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A Systematic Approach to Standardizing the Isolation Process and Characterizing Starch from *Chenopodium album*

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Abstract: Being novel and small in structure 3-4mm there have been always a scarcity in studies of methods for starch extraction from *Chenopodium album*, therefore in this study the process was optimized and modified to get better starch yield and purity. To get better yield of starch process was optimized by combination of alkaline steeping and alcohol precipitation method. Alcohol precipitation method resulted in higher starch yield from 24 to 28 % in Pusa1 and from 18 to 21 %in IC415477. Also, purity and proximate analysis of extracted starches verified application of optimized extraction process. Transmittance level was 40% lower for Pusa1 from IC415477. Both the viscosity and pasting temperature were lower for IC415477. Water absorption capacity was higher (125%) for pusa1 then 110% for Ic415477 and lower for Oil binding capacity (225%) then IC415477 (248%). The variation observed in yield and purity value is because of the varietal differences. These all analyzed reports showed potential application for *C.album* starch as thickener in food formulation where high viscosity is required and also an economic, safe and environment friendly material.
General Terms: Chenopodium album, starch.

Keywords: *Chenopodium Album*, Viscosity, Starch.

Research Paper

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INTRODUCTION

Chenopodium album belongs to genus *Chenopodium* which comprises around 150 species, out of them *C. album* and *C. quinoa* are rich in nutrition. *C. album* is an edible wild weed which is most widely distributed in the world. It is valuable, summer annual, fast growing, underutilized, pseudo cereal which is rich in numerous nutritional properties. *C. album* is cultivated as a leafy vegetable, grain crop for human as well as animal food-stuff due to high-protein and a balanced amino-acid contents [1]. In India *C. album* is known as Bathua in Hindi, and notorious by many diverse names in different parts of India, Cultivated throughout the India majorly in Kullu, Shimla, Punjab, and Rajasthan. Its seeds are rich in Starch and small with diameter ranging from 1 to 2.5 mm and can grow in areas with low rainfall of 300 to 400 mm have proven the focus of attention in many scientific studies. Recently, the demand for gluten free food products due to their health benefits to reduce risk of allergic reaction and celiac disease. Hence, due to hypo allergic property and absence of gluten in *C. album* the demand of *C. album* starch for formulation of gluten-free food products is coming into considerations [2].

The wide industrial application of starch, specially modified starch, makes it well preferred for a wide variety of foods. It has been estimated that the overall production of starch will be 1.25 million tones by the year 2020 and the Asian pacific market are going to be biggest contributor with CAGR 8.8% throughout period [3]. Starch contributes to morphological, rheological, thermal, adhesion, binding and textural properties of products that are of major interest in food industry, but for storage, handling and transport, mechanical phenomena such as friction or flowability are important as well [4]. The rheology of bulk powder can be measured with shear tester known as yield loci**.** Commercially starches are majorly extracted from cereals such as wheat, corn, maize and different variety of rice and from Pseudo cereals such as adzuki beans, *quinoa*, *Amaranthus* etc. and from roots also such as potato, cassava. *C album* starch shows gel texture with hardness which shows ample intrinsic properties promoting the applications in food and non-food products.

MATERIALS AND METHODS

Raw Material

Chenopodium album (Pusa1) seed was obtained from the Indian Agriculture Research Institute (IARI) Delhi and *Chenopodium album* (IC415477) seeds were obtained from the local farmers of Sangrur, Punjab, India. Deionized water used for the analysis was

obtained from the Millipore LXR. All other chemicals used for the analysis was obtained from Sigma Aldrich Mumbai.

Methods

Alkaline Method

Industrial level extraction of starch from different sources is carried out mostly by alkali method in which the extraction of starch is done either directly from seeds or seed flour. The same process was followed for extraction of starch from *Chenopodium album* in which *Chenopodium album* grains were steeped into solution of NaOH and varied from the range of 0.5 to 1.5N. Double deionized water was used during steeping. For the optimisation of starch extraction method sample was steeped from the range of 12-48 hour under different concentration range of sodium hydroxide at centrifugation speed of 5000rpm.

Alcohol Separation Method

A new method practised to overcome the problems observed in existing Alkali method for extraction of pure starch from *Chenopodium album* with high yield. *Chenopodium album* seeds (100 g) were steeped in 100 ml of NaOH (0.25 g/100 ml) at 4 °C for 24 h. Double deionized water was used during steeping. After steeping the supernatant was decanted from the container. The steeped grains were again ground in a grinder (wet grinding). The paste obtained from grinding was mixed with (1:5 ratio) water to form a slurry. The slurry was filtered through 240 and 300 sieve, with muslin cloth over it. The filtrate was centrifuged at 5000 rpm for 10 min. After centrifugation overnight soaking of the sample resulted in separation of starch and protein, clear demarcation between the layers was observed.

