process adopted according to reference [7], it was further subjected to enzymatic hydrolysis with α amylase enzyme at pH 6.8 with phosphate buffer. The dextrin content was measured with 0.05 (N) iodine solution by taking the hydrolyzed starch aliquot in an interval of 30 minutes at 620nm in UV- VIS spectrophotometer. The unknown solution of dextrin was calculated from standard curve of dextrin.

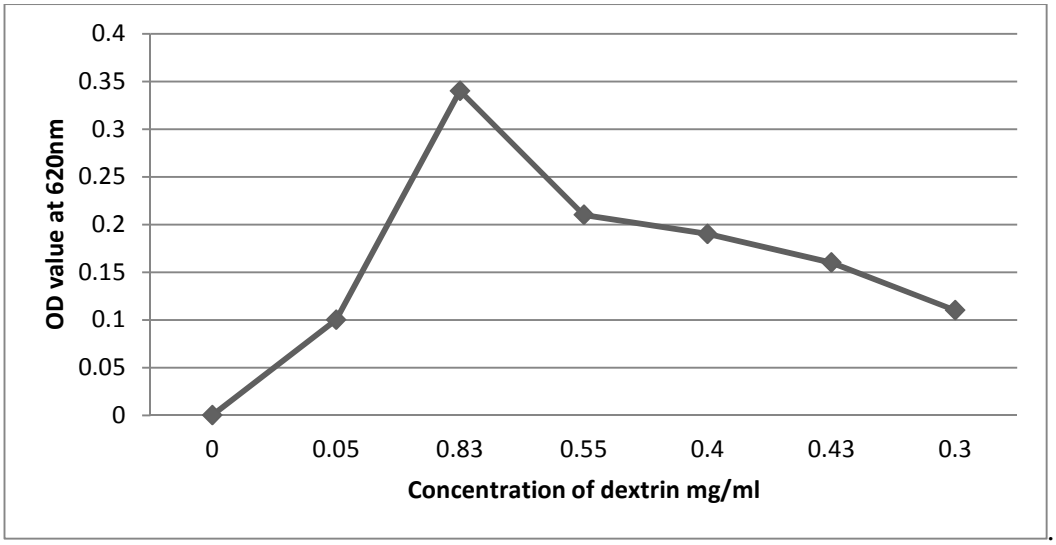

Figure 3: Dextrin concentration obtained from hydrolyzed Sal starch. Sample evaluation is done in triplicate. Values are calculated as Mean±SD (n=3)

The calculated amount of dextrin obtained after partial hydrolysis of Sal starch was found to be 8.13%. The percentage of resultant quantity of dextrin may be improved or the yield may be enhanced with the removal of fiber. Thus, more critical investigation is required for improving the yield.

CONCLUSION

Sal De-oiled Cake is starch enriched product and its use as food is very limited till date. The scope of process development for production of dextrin using enzymatic hydrolysis will increase the economic feasibility of Sal De-oiled Cake and will help to formulate value added products. Dextrin is an important food product from nutritional point of view. Thus, from this investigation it is concluded that modification of Sal starch by enzymatic conversion process holds promise but a more critical investigation is needed to increase the productivity of dextrin.

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