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Extraction, modification, and characterization of starch from two barley (*Hordeum vulgare* L.) cultivars

Shweta Dhiman[1], Krishan Kumar[2*], Naseer Ahmed[1*], Tajendra Pal Singh[1], Divya Chauhan[1], Sachin Kumar[1], Paras Sharma[3], Ajar Nath Yadav[4,5]

[1]Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, India.
[2]Department of Food Technology, Rajiv Gandhi University, Doimukh, Arunachal Pradesh, India.
[3]Department of Food Technology, Mizoram University, Aizawl, India.
[4]Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, India.
[5]Research and Innovation Cell, Rayat Bahra University, Mohali-140301, Punjab, India.

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ABSTRACT

Barley (*Hordeum vulgare* L.) is ranked fourth amongst the cereals after maize, rice, and wheat and is mostly used for malting and animal feed. The use of barley in processed food products has gained popularity in recent years. The majority of the kernel or more than 70% of its dry weight is made up of starch. The native starch is unstable to a wide range of conditions such as temperature, pH, shear forces, and so on, and thus, limits its usage to a wide range of food and non-food industrial applications. The objective of this study was to modify the starch isolated from two cultivars of barley, i.e., PL-751 and PL-830 using physical (annealing) and enzymatic (amylase) methods and to investigate the differences in functional properties of native and modified starch. The functional properties such as syneresis, light transmittance, swelling, and solubility index changed significantly in modified starches as compared to native starch. The syneresis of annealed starch for PL-751 and PL-830 increased from 85.5%–96.67% and 81.0%–94.50%, respectively. The syneresis values of amylase-treated starch for PL-830 and PL-751 at enzymatic concentration of 7.5 U increased from 86.5%–92.0% and 84.5%–89.83%, respectively. Similarly, the values increased from 88.17%–91.83% and 79.5%–87.5% for PL-830 and PL-751 at 15 U enzymatic concentration, respectively. The light transmittance of annealed starch for both PL-830 and PL-751 decreased significantly ($p \leq 0.05$) during storage for 5 days. The swelling and solubility index also increased significantly ($p \leq 0.05$) with an increase in temperature both in native as well as modified starch. However, a significant ($p \leq 0.05$) drop in swelling and solubility index of an annealed and enzyme-modified starch was observed as compared to native starch. Starch modification is a dynamic field with vast potential for future applications in both the food and non-food industries. The physical and enzymatic modification in starch enhanced the thermal stability, swelling power, and solubility index of the starch with reduced syneresis characteristics resulting in stable gels and a better shelf life. With the help of modification techniques, minimally processed foods can be developed with an increase in consumer demand. Modified starch helps in improving the shelf life of food products by enhancing moisture retention, and inhibiting retrogradation, and thus, the texture and freshness of the product are maintained for a longer period of time.

1. INTRODUCTION

Cereal crops are a fundamental part of the human diet, providing vital nutrients and energy, and are also the main ingredient in livestock feed. Barley, scientifically known as *H. vulgare*, is a grass that belongs to the family Poaceae, and the genus *Hordeum*. It is an important cereal crop and ranks fourth worldwide after wheat, rice, and corn. Presently, it is mainly used for livestock feeding and little is used for the consumption of humans and value-added products [1,2]. In India, barley is cultivated in nearly 7.604 lakh hectares of area with a total production of 13.30 million metric tons with productivity of 1,888 kg/ha [3].

Whole grain of barley contains nearly 70% starch, 10%–20% protein, 2%–3% free lipids, 2.5% minerals, 11%–34% dietary fibres, and 5%–10% β-glucan depending upon the type of variety [4]. Starch is a naturally occurring substance in most plants and is the energy storage house in green plants. Barley is utilized as a source of starch because it is a very versatile crop containing a high amount of starch and has large starch granules as compared to other cereal crops [5]. Starch in its natural or native form has some undesirable characteristics that make its application limited to a wide range of applications in the food and non-food industry. These industries show interest in the utilization

**Corresponding Author*
Krishan Kumar, Department of Food Technology, Rajiv Gandhi University, Doimukh, Arunachal Pradesh, India. E-mail: krishan.kumar @ rgu.ac.in; Naseer Ahmed, Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, India. E-mail: naseerfst @ gmail.com

of starch due to its abundance, cheapness, biodegradability, and non-toxic behavior [6].

Native starch is inert, water-insoluble, and retrograde with syneresis and is unstable to temperature, pH, and shear forces [7]. Therefore, they are subjected to various modifications such as physical, chemical, or enzymatic methods to enhance their functionality and overcome their shortcomings [8]. The physical methods use thermal and non-thermal techniques whereas chemical modification introduces functional groups in starch molecules using derivatization reactions such as esterification, etherification, cross-linking, and modifications involving the use of enzymes [9]. Modified starch can be used as a binding agent, gelling agent, thickener, and emulsifier in bakery and confectionary sectors [10]. Physical modification has gained acceptance over a few decades due to its low cost, green (no use of chemical reagents), effective characteristics, improved starch applicability, and enhancement in specific properties of starch molecules depending on its applicability [11–13]. Modification by annealing starch from various barley cultivars increased gelatinization transition temperature and decreased gelatinization temperature Waduge *et al.* [14].

The use of enzymatic modifications is being adopted partly replacing physical and chemical modification as enzymes are cleaner, safer, more accurate, and healthier options than opting for chemical ways for the modification of starch [15–17]. One of the main objectives of enzymatic modification is to enhance the functional characteristics and nutritional value of the product for broader industrial applications by reducing starch molecules into different oligosaccharides [18,19]. In food industries, modified starch is used as a food additive to regulate the uniformity, stability, and texture of soup and sauces, inhibit syneresis, and increase the shelf life of products [5,20]. It can also be used as a binding agent, gelling agent, thickener, and emulsifier in bakery and confectionary sectors thus providing the desired consistency and mouth feel [10,21,22]. Modified starch can also help in improving the shelf life of food products by enhancing moisture retention, and inhibiting retrogradation thereby maintaining the texture and freshness of the product for a longer period [23].

