International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 11-04-2023, Accepted: 26-04-2023, Published: 11-05-2023
Volume 8, Issue 2, 2023, Page No. 30-35

Physiochemical and functional properties of barley (*Hordeum vulgare L)* starch modified with different chemical methods

Prixit Guleria, Jyotika Dhankhar*
Department of Food Technology, Maharshi Dayanand University Rohtak, Haryana, India

Abstract
This study examined the physiochemical and functional characteristics of barley (*Hordeum vulgare L*) starch modified with different chemical methods. The physicochemical properties, such as swelling power and solubility, amylose content, color evaluation, pasting properties, and gel hardness, are greatly impacted by modifications. Native, cross-linked, oxidized, and hydroxypropylated starches have been found to contain different amounts of amylose content: 14.68%, 18.60%, 8.83%, and 22.34% respectively. In comparison to native starch, the modified starch had reduced swelling volume, swelling power, and solubility. The L* of oxidized starch was higher than the native and modified starches. All starch samples after the modification had lower value of gel hardness. All chemically modified starches showed decreased in peak viscosity, cold paste, and hot paste viscosity. These results confirmed that the undesirable properties of barley starch can be modified by the use of chemical methods.

Keywords: amylose, barley, cross-linking, swelling power, peak viscosity

Introduction

Barley (Hordeum vulgare L.) is a cereal crop of the *Poaceae* grass family. In terms of productivity and area under cultivation, barley ranks fourth after wheat, rice, and maize (Morten *et al*., 2011) [1]. Barley is generally consumed around the world and is mostly grown for animal feed, with 20% malted for use in alcoholic and non-alcoholic beverages and 5% used to make various food products. There are three types of barley used as food and feed. 1) *Hordeum vulgare*: Six-row barley with three spike lets on each notch and a spike notched on opposite sides. 2) *Hordeum distichum*: a two-row barley with fertile central florets and sterile lateral florets; and 3) *Hordeum irregular*: the least cultivated, with fertile central florets and varied proportions of fertile and sterile lateral florets (Zhou, 2010). Approximately 65-68% starch, 10-17% protein, 4-9% -glucan, 2-3% free lipids, and 1.5-2.5% minerals are found in whole barley grain (Izydorczyk *et al*., 2000) [20]. Fastnaught (2001) [10] reports that total dietary fibre levels range from 11 to 34%, with soluble fibre levels ranging from 3 to 20%. The largest and most important component of barley kernel is starch, which accounts for more than 70% of the dry weight (Asare *et al*., 2011) [5]. Barley starch amylose content ranges from 0% in zero amylose waxy to 5% in waxy, 20-30% in normal, and up to 45% in high-amylose barley (Bhatty and Rossnagel, 1997; Henry, 1988; Morrison *et al*., 1986) [6, 14, 24]. Low amylose starches, such as barley, wheat, and corn, have lower pasting temperatures and higher hot-paste viscosity, swelling power, granule fragility, and freeze-thaw stability than higher amylose starches (Zheng and Sosulski, 1998). These properties of barley starch can be altered by introducing functional groups into the starch molecule via derivatization (etherification, esterification, and cross-linking) or decomposition (oxidation and acid hydrolysis) reactions (Singh *et al*., 2007) [30]. Starches modified with cross-linking, hydroxypropylation, and oxidation improve durability, freeze-thaw stability, low-temperature storage stability, paste clarity and texture, and reduce gelatinization temperature (Pal *et al*., 2002) [25], as well as increase swelling and improve starch stability, binding, film formation, and adhesion properties (Sangseethong *et al*., 2010). The primary goal of this research was to determine the changes in the functional properties of barley starch after cross-linking, hydroxypropylation, and oxidation to obtain modified barley (Hordeum vulgare L.) starch with appropriate properties for use in the food processing industry for the manufacture of various types of food products.

Materials and methods

Barley (*Hordeum vulgare L*.) seeds were purchased from a local market in Rohtak, Haryana (India). All the reagents and chemicals used for research purposes were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