Physiochemical Properties Characterization

Proximate

Moisture content, protein content, total starch and pasting properties were determined according to AACC Approved Method 44-15A, 46-13, 76-13, 76-30, and 61-02, respectively (AACC 2000). Starch purity calculated as total content by excluding protein, fat, fiber and Ash from total extracted starch.

Water and Oil Absorption Capacity

Water and Oil absorption capacity for extracted starch was determined by Anderson [5], method with little modification. In this method samples were prepared by mixing of 5g starch samples with 75ml oil for oil absorption and 50ml water for water absorption, agitating the same for 1 hr. Agitated starch sample then centrifuged at 3000 rpm for 10 min. then free oil and free water was drained properly and weight of residues calculated.

Amylose Content

Amylose content of starch samples was determined by a precise and improved colorimetric method of Morrison) [6]. In which a distinction between apparent amylose and total amylose is made. In this method 10ml UDMSO solution (Solution of Urea and DMSO (Dimethyl Sulfoxide) in 1:9 ratio) mixed with 70gm starch sample and heated for 10 min at 100°C along with continuous stirring. This mixed composition sample fist incubated for 1 hour at 100°C and then cooled to room temperature. A solution with 25ml distilled water with 1ml I-KI solution (0.2 g Iodine (I) and 2 g Potassium Iodide (KI) in 100 mL distilled water and 2 ml UDMSO was made as blank sample. Another sample by subsequent addition of 0.5ml of above mixed incubated sample (Starch-UDMSO) with 25ml distilled water and 1ml I-KI Solution (Iodine (I) and Potassium Iodide (KI)) was made. Absorbance of solution was taken after 15 min of addition of I-KI solution at 635 nm. The spectrophotometer was balanced with water in reference cell and UDMSO-I-KI Blank sample in sample cell.

Amylose content = 28.414 x Blue value

$$\text{Blue Value (\%)} = \frac{\text{Absorbance} \times 100}{2 \times g \text{ solution} \times \text{mg starch}} \times 10$$

pH value and Bulk Density

The pH value of starch solution is determined by measuring the potential difference of two immersed electrode. The Bulk density of starch sample calculated by filling of venkle's design standard graduated cylinder (100 ml) (Standard Instrument Corporation, Patiala, India). Starch sample was filled up to 100 ml mark of cylinder. At this initial point, sample was weighed to calculate Aerated Bulk density.

Swelling Power

In this method starch slurry (2g/100ml dry basis) was prepared in glass tubes. The slurry was heated over a temperature range of 55, 65, 75, 85, 95 °C for 30 min. after cooling the tubes were centrifuged at 112 ×g for 20 min (C24, BL; M/s. Remi laboratory industries, Mumbai, India). The supernatant was carefully decanted, evaporated and dried in Petri plate at 105°C. The residue was measured for swelling power estimation.

Pasting Property

Pasting Properties of both variety of starch samples were analysed by Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia). In RVA, Pasting characteristics of starch sample were obtained through Viscoamylogram in terms of peaks for viscosity, total set back, breakdown, and final viscosity. An aluminum canister containing suspension of 3.0±0.2 g starch (12% moisture) and 25 ml distilled water was mounted on RVA. The RVA was programmed with heating and cooling cycles with a constant speed of stirrer at 160 rpm. Starch samples were hold at 50 °C for 1 min followed by progressive heating at 12 °C/min to 95 °C and held for 2.5 min. Samples were than cooled down at same rate of 12 °C/min to 50 °C and held for 2 min. Viscoamylogram were then analyzed to study pasting characteristics of starch samples.

Statistical Analysis

Statistical analysis was done by using Statistica-log software package version 7 (M/s. StatSoft Inc., OK, USA). The significant differences were obtained by a one-way analysis of variance test (ANOVA) followed by Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

Starch Yield, Purity and Proximate

The starch yield and purity of IC415477 and Pusa1 variety are shown in Table 1. Significant variation of yield in starches was observed. Starch yield was observed [7], to be vary from 8.65 to 24 and 4.3 to 16.89% for pusa1 and IC415477. Purity of starches was observed in the range of82 to 87% for IC415477 and Pusa1. Maximum yield of starch in alkaline method at 0.25 N NaOH for 24hour steeping time was evaluated on the basis of protein, moisture and fiber [8]. Due to absence of ash content it is not mention in table. Alkaline steeping condition was less effective compare to alcohol precipitation method. In case of alcohol precipitation *Chenopodium album* starch yield was increased up to 28% in pusa 1 and 22% in IC415477. The difference between yield of starch is may be because of the varietal difference and harvesting of these seed from different geological region.