The novelty of this research is to create tailored ways to modify starch and develop various innovative ways to enhance starch functionality. These techniques can help to enhance the functional characteristics of starch such as gelatinization temperature, freeze-thaw stability, thermal stability, and water-holding capacity thereby elevating specific characteristics of a starch for making value-added products.

2. MATERIALS AND METHODS

2.1. Materials

The two barley cultivars, i.e., PL-751 and PL-830 used in the current research were procured from Eternal University, Baru Sahib, Distt Sirmaur, HP. The chemicals used were bought from standard companies such as Hi-Media, SRL, and Sigma.

2.2. Physicochemical Characteristics

The physiochemical assessment of two cultivars of barley, i.e., PL-751 and PL-830 were carried out at the laboratories of Eternal University, Baru Sahib, Distt Sirmaur, HP, India. The moisture content was estimated by following the air oven drying method [11]. The crude protein content was estimated using Kjeloplus (Pelican Inc.), crude fiber by Fibroplus (Pelican Inc.), and crude fat using Soxoplus (Pelican Inc.). The ash content was measured as per the method described by Ranganna [24]. The total carbohydrates were calculated by deducting the sum of moisture, protein, fat, fiber, and ash content out of 100. The β-glucan content was estimated as per the method described by Vizhi and Many [25]. The color of all samples involved in the present study was measured in the form of L, a, and b values using a chromameter (CR-400, Konica Minolta, Japan. The term indicates Hunter L, i.e., lightness ranging from 0 to 100, indicating black to white. The values for "a" denote green (-) to red (+) and values for "b" denote blue (-) to yellow (+). The 1,000 kernel weight was measured according to the method given by AOAC International [26]. Bulk density was estimated by the method described by Huang *et al.* [27] and tap density as per the methodology described by Jones *et al.* [28]. The average size of the kernel, i.e., length, width, and height of barley kernels was evaluated by Vernier Calliper. The functional characteristics of barley grains such as hydration capacity and swelling power were evaluated by the method prescribed by Williams *et al.* [29]. Syneresis was estimated according to the method described by Shaikh *et al.* [30], swelling and solubility index by Sofi *et al.* [31], and light transmittance as per the methodology described by Ashwar *et al.* [32].

2.3. Isolation of Starch From Barley

Starch was isolated according to the method prescribed by Pérez *et al.* [33]. The 100 g of barley grains were weighed and soaked in 500 ml of 0.25% NaOH for 24 hours. After 24 hours, the grains were washed with distilled water until the water became colorless. The soaked grains were then blended in 200 ml distilled water for 2 minutes. The blended mix was sieved and the filtrate collected was again blended in 200 ml distilled water and this process was repeated 3 times. The starch collected was neutralized to pH 7 using 0.2N HCL. The collected starch solution was kept undisturbed for 4–8 hours. After settling, the supernatant was discarded and the settled portion was centrifuged at 3,000 rpm for 20 minutes. After centrifugation, the blackish layer obtained was discarded and starch was collected and freeze-dried. After drying, starch was ground and packed in polypropylene bags and stored in the refrigerator.

2.4. Physical Modification of Starch

Single-step annealing was done as per the methodology described by Devi and Sit [34]. 150 g of starch sample was weighed and their moisture content was increased by adding 75% distilled water. After that, the sample was sealed in a retort pouch and was heated in a hot water bath at 50ºC for 72 hours. After 72 hours, the sample was centrifuged at 2,000 rpm for 10 minutes and the residual starch obtained was dried at 45ºC for 24 hours.

2.5. Enzymatic Modification of Starch

The enzymatic modification was performed by the method described by Zhai *et al.* [35]. The 50 g wheat starch sample was taken and mixed with in 200 ml sodium acetate buffer (50 mM, pH 5.0). The slurry was preheated in the hot water bath at 50ºC for 10 minutes. After 10 minutes, added amylase enzyme at different dosages, i.e., 7.5 U and 15 U/g to the slurry and heated for another 1 hour at 50ºC in a shaking hot water bath. After 1 hour, the reaction was terminated by the addition of 1M NaOH and pH was adjusted to 4.5 using 1M HCl. After this, the slurry was centrifuged at 8,000 rpm for 10 minutes. The supernatant was then collected for analysis of the degree of hydrolysis and the residual starch was washed with deionized water at least 3 times and centrifuged. Then, the sample was dried at 40ºC overnight.

2.6. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The FTIR spectroscopic analysis was carried out using a Fourier–transform spectrophotometer (Perkin Elmer, USA) at Eternal University, Baru Sahib in the range of 400–4,000^{cm-1}.

2.7. Mineral Contents

Mineral profiling of two cultivars of barley, i.e., PL-751 and PL-830 was carried out using Inductively coupled plasma optical emission spectroscopy (PerkinElmer, USA) at Eternal University, Baru Sahib.

2.8. Statistical Analysis

Each experiment was conducted in triplicate and the mean standard deviation was used to represent the findings. The IBM SPSS Statistics Developer software, version 16.0, was used to compute means using an embedded one-way analysis of variance. Significant differences were taken into consideration at a significance level of $p \leq 0.05\%$.

3. RESULTS

3.1. Physico-Chemical Characteristics of Different Cultivars of Barley

The data on nutritional and functional characteristics of barley grains is presented in Table 1. The PL-751 and PL-830 varieties contained moisture content of 6.43% and 6.23%; crude protein of 11.50% and 10.50%; crude fiber of 0.93% and 1.05%; crude fat of 1.03% and 1.05%, ash content of 2.67% and 2.63%; and carbohydrates 77.43% and 78.48%, respectively. The β-glucan content in PL-751 and PL-830 varieties was 4.98% and 4.03%, respectively. The bulk and tap density of the PL-751 variety were 80.33 and 75.33 g/ml, respectively, and that of the PL-830 variety were 84.6 and 83 g/ml, respectively. The average size of the kernel of the PL-751 variety in terms of length, width, and height were found to be 0.87, 0.27, and 0.23 mm, respectively, and that of the PL-830 variety were 1.03, 0.33, and 0.17 mm, respectively. The hydration capacity and swelling capacity of the PL-751 variety were 1.85% and 0.072 ml/seed, respectively, and that of the PL-830 variety was 2.01% and 0.08 ml/seed, respectively. The 1,000 kernel weight of PL-751 variety was 39.25 g and that of PL-830 was 35.31 g. The color characteristics of the PL-751 variety in terms of L, a, and b values were 59.79, 3.72, and 19.10, respectively, and those of the PL-830 variety were 60.32, 3.65, and 19.07, respectively.