1. Starch isolation

Barley starch was extracted from barley kernels using a slightly modified method developed by Subaric *et al*. (2011) [32]. Barley kernels were thoroughly cleaned to remove all dust particles before being milled into fine flour in a laboratory flour mill (Milcent Appliances, Gujarat, India). To make slurry, barley flour was mixed with distilled water in a 1:2 (w/v) ratio. 1M NaOH was added to the slurry to achieve a pH of 11 and the mixture was agitated for 20 hours on a rotatory shaker (Sigma). The slurry was then filtered via a 200 mm sieve with extra distilled water added. After allowing the starch suspension to stand for 1 h, the supernatant was decanted and centrifuged at 3000 rpm for 10 min. With a spatula, the upper greyish layer of starch was removed, and the residual starch was suspended in distilled water. Again, the starch slurry was sieved to remove coarse bran particles. The starch milk was centrifuged again for 10

min at 3000 rpm, and the top greyish layer was scraped off with a spatula. This procedure was repeated 2-3 times until the starch appeared clean. The leftover starch was then suspended in distilled water, neutralised with 1.0 M HCl, and centrifuged once more. The resulting starch was oven-dried for 12 h at 45°C, powdered with a pestle and mortar, and stored in an airtight container for further study.

2. Chemical composition of starch

Moisture, ash, fat, crude protein and carbohydrate content of native barley starch was determined by using standard methods of AOAC (1995). The amylose content of a starch sample was measured by the method adopted by Hoover and Ratnayake (2001) [27].

3. Cross-linking

The cross-linking of starch was accomplished using the method described by Chung *et al*. (2008), with minor modifications. Cross-linking of starch was achieved by adding the mixture of STMP and STPP (99/1% w/w, 2g) to the starch slurry. In a beaker, 50 g starch was placed, 1.98g STMP and 0.02g STPP was added with 100 ml of distilled water, and the pH of the slurry was adjusted to 11 (with 0.1M NaOH) before being placed in a hot water bath with continuous stirring for 3 hours at 45°C. The pH of the slurry was retained at 11 by adding 0.1M sodium hydroxide, neutralizing it with 1M hydrochloric acid, and washing it 4-5 times with demineralized water. The obtained wet starch was oven-dried overnight at 40 ° C and preserved in an airtight polybag for further research.

4. Oxidation

Forssel *et al.* (1995) [11] method was slightly modified to produce an oxidized starch. To make 50 % starch slurry, 100 ml of distilled water was used, and 2M NaOH was used to bring the pH level to 9.5. 20 g of NaOCl (4% active chlorine) was added dropwise to the slurry over 30 minutes while maintaining a pH range of 9.0-9.5 and constant stirring at 30±2°C. After the addition of NaOCl, the reaction was kept going for another 10 minutes. Now, again pH was maintained at 7 with 1M HCL, and starch was washed 3-4 times with distilled water and centrifuged. The obtained starch was oven-dried at 45˚C for 12 h, pulverized with a pestle and mortar, and stored in an airtight polybag for further research.

5. Hydroxypropylation

The starch was hydroxypropylated by using the method Hjermstad's (1992). 20 % starch slurry (w/v) was prepared with the addition of 10% Na2SO4 (w/w, dwb) in it and stirred continuously. 10.5 pH of the starch slurry was maintained with the addition of 5% NaOH and 10% propylene oxide was added to the slurry and stirred for 30 min at room temperature on a shaker. Then the starch suspension was incubated at 40 °C for 24 h in an incubator shaker (Scigenics orbitek, India) at 200 rpm to prevent sedimentation. After incubation, 10% HCl was used to adjust the slurry's 5.5 pH. Distilled water was used to neutralize the slurry and filter. The obtained starch was oven-dried at 40˚C for 24 h, pulverized with a pestle and mortar, and stored in an airtight polybag for further research.

6. Color evaluation

Hunter colorFlex EZ 45/0 (Hunter Associates Laboratory, Reston, Virginia 20190 USA) was used to measure the change in color in native and modified barley starches. The croma meter was calculated with a white tile and the L* (whiteness) parameter value was then obtained.

7. Swelling power and solubility

The swelling power and solubility of starch samples were determined by using the method used by Lauzon *et al.* (1995) [18] with some modifications. Take 0.5 g starch in a centrifuge tube and add 25 ml distilled water to it. Tubes containing samples were heated differently at temperatures of 55, 65, 75, and 85 ˚C for 1 h with continuous shaking followed by rapid cooling to room temperature and centrifugation at 1900×g for 15 min. The % solubility and swelling power were calculated as given below

$$\% \text{ Solubility} = \frac{\text{Weight of dried sample}}{\text{Sample weight}} \times 100$$

$$\% \text{ Swelling power} = \frac{\text{Weight of wet sample}}{\text{Sample weight} \times (100\text{-}\% \text{ solubility})} \times 100$$

8. Swelling volume

The swelling volume of starch sample was determined by using the method of Crosbie (1991) [9]. The dried starch samples (0.5 g) were placed in a falcon tube (15 ml), distilled water (10 ml) was added slowly, and the tube was kept at room temperature for 20 h. The swelling volume was calculated by dividing the total volume of the swollen starch by the original dry weight of the initial starch**.**