Table 1

Time (h)	Concentration (%)	Starch Yield (%)		Moisture (%)		Fiber (%)		Protein (%)		Carbohydrates (%)	
		Pusa 1	IC415477	Pusa 1	IC415477	Pusa 1	IC415477	Pusa 1	IC415477	Pusa 1	IC415477
12	0.05	8.30a	3.00a	10.55a	10.12a	2.24a	2.43a	2.25a	4.36a	84.96a	83.10a
	0.25	9.74b	4.35bd	10.18b	10.19b	2.05b	2.44b	2.43a	5.40a	85.35ab	81.98bc
	0.45	9.00c	4.43b	10.24b	10.06a	2.08ab	2.39bd	2.94a	5.46a	84.76ab	82.11c
	0.65	7.91ad	3.75c	10.14b	10.26c	2.50ab	2.32c	2.96a	5.52a	84.41ab	81.90b
	0.85	7.61d	3.25a	9.16c	9.61d	2.23b	2.18cd	3.53a	5.56a	85.09ab	82.66d
	1.5	8.45a	4.00cd	9.89d	9.74e	2.41b	3.32c	3.48a	5.51b	84.22b	81.44e
24	0.05	22.35a	11.86a	9.55a	9.71a	1.88a	2.11a	3.03a	4.11a	85.55a	84.08a
	0.25	24.80b	12.15a	8.84b	9.88b	1.14b	1.06a	2.21b	4.09b	87.82b	84.98b
	0.45	16.05c	16.80b	9.02c	9.53c	1.76cd	2.17ab	3.20c	4.13ac	86.03c	84.18a
	0.65	17.75d	15.51c	10.25d	10.36d	1.36cd	2.16a	3.27d	4.10ac	85.13d	83.39cd
	0.85	18.70e	13.30d	10.44e	10.16e	1.70de	2.19bc	3.48c	4.21cd	84.39e	83.45c
	1.5	19.10f	13.01d	10.27d	10.29d	2.03e	2.23c	3.64e	4.18d	84.07e	83.31d
36	0.05	14.99a	12.24a	10.71a	10.76a	1.64a	2.27a	4.75a	5.20a	82.91ac	81.78a
	0.25	15.67ab	13.41b	10.56b	10.53b	1.41b	2.36b	5.01b	5.42b	83.03bc	81.70b
	0.45	17.51b	15.75c	10.75c	10.76a	1.56a	2.37c	4.83c	5.50b	82.86	81.38c
	0.65	17.87b	13.00d	10.49d	10.52b	1.48ab	2.35d	4.89de	5.55b	83.16c	81.58d
	0.85	17.22ab	10.89e	10.82e	10.86c	1.53ab	2.35e	4.90cd	5.60ab	82.77b	81.21e
	1.5	17.95b	11.08f	10.80e	10.82c	1.46ab	2.34e	4.88de	5.54ab	82.87bc	81.30f
48	0.05	14.80a	8.57a	10.93a	10.91a	1.77a	2.40ab	3.20ad	5.36a	84.10a	81.34a
	0.25	14.88ab	10.63b	8.34b	8.36b	1.23bc	2.42ab	3.42b	5.37a	87.02b	83.86b
	0.45	15.05bc	9.51c	9.05c	9.06c	1.41ac	2.45ab	3.24c	5.40a	86.31c	83.10c
	0.65	15.06bc	8.75a	9.55d	9.52d	1.76c	2.51a	3.51a	5.46bc	85.19e	82.52a
	0.85	15.11c	7.55dd	9.32e	9.35e	1.76abc	2.53ab	3.37ad	5.30c	85.56d	82.83d
	1.5	15.14c	7.61d	9.23ce	9.34e	1.85c	2.56b	3.49d	5.35c	85.44de	82.76d

Amylose Content

Amylose content is the condensation product of D-glucopyranose from alpha 1,4 glyosidic bond, which makes long glucose linear chain of variable degree of polymerisation and molecular weights up to 106 Daltons [9]. Amylose content of *C. album* starches differs significantly ($p \leq 0.05$) with higher mean value of14.21 g/100 g inPusa1 followed by 16.71 g/100 g in IC415477 [10]. Amylose content affects the functional and physicochemical properties of starch, including its pasting, gelatinization, retrogradation and swelling characteristic. Also the factors such as botanical sources, climatic conditions, harvest time and different types of soil during cultivation affects the variability in amylose and amylopectin ratio within the same species.

pH Value and Bulk Density

The pH values for extracted starches were almost similar with *C.album* IC415477 starch having slightly lower pH (6.43) then pusa1 starch pH (6.09). The pH values of starch play an important role in deciding conversion rate of starches into Dextrin. There was a significant difference observed in extracted starches.

The Bulk [11]. Densities of extracted starches varies significantly. Bulk density plays an important role in deciding material handling [12], and packaging requirements of starch in food industry. The BD values for IC415477 was (0.39 g/ml) which is lower than the Pusa1 (0.41 g/ml). High Bulk density signify its maximum volume reduction which may be result of polygonal shape, fine texture and similar particle size having closer packing of particles.

WBC and OAC

Water absorption for food product can be defined as an index of the maximum amount of water absorbed and retained by product important to increase digestibility and softness of food product [13]. The values of WAI and WSI observed were higher for Pusa1 125% variety than IC415477 110%. Capacity of oil absorption is an important parameter for food product in terms of energy and nutrients density for infants and

teenagers [14]. Values for OAC was higher (248%) in IC415477 than Pusa1(225%). In food products where oil holding capacities is an essential parameter this high oil-binding capacity for IC415477 will be useful for such food formations.

Swelling Power

Swelling power of the *IC415477* starch ranged from 8.82, 8.86, 8.94, 9.30, 9.34 g/g whereas, in case of pusa1 the swelling power was found to be 10.15, 10.18, 10.25, 10.32, 10.36 g/g. The swelling power of starches is the representation of properties of amylopectin content while amylose [15] act as diluent. Higher value of Swelling Power in Pusa1 might be due to presence of High amylopectin content that dominate starch swelling power. Thus, the ratio of amylopectin and amylose in starch and the way it is arranged, affect swelling power of starches.

Transmittance

Transmittance reflects clarity of starch solutions. Pusa1 (8.78, 6.74, 5.23, 5.10, 4.93)% showed higher transmittance than IC415477 (3.73, 3.70, 3.62, 3.49, 3.36)%. The clarity of starches plays an important role in selection of [16], starch for use as thickeners. Transparent thickeners are always preferable in food industry which can be used as thickener for food pie filling. Whereas [17], opaque thickeners also have applications in salad dressing. Opaqueness in I415477 may be due to refraction of light by swollen granules. The transmittance level of starches show inverse relationship with storage period but transmittance for IC415477 decreased at slower rate than Pusa1 which may be due to lower tendency for retro gradation. The clarity of starch solution attributes different [18], factors like amylose, amylopectin, chain length, granule size and granule swelling.

Pasting Properties

RVA pasting curve was used for calculation of pasting parameters of pusa1 and IC415477 starches. There was significant difference in pasting properties. IC415477 showed lower Peak viscosity than Pusa1. Break down viscosity (BD) (measure of the resistance of starch paste to heat and shear) and set back (SB) viscosity which indicates the tendency of starch paste to retrograde were found to be lower for IC415477 starch in comparison to *C.album* starch.

Pasting temperature (PT) which indicates the minimum temperature required to cook was lower for IC415477 starch (79.15°C) than Pusa1 starch (80.05°C). Aggregation of the amylose molecules has been considered to be the reason for increase in final viscosity [19]. Higher PV, TV and FV of pusa 1 starch than IC415477 starch indicates its capability to resist swelling, rupture towards heat and shear and its suitability for products requiring high elasticity and gel strength.

CONCLUSION

Starch extracted from IC415477 and Pusa1 from alkaline steeping showed higher yield value at 24 hour of steeping and 0.25N NaOH in comparison toother parameter of steeping and concentration of NaOH. After alkaline method the fiber content of the starch was higher because of the strong interaction between starch and fiber which need to get week, for the higher yield and purity fiber should get dissolve in the solution to which alcohol will act like a bridging liquid. Hence, alcohol precipitation method was used. Problem faced during extraction of starch from *Chenopodium album* with alkali method was removal of *Chenopodium album* husk and due to its small and fine nature. In the case of *Chenopodium album* interaction between starch and protein is very strong due to which extraction of pure starch from *Chenopodium album* becomes very difficult. Alcohol precipitation weakens the interaction between starch and fiber which gives higher yield and purity of starch and showed lower residual protein for both variety.

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