The PL-751 and PL-830 barley cultivars contained minerals such as Se 1.16 and1.37 ppm, Zn 12.23 and 13.59 ppm, Cd 1.72 and 1.75 ppm, Fe 39.78 and 46.64 ppm, Mn 6.72 and 7.63 ppm, Mg 318.2 and 366.9 ppm, Ca 223 and 163.8 ppm, Cu 3.47 and 4.81 ppm and K 1987 and 2209 ppm, respectively.

3.2. Changes in Syneresis, Light Transmittance, Swelling Power, and Solubility Index of Starch Extracted From PL-751 and PL-830 Cultivar

The changes in syneresis of starch extracted from barley cultivars were observed for 5 days and are presented in Table 2. The values increased from 85.99% to 91.0% during 5 days in starch from the PL-751 variety and for the PL-830 variety, the values increased from 90.67% to 97%. Whereas, the light transmittance recorded for 5 days was found to decrease from 2.30% to 2.00% for the PL-751 variety and that for PL-830 declined from 3.63% to 1.90%.

The changes in swelling power and solubility index of native starch of PL-751 and Pl-830 varieties are shown in Table 3. The swelling power was observed at different temperature ranges, i.e., 50ºC, 60ºC, 70ºC, 80ºC, and 90ºC, and values increased from 2.74 to 9.07 g/g for the PL-751 variety and that for the PL-830 variety decreased from 3.07 to 8.60 g/g. The solubility index of starch was also observed at different temperature ranges and values observed for the PL-751 variety were 0.47, 3.63, 4.67, 5.50, and 14.50, respectively, at 50ºC, 60ºC, 70ºC, 80ºC, and 90ºC and that for the PL-830 variety were 0.67, 3.00, 3.50, 7.50, and 15.17, respectively.

Table 1. Physico-chemical and functional characteristics of different cultivars of barley.

Parameters		Barley cultivars	
		PL-751	PL- 830
Dimensional characteristics	Length	0.87 ± 0.06^{b}	1.03 ± 0.06^{a}
	Width	0.27 ± 0.06^{a}	0.33 ± 0.06^{a}
	Height	0.23 ± 0.06^{a}	0.17 ± 0.06^{a}
Moisture content (%)		6.43 ± 0.75^{a}	6.23 ± 0.06^{a}
Crude protein (%)		11.50 ± 0.50	10.50 ± 0.50
Crude fibre (%)		0.93 ± 0.21^{a}	1.05 ± 0.05^{a}
Crude fat (%)		1.03 ± 0.15^{a}	1.10 ± 0.10^{a}
Ash content (%)		2.67 ± 0.06^{a}	2.63 ± 0.12^{a}
Carbohydrates (%)		77.43 ± 1.25^{a}	78.48 ± 0.39^{a}
Beta glucan content (%)		4.98 ± 0.06^{a}	4.03 ± 0.07^{b}
Colour characteristics	L	59.79 ± 1.35^{a}	60.32 ± 0.94^{a}
	a	3.72 ± 0.33^{a}	3.65 ± 0.35^{a}
	b	19.10 ± 0.03^{a}	19.07 ± 0.35^{a}
Physical characteristics			
1,000 kernel weight (g)		39.25 ± 0.22^{a}	35.31 ± 0.27^{a}
Bulk density (g/ml)		80.33 ± 0.58^{a}	84.6 ± 0.58^{b}
Tap density (g/ml)		75.33 ± 0.58^{a}	83 ± 0.58^{b}
Minerals (ppm)			
Selenium (Se)		1.16 ± 0.25^{b}	1.37 ± 0.14^{a}
Zinc (Zn)		12.23 ± 0.06^{b}	13.59 ± 0.13^{a}
Cadmium (Cd)		1.72 ± 0.02^{a}	1.75 ± 0.03^{a}
Iron (Fe)		39.78 ± 0.15^{b}	46.64 ± 0.45^{a}
Manganese (Mn)		6.72 ± 0.01^{b}	7.63 ± 0.04^{a}
Magnesium (Mg)		318.2 ± 3.98^{b}	366.9 ± 1.46^{a}
Calcium (Ca)		223 ± 2.86^{a}	163.8 ± 0.57^{b}
Copper (Cu)		3.47 ± 0.02^{b}	4.81 ± 0.02^{a}
Potassium (K)		1987 ± 22.95^{b}	2209 ± 6.95^{a}
Functional characteristics			
Hydration capacity (%)		1.85 ± 0.01^{b}	2.01 ± 0.01^{a}
Swelling capacity (ml/seed)		0.072 ± 0.01^{a}	0.08 ± 0.01^{a}

Values in the table are presented as mean ± SD. Duncan's LSD *post hoc* analysis at $p \leq 0.05$ indicates that values within rows sharing the same letter are not significantly different.

3.3. Changes in Syneresis, Light Transmittance, Swelling Power, and Solubility Index of Starch From PL-751 and PL-830 Barley Cultivars Modified by Annealing

The syneresis and light transmittance of starch modified by annealing are presented in Table 2. The changes in the syneresis were observed for 5 days and values increased from 85.50% to 96.67% for the PL-

Table 2. Changes in syneresis and light transmittance of native and modified starch extracted from PL-751 and PL-830 cultivars of barley.

Cultivar	Treatment	Syneresis (%) (Days)				
		1	2	3	4	5
PL-751	Native	85.33 ± 0.58c	88.17 ± 0.76b	89.33 ± 0.58b	90.67 ± 0.58a	91.00 ± 1.00a
	Annealing	85.50 ± 0.50e	89.00 ± 1.00d	90.67 ± 0.58c	95.50 ± 0.50b	96.67 ± 1.53a
	7.5 U	84.50 ± 0.50d	86.50 ± 1.32c	88.00 ± 1.00bc	89.00 ± 1.00ab	89.83 ± 0.76a
	15U	79.50 ± 0.50e	82.17 ± 1.61d	83.50 ± 1.32c	86.00 ± 1.00b	87.50 ± 1.32a
PL-830	Native	90.67 ± 0.58c	92.67 ± 0.58b	93.50 ± 0.87b	96.83 ± 0.76a	97.00 ± 0.87a
	Annealing	81.00 ± 1.0e	88.17 ± 0.8d	90.33 ± 0.6c	92.17 ± 1.0b	94.50 ± 0.9a
	7.5U	86.50 ± 0.50c	88.00 ± 1.00bc	89.00 ± 1.00b	90.83 ± 1.04a	92.00 ± 1.00a
	15U	88.17 ± 0.76c	88.83 ± 0.76c	90.17 ± 0.29b	91.17 ± 0.29a	91.83 ± 0.29a
		Light transmittance (%)				
PL-751	Native	2.30 ± 0.10a	2.17 ± 0.06ab	2.10 ± 0.10ab	2.03 ± 0.06b	2.00 ± 0.10b
	Annealing	9.10 ± 0.10a	8.10 ± 0.10b	7.27 ± 0.15c	7.23 ± 0.06c	7.17 ± 0.06c
	7.5U	2.90 ± 0.10a	2.50 ± 0.10b	2.27 ± 0.06c	2.10 ± 0.10c	1.80 ± 0.10d
	15U	2.30 ± 0.10a	2.10 ± 0.10b	2.00 ± 0.10b	1.77 ± 0.12c	1.40 ± 0.10d
PL-830	Native	3.63 ± 0.15a	3.23 ± 0.25b	2.30 ± 0.26c	2.17 ± 0.12cd	1.90 ± 0.10d
	Annealing	10.40 ± 0.2a	8.33 ± 0.2b	7.13 ± 0.1c	7.10 ± 0.1c	5.13 ± 0.1c
	7.5U	2.10 ± 0.10a	2.00 ± 0.10a	1.73 ± 0.15b	1.47 ± 0.15c	1.10 ± 0.10d
	15U	3.37 ± 0.15a	3.00 ± 0.10b	2.90 ± 0.10bc	2.60 ± 0.26c	2.27 ± 0.21d

The values in the table are displayed as mean ± standard deviation; Duncan's LSD *post hoc* analysis at $p \leq 0.05$ indicates that values within rows sharing the same letter are not significantly different.

Table 3. Changes in swelling power and solubility index of native and modified starch extracted from PL-751 and PL-830 cultivars of barley.

Cultivar	Treatment	Swelling power (g/g) at different temperatures (ºC)				
		50ºC	60ºC	70ºC	80ºC	90ºC
PL-751	Native	2.74 ± 0.01e	3.50 ± 0.10d	5.97 ± 0.09c	6.32 ± 0.02b	9.07 ± 0.31a
	Annealing	2.78 ± 0.24c	4.45 ± 0.22b	4.49 ± 0.36b	6.56 ± 0.17a	6.71 ± 0.49a
	7.5U	3.10 ± 0.34d	3.25 ± 0.07d	5.05 ± 0.20c	6.18 ± 0.17b	10.53 ± 0.28a
	15U	2.48 ± 0.12d	2.59 ± 0.20d	4.65 ± 0.29c	6.20 ± 0.08a	10.62 ± 0.21a
PL-830	Native	3.07 ± 0.06e	4.05 ± 0.13d	6.13 ± 0.11c	6.79 ± 0.26b	8.60 ± 0.64a
	Annealing	2.99 ± 0.2d	4.28 ± 0.2c	4.47 ± 0.3c	5.77 ± 0.2b	7.31 ± 0.3a
	7.5U	2.73 ± 0.08e	3.25 ± 0.18d	4.67 ± 0.21c	5.62 ± 0.21b	6.30 ± 0.12a
	15U	2.60 ± 0.03e	3.68 ± 0.07d	4.50 ± 0.20c	5.48 ± 0.19b	6.07 ± 0.02a
		Solubility index (%) at different temperature (ºC)				
PL-751	Native	0.47 ± 0.06e	3.63 ± 0.32d	4.67 ± 0.29c	5.50 ± 0.50b	14.50 ± 0.50a
	Annealing	1.17 ± 0.29c	2.30 ± 0.26c	3.83 ± 0.29b	5.50 ± 0.50a	6.17 ± 1.26a
	7.5 U	4.20 ± 0.26d	6.50 ± 0.50c	8.67 ± 1.76b	9.50 ± 0.50b	11.67 ± 0.76a
	15 U	5.23 ± 0.25d	6.17 ± 0.76d	8.50 ± 0.50c	11.50 ± 0.50b	12.50 ± 0.50a
PL-830	Native	0.67 ± 0.29d	3.00 ± 0.50c	3.50 ± 0.50c	7.50 ± 0.50b	15.17 ± 0.76a
	Annealing	1.50 ± 0.5d	3.00 ± 0.5c	3.17 ± 1.0c	5.17 ± 1.0b	6.83 ± 0.3a
	7.5 U	3.83 ± 0.76d	4.50 ± 0.50d	7.50 ± 0.50c	10.83 ± 0.29b	14.33 ± 0.29a
	15 U	5.37 ± 0.23c	6.17 ± 0.76c	7.37 ± 0.23b	13.83 ± 0.76a	14.00 ± 0.50a

The values in the table are displayed as mean ± standard deviation; Duncan's LSD *post hoc* analysis at $p \leq 0.05$ indicates that values within rows sharing the same letter are not significantly different.

751 cultivar and that for the PL-830 variety enhanced from 81% to 94.50%. The light transmittance was observed for 5 days and values obtained for the PL-751 variety decreased from 9.10% to 7.17% and that for the PL-830 variety declined from 10.40% to 5.13%.