9. Pasting characteristics

Rapid Visco Analyser (RVA Techmaster, Perten instruments, Newport Scientific, Australia) was used to measure the pasting properties of starch samples. The starch sample was directly taken into a canister, and 25 ml of distilled water was added to it making a final weight of 28 g (10% w/w). A sample heating and cooling cycle of 13 min was used and the sample was heated from 50°C to 95°C over 3.5 min, then held at 95°C for 2.5 min, cooled from 95°C to 50°C over 4 min, and then held at 50°C for 1 min. The pasting characteristics include peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (BD), setback (SB), peak time (Ptime), and pasting temperature (Ptemp) were determined.

10. Gel hardness

The gel hardness of native and modified starch gels was determined with a texture analyzer TA-XTi (Stable Micro Systems, Godalming, UK). Pastes obtained after pasting measurements in a canister were sealed with parafilm to avoid moisture loss and stored in the refrigerator for 24 h at 4˚C. The gel samples were left for 4 h at room temperature before the measurement. The gels were punctured using a cylindrical probe up to a distance of 10.0 mm with a speed of 1.0 mm/s. The gel hardness was determined by measuring the peak force of the first penetration and termed gel hardness.

11. Statistical analysis

All results of the analysis were taken in triplicate and the mean values ± SD were reported to get accurate results. Analysis of variance was performed by one and two-way ANOVA analysis (SPSS 19.0) at a significant difference ($p<0.05$).

Results and discussion

1. Proximate composition

The proximate composition provides information on the chemical components that are present in food samples. The barley starch was isolated and its proximate composition was determined. Moisture, lipid, crude fiber, and protein content were all examined and data is presented in Table 1. The yield of barley starch was 45.14% (w/w, based on the weight of dried seeds). Moreover, Asare *et al*. (2011) [5] found between 58.1% to 72.2% starch content in 10 different Canadian genotypes. The variation in starch yield may be attributed to the type of species and cultivars used, the associations of starch granules with other components, and the methods employed for estimation. The native barley starch contained 11.26% moisture, 0.98% protein, 0.43% ash, and 0.79% lipid content. Similarly, Pycia *et al*. (2015), found between 0.10 to 061% lipids and 0.50 to 1.26% protein content in their research. The purity of the starch was reflected by the lower percentages of ash, protein, and fat in the starch. The crude fiber content in barley starch was 0.38%. Crude fiber is considered a bioactive substance that exhibits various functional properties and acts as a nutraceutical, improving human physiological performance by aiding in the prevention of constipation and mitigating the risk of different diseases. By using the difference method, the carbohydrate content of barley starch was determined and found to be 86.59%. The carbohydrate content of barley starch was determined to be 86.59%. Carbohydrate plays an essential role in determining the nutritional value of food and can have significant implications for individuals with specific dietary requirements or health concerns.

Table 1: Proximate composition of barley starch

Sr no.	Constituents	(%) content
1	Moisture	11.26±0.06
2	Ash Ash	0.43±0.00
3	Protein	0.98±0.03
4	Lipid	0.36±0.02
5	Crude fiber	0.38±0.15
6	Carbohydrate	86.59±0.14

2. Color evaluation, amylose content, and swelling volume

The color is a major criterion whiles the modification of different types of starches. The color of native and modified pigeon pea starch samples were observed by Hunter color Flex colorimetric assay and data is presented in Table 2. The L^* value of the native, oxidized, cross-linked, and hydroxypropylated barley starch samples were 94.34, 96.10, 94.66, and 94.78 respectively. Native, cross-linked, and hydroxypropylated modified starch showed a similar trend but oxidized starch has more whiteness than native starch. Similar findings were also reported by Sanchez-Rivera *et al*. (2005) [28] for oxidized banana starch and they concluded that the L^* value of starch will be increased with the increase in chlorine concentration. In an oxidation reaction process, a few pigments and proteins are oxidized before the glucose units and thus produce starch brighter (Sanchez-Rivera *et al*., 2005) [28]. The amylose content of native and modified barley starch samples is presented in Table 2. The amylose content in native, cross-linked, oxidized and hydroxypropylation starch was found to be 14.68%, 18.60%, 8.83%, and 22.34% respectively. Previously, different researchers found variations in barley starch amylose content, which varies from 0 % in zero amylose waxy to 5% in waxy, 20–30% in normal, and up to 45% in high-amylose barley (Bhatty and Rossnagel, 1997; Henry, 1988; Morrison *et al*., 1986) [14, 24]. Starch modifications significantly ($p<0.05$), affect the amylose content in all modified samples. The amylose content was significantly reduced ($p<0.05$) after oxidation and increased after cross-linking and hydroxypropylation modification. The swelling volume of native and modified barley starches varied from 1.49 to 1.66 ml/g and being highest for the oxidized starch (1.66 ml/g) and lowest for the native starch (1.49 ml/g). The hydroxypropylated (1.51 ml/g) and cross-linked (1.63 ml/g) starch showed intermediate swelling volumes between the native and oxidized starch.