The swelling power and solubility index of annealed starch are mentioned in Table 3. The swelling power observed at different temperature ranges was 2.78, 4.45, 4.49, 6.56, and 6.71 g/g for the PL-751 variety and that for the PL-830 variety was 2.99, 4.28, 4.47, 5.77, and 7.31 g/g at 50ºC, 60ºC, 70ºC, 80ºC, and 90ºC, respectively. The solubility index observed at the same temperature ranges for the PL-751 variety was 6.17%, 5.50%, 3.83%, 2.30%, and 1.17%, and that for the PL-830 variety was 6.83, 5.17, 3.17, 3.00, and 1.50, respectively.

3.4. Changes in Syneresis, Light Transmittance, Swelling Power, and Solubility Index of Starch Modified With Enzymatic Treatment

The syneresis and light transmittance of amylase-modified starches are presented in Table 2. The syneresis in starch modified by 7.5U enzymatic concentration was observed for 5 days for the PL-751 variety and it was found to increase from 84.50% to 89% and that for 15U enzymatic concentration from 79.50% to 87.50%. Similarly for PL-830 variety, the values increased from 86.50% to 92% for 7.5U enzymatic concentration and from 88.17% to 91.83% for 15U enzymatic concentration. The light transmittance of starch was modified by 7.5U enzymatic concentration for the PL-751 variety and it was found to decline from 2.09% to 1.80% and that for 15 U enzymatic concentration decreased from 2.3% to 1.40%. Similarly for PL-830 variety, the values decreased from 2.10% to 1.10% for 7.5 U enzymatic concentration and from 3.37% to 2.27% for 15 U enzymatic concentration.

The swelling power of amylase-treated starch of PL-751 variety at 7.5U enzymatic concentration at temperatures ranging from 50ºC to 90ºC increased from 3.10 to 10.53 g/g and from 2.48 to 10.62 g/g at 15 U enzymatic concentration (Table 3). Similarly for PL-830 variety, the values increased from 2.73% to 6.30% for 7.5 U enzymatic concentration and from 2.60% to 6.07% for 15 U enzymatic concentration. The solubility index of amylase-treated starch of PL-751 variety at 7.5 U enzymatic concentration at temperatures ranging from 50ºC to 90ºC increased from 4.20 to 11.67 g and from 5.23 to 12.50 g g/g at 1 5U enzymatic concentration (Table 3). Similarly for PL-830 variety, the values increased from 3.83% to 14.33% for 7.5 U enzymatic concentration and from 5.37% to 14.00% for 15 U enzymatic concentration.

To modify the starch, the amylase enzyme was taken at two different concentrations, i.e., 7.5 and 15 U because as per the literature notable difference was observed at these concentrations and better results were obtained due to the formation of more short-chain amylopectin, and less long chain amylopectin.

3.5. FTIR Spectroscopic Analysis

The FT-IR spectra of native, annealed, and amylase enzyme-treated starch of the PL-830 barley variety are presented in Figure 1. The band assignment of native, annealed, and enzyme-treated starch extracted from PL-751 and PL-830 cultivars of barley is presented in Table 4.

The appearance of OD band is shown in the region 3,300–2,500, 1,275–1,200, 1,205–1,124, 1,085–1,050, and 1,150–911 in the three spectra that show that these have O-H, C-O, and C-O-C functional groups, respectively. The absorbance peak between wave range 3,300–2,500 cm^{-1} shows O-H stretching; 1,275–1,200, 1,205–1,124, and 1,085–

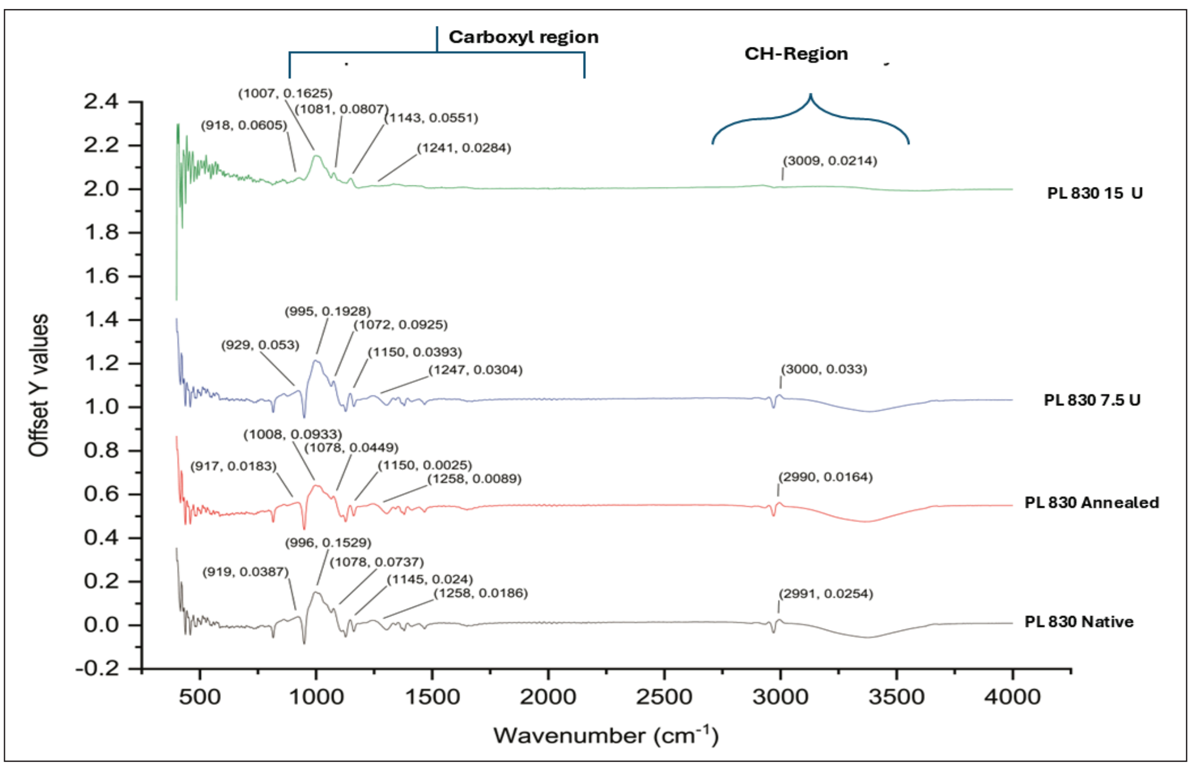

Figure 1. FTIR spectrum of native and modified starches of barley PL 830 cultivar.

Table 4. Band assignment of native, annealed, and enzyme-treated starch extracted from PL-751 and PL-830 cultivars of barley.

Cultivars	Wave range (cm^{-1})	Functional Group	Compound class	Native starch	Annealed starch	Enzyme treated starch	
						7.5 U	15 U
PL-751	3,300–2,500	O-H stretching	Carboxylic acid	2,955	2,988	2,936	2,940
PL-830				2,991	2,990	3,000	3,009
PL-751	1,275–1,200	C-O stretching	Alkyl aryl ether	-	-	-	-
PL-830				1,258	1,258	1,247	1,241
PL-751	1,205–1,124	C-O stretching	Tertiary alcohol	1,148	1,151	1,148	1,151
PL-830				1,145	1,150	1,150	1,143
PL-751	1,085–1,050	C-O stretching	Primary alcohol	1,076	1,076	1,075	1,076
PL-830				1,078	1,078	1,072	1,081
PL-751	1,150–911	C-O-C group	Ether	996	1,001	1,001	1,001
PL-830				919	917	929	918

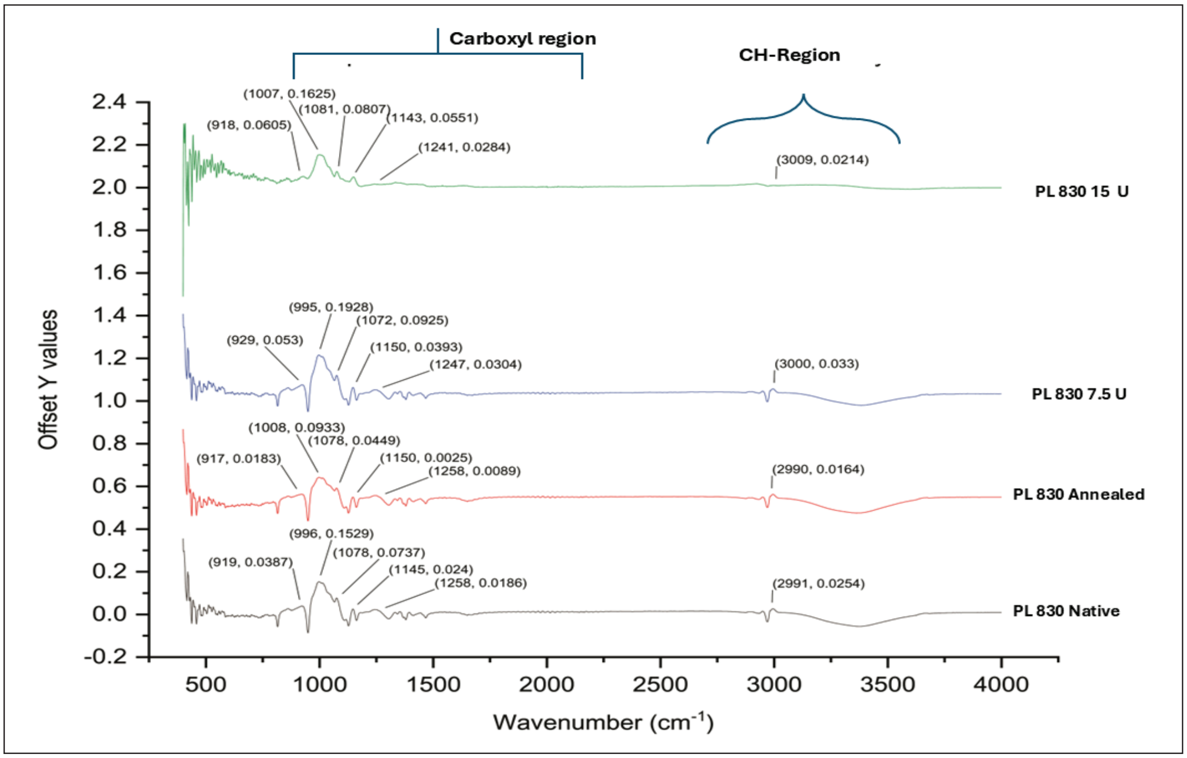

Figure 2. FTIR spectrum of native and modified starches of barley PL 751 cultivars.

1,050 cm^{-1} shows C-O stretching and 1,150–911 cm^{-1} shows C-O-C Group.

The present study stated that native starch showed high O-H stretching and C-O stretching groups as compared to modified starches but high C-O stretching and C-O-C groups were found in both annealed and enzyme-treated starch.

The FT-IR spectra of native, annealed and amylase enzyme-treated PL-751 barley variety are given in Figure 2. The appearance of OD band is shown in the region 3,300–2,500, 1,205–1,124, 1,085–1,050, and 1,150–911 in the three spectra that show that these have O-H, C-O, and C-O-C functional groups, respectively. The absorbance peak between wave range 3,300–2,500 cm^{-1} shows O-H stretching, 1,205–1,124 cm^{-1} and 1,085–1,050 cm^{-1} shows C-O stretching and 1,150–911 cm^{-1} shows C-O-C Group.

The high O-H stretching was seen in annealed starch as compared to native and enzyme-treated starch. The high C-O stretching and C-O-C group were found in both modified starches as compared to native starches but C-O stretching in wave range 1,085–1,050 cm^{-1} was nearly equal to native starch of PL-751 variety.

4. DISCUSSION

Barley (*Hordeum vulgare* L.) is a versatile cereal crop known for its nutritional value and functional properties, with starch being a key component used in food and industrial applications. The physicochemical properties of barley starch, influenced by its cultivar-specific characteristics, can be further enhanced through targeted modifications to improve its functionality for applications such as thickeners, stabilizers, and biodegradable materials. This study aims to address the limited understanding of barley starch by investigating two distinct cultivars, focusing on optimizing extraction methods, applying modification techniques, and characterizing the resulting starch. By exploring these cultivar-specific variations, the research seeks to expand the potential applications of barley starch, supporting the demand for sustainable and functional biomaterials.

The grains of PL-751 and PL-830 barley varieties contained moisture content in the range of 6.23%–6.43%; crude protein 10.50%–11.50%; crude fiber 0.93%–1.05%; crude fat 1.03%–1.05%; ash content 2.63%–2.67%; and carbohydrates 77.43%–78.48%, respectively. As reported by Bai *et al.* [36], the highland barley contained 12.21% moisture content, 13.11% protein and 65.53% carbohydrate content. As per the results given by Obadi *et al.* [37], the different highland barley varieties contained crude protein 10.15%–14.5%, crude fat 1.55%–4,81%, crude fiber 1.75%–5.51%, and ash content in the range of 0.79%–2.63%. Similarly, the study conducted by Alijošius *et al.* [38] reported that ash content ranged from 1.94% to 2.40%, crude fiber 3.57%–5.12%, and crude fat 1.09%–1.93% in different barley cultivars that conformed to the findings of current investigation. The β-glucan content in PL-751 and PL-830 varieties was 4.98% and 4.03%, respectively. Similar results were reported by Storsley *et al.* [39], they extracted β-glucan from eight different varieties of barley and it ranged from 3.64% to 7.96%. Similarly, Vizhi and Many [25] reported that different barley flour varieties contained β-glucan in the range of 2.95%–4.83%. The bulk density of both varieties ranged from 80.33 to 84.6 g/ml and tap density ranged from 75.33 to 83 g/ml. The dimensional characteristics such as length, width, and height ranged from 0.87-1.03 mm, 0.27–0.33 mm, and 0.17–0.23 mm, respectively, in both barley cultivars. The hydration and swelling capacity ranged from 1.85% to 2.01% and 0.072–0.08 ml/seed, the 1,000 kernel weight ranged from 35.31 to 39.25 g. As reported by Chen *et al.* [40], the 1,000 kernel weight of waxy and non-waxy barley varieties ranged from 42.77 to 52.20 g. The color characteristics, i.e., L, a, b ranged from 59.79–60.32, 3.65–3.72, and 19.07–19.10. The color values of PL-751 and PL-830 varieties conformed to the findings of Bai *et al.* [36] who reported the color values of the highland barley variety in terms of L, a, and b values were 70.16, 2.60, and 13.97, respectively.

The PL-751 and PL-830 barley cultivars contained minerals such as Se in the range of 1.16–1.37 ppm, Zn 12.23–13.59 ppm, Cd 1.72–1.75 ppm, Fe 39.78–46.64 ppm, Mn 6.72–7.63 ppm, Mg 318.2–366.9 ppm, Ca 163.8–223 ppm, Cu 3.47–4.81 ppm, and K 1,987–2,209 ppm. According to the study conducted by Šterna *et al.* [41], the zinc content in hull and hull-less barley varieties ranged from 17.60 to 23.80 mg kg^{-1}, manganese 11.80–16.37 mg kg^{-1}, copper 2.00–3.42 mg kg^{-1}, and potassium 3,723–4,128.33 mg kg^{-1}. Panizo-Casado *et al.* [42] reported that barley contained calcium in the range of 381–483 mg kg^{-1}, potassium 3,220–4,747 mg kg^{-1}, magnesium 1,101–1,461 mg kg^{-1}, iron 34.8–59.6 mg kg^{-1}, manganese 12.4–17.9 mg kg^{-1}, zinc 38.3–54.3 mg kg^{-1}, and copper 5.32–8.44 mg kg^{-1}.

The functional properties of native starch of PL-751 and PL-830 varieties were studied, such as syneresis, light transmittance, swelling power, and solubility index. The syneresis of native starch of PL-751 and PL-830 varieties increased from 85.33% to 97%, and light transmittance decreased from 2.30% to 1.90%. As per the study conducted by Singh *et al.* [43], light transmittance was found to decrease from 5.8% to 1.6%. Gupta *et al.* [44] found that light transmittance of barley starch decreased significantly during storage. The swelling power of native starch of both PL-751 and PL-830 varieties ranged from 2.74 to 9.07 g/g, and the solubility index ranged from 0.47% to 15.17%. Similar results were reported by Li *et al.* [45], who found that the swelling power of starch isolated from barley increased from 2.55 to 17.35 g/g and the solubility index from 0.44% to 21.64%. Results were also comparable to the study conducted by Waleed *et al.* [46] on highland barley varieties.

The annealed starch of PL-751 and PL-830 barley varieties showed syneresis increased from 81% to 96.67% and light transmittance decreased from 9.10% to 5.13%. In the study conducted by Devi and Sit [34], they reported that the annealed barley starch showed syneresis in the range of 68.23% to 84.99%. Similar results were found by Ali and Hasnain [47], that the syneresis of white sorghum annealed starch ranged from 83.6% to 84.0%. The increase in syneresis of annealed starch in comparison to native starch may be due to the improved stability of starch molecules. As per the findings of Mendes *et al.* [48], the light transmittance of annealed corn starch declined from 0.64% to 0.32%. Similar results were reported by Sudheesh *et al.* [49] for light transmittance of *kithul* starch that decreased from 6.5% to 1.1%. Light transmittance depends on the rate of retrogradation and the reassociation of starch chains leading to restriction of transmittance of light thus resulting in opaque paste. The annealed starch of PL-751 and PL-830 barley varieties showed swelling power varying from 2.78 to 7.31 g/g and solubility index varying from 1.17% to 6.83%. Similar results were obtained by Ali and Hasnain [47] for the swelling power of white sorghum annealed starch that ranged from 2.5 to 10.6 g/g and solubility index ranged from 1.40% to 5.6%. Similarly, Ji [50] reported that annealed corn starch had a swelling power up to 14.2 g/g and a solubility index up to 9.7%. The reason for the increase in swelling power and solubility index may be due to enhanced gelatinization and improved dispersion of starch molecules that resulted in increased swelling power and solubility index.

The amylase starch of both PL-751 and PL-830 barley varieties showed syneresis at 7.5 U of enzymatic concentration varying from 84.50% to 92% and at 15 U enzymatic concentration was 79.5%–91.83%. Similarly, the light transmittance of both varieties at 7.5 U of enzymatic concentration varied from 2.9% to 1.10%, and at 15 U enzymatic concentration was 3.37% to 1.40%. Zhong *et al.* [51], reported the increase in syneresis of corn starch modified by amylase treatment. As reported by Balasubramanian *et al.* [52], the light transmittance of amylase-modified pearl millet starch reduced from 99% to 97.5%. The increase in syneresis and decrease in light transmittance may be due to the breakdown of starch into smaller molecular weight fragments such as oligosaccharides and sugars through hydrolysis and these smaller fragments have high solubility in water thus contributing to greater syneresis and lower light transmittance [51]. The swelling power of amylase-treated starch of PL-751 variety at 7.5 U enzymatic concentration at temperatures ranging from 50ºC to 90ºC increased from 3.10 to 10.53 g/g and from 2.48 to 10.62 g/g at 15 U enzymatic concentration (Table 3). Similarly for PL-830 variety, the values increased from 2.73% to 6.30% for 7.5U enzymatic concentration and from 2.60% to 6.07% for 15 U enzymatic concentration. The solubility index of amylase-treated starch of PL-751 variety at 7.5 U enzymatic concentration at temperatures ranging from 50ºC to 90ºC increased from 4.20 to 11.67 g and from 5.23 to 12.50 g g/g at 15 U enzymatic concentration (Table 3). Similarly for PL-830 variety, the values increased from 3.83% to 14.33% for 7.5 U enzymatic concentration and from 5.37% to 14.00% for 15 U enzymatic concentration. Similar results were reported by Hutabarat and Stevensen [17], they modified starch from different sources such as rice, corn, pea, potato, and sweet potato and the swelling power of modified starch ranged from 2.87 to 10.03 g/g, and the solubility index from 0.10% to 8.37%. Keeratiburana *et al.* [53] depicted that the swelling power of modified rice starch increased from 2.74% to

2.76% and the solubility index from 1.45% to 2.56%. Similar results at different enzymatic concentrations were given by Zhai *et al.* [35]. The slight decrease in swelling of modified starch could be due to the reason that the hydrolysis has deformed the starch granules. Similarly, the slight increase in solubility could be the result of an increase in the number of short chains caused by the formation of small starch fragments, as amylase acts as an endo-acting enzyme, increasing the natural solubility of the starch [17].

The amylase enzyme acted on the crystalline and granular structure of barley starch which thus led to the formation of more short-chain amylopectin molecules which reduced the rate of retrogradation and gelatinization enthalpy thus improving its functional properties whereas annealing causes the rearrangement of starch molecules which leads to the formation of more uniform and stable starch molecular structure thus making it more resistant to gelatinization and better functional properties.

The FT-IR analysis of native, annealed, and amylase starch showed the appearance of OD band in the region 3,300–2,500, 1,275–1,200, 1,205–1,124, 1,085–1,050, and 1,150–911 having O-H, C-O, and C-O-C functional groups, respectively. The absorbance peak between wave range 3,300–2,500 cm^{-1} shows O-H stretching; 1,275–1,200 cm^{-1}, 1,205–1,124 cm^{-1} and 1,085–1,050 cm^{-1} shows C-O stretching and 1,150–911 cm^{-1} shows C-O-C Group. Ji *et al.* [54] reported that annealed waxy starch showed maximum peak absorbance at 1,047–1,022 cm^{-1}, which may be due to its crystalline and amorphous structure of starch and Ji *et al.* [55] reported that waxy starch showed maximum absorbance peak at 3,700–3,100 cm^{-1} due to presence of O-H stretching group. Khatoon *et al.* [56] reported that starch modified through α-amylase showed maximum absorbance peaks at 1,156, 1,080, and 1,020 cm^{-1} which is due to the formation of more ordered and amorphous regions in starch molecules.

Modifying barley starch from PL-751 and PL-830 cultivars through annealing and enzymatic methods significantly improved its functional properties, including thermal stability, reduced syneresis, and enhanced swelling power and solubility. These changes address the limitations of native starch, making it suitable for diverse food and industrial applications. This study underscores the potential of starch modification to create innovative, shelf-stable products that meet consumer demands for quality and functionality.

5. CONCLUSION

The study examined the starch modification in two barley cultivars, i.e., PL-751 and PL-830. As the native starch is unstable to a wide range of temperature, pH, and shear forces, therefore in the current investigation starch was modified through physical (annealing) and enzymatic (amylase) methods. Various functional properties of modified starch such as syneresis, light transmittance, swelling, and solubility index were analyzed and it was observed that modification by annealing and enzymatic treatments improved the functional characteristics of starch as compared to native starch. The enhancement in functional properties of starch was observed due to the rearrangement of molecules which led to reduced retrogradation and gelatinization and the formation of high short-chain amylopectin molecules. The thermal stability, reduced syneresis, increased swelling, and solubility index of the starch was improved by physical and enzymatic modification, which resulted in stable gels, and better shelf life and thus can be used as thickening agents in soups, sauces, gravies, dairy and confectionary in food industries. Starch modification is a dynamic field that holds vast potential for future applications in both the food and non-food industries. Modified starch can be used for the development of functional foods with enhanced texture, stability, and nutritional profile and can be useful in formulating gluten-free, low glycemic foods or minimally processed foods. Through inhibition of retrogradation and improved moisture retention, modified starch also assists in the extension of the shelf life of the product by maintaining the texture and freshness of the product over time. Thus, it can be concluded that modification offers numerous advantages and opportunities across various industries as it enhances the functional properties of the starch, and starch after modification can be utilized in food and non-food industries for a wide range of applications. Through the modification of starch, various innovative products can be developed that can meet consumers' demands and improve the market value of products.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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