Table 2: Color evaluation, amylose content, and swelling volume of native and modified barley starches

Samples	L*	Amylose content (%)	Swelling volume (ml/g)
Native	94.34 ± 0.02^{a}	14.68 ± 0.38^{b}	1.49 ± 0.02^{a}
Oxidized	96.10 ± 0.01^{d}	8.83 ± 0.24^{a}	1.66 ± 0.06^{d}
Cross-linked	94.66 ± 0.04^{b}	18.60 ± 0.21^{c}	1.63 ± 0.04^{c}
Hydroxypropylated	94.78 ± 0.03^{c}	22.34 ± 0.34^{d}	1.51 ± 0.03^{b}

3. Swelling power and solubility

The swelling power and solubility of native and modified barley starches are presented in Table 3. Th e swelling power of starch depends upon amylose content and the water-holding capacity of starch molecules by hydrogen bonding (Liang and King, 2006). The swelling power of all starch samples showed increased with the rise in temperature and maximum swelling occurs between 75°C to 85°C. At 85°C, the swelling power of all starch samples was increased in comparison to native starch, and the maximum rise occurred in hydroxypropylated starch (14.63%). Increased swelling power in hydroxypropylated starch was also reported by Majzoobi *et al*. (2014) [] for wheat starch and Lawal, (2011) for pigeon pea starch. During hydroxypropylation, alkaline treatment increased the pH which ionizes the hydroxyl groups in starch chains and disrupts the hydrogen bonds within the molecules increasing the granular swelling (Gray and BeMiller, 2005; Gunaratne and Corke, 2007). With the increase in temperature, the solubility of the starch samples was increased and maximum solubility occurs between 75°C to 85°C. At 85°C, the solubility in all modified starch samples was increased in comparison to native starch, and the maximum rise has occurred in cross-linked starch (16.11%). An increase in the solubility of starch after hydroxypropylation was due to the leached amylose content from the amorphous region (Lawal, 2011) [19]. The higher solubility of oxidized starch could be due to the weakening of the starch granule structure and de-polymerization, thereby increasing the leaching of amylose from the starch granules (Ali *et al*., 2014). The higher solubility of oxidized starch as compared to other starch samples indicated that the granular structure of starch becomes weakened after oxidation.

4. Pasting characteristics

The pasting characteristics are an essential parameter to determine the use of starch in different food products. The pasting characteristics of native and chemically modified barley starches obtained by RVA are results are presented in Table 4. The pasting temperature of native and chemically modified barley starch samples varied from 73.47 to 91.32˚C. After chemical modification, the pasting temperature in all modified starches was increased and the highest increase was occurred in cross-linked starch. This behavior is different from the results of Spier *et al.* (2013) [31]. They found that the pasting temperatures of sodium hypochlorite-oxidized corn starch were lower than that of the native corn starch. The peak time of native and chemically modified barley starches varied from 4.86 to 6.21 min, being lowest for hydroxypropylated starch and highest for cross-linked starch. Starches having lower peak times are easy to cook and starches having higher peak times need more time for cooking (Yamani *et al*., 2013) [34]. The peak viscosity of native and modified barley starches varied from 1098 to 3350cP. The lowest value of peak viscosity was obtained for cross-linked starch (1098cP), while the highest value was obtained for native starch (3350cP). The high peak viscosity in native starch gel paste might be due to the formation of the firm hydrogel as compared to other modified starch pastes. A reduction in peak, cold paste, and hot paste was observed for all chemically modified starches and cross-linked starch exhibited the lowest values for all pasting parameters except peak time. The reduction in the pasting viscosities of modified starches could be ascribed to the fact that reorganization occurred within the starch granules after chemical modification, which restricted the swelling capacity of starch and less amount of amylose leached into the medium to increase its viscosity. The breakdown viscosity of starch was used to determine its fragility. (Vanier *et al*. 2012) [33]. The observed breakdown viscosity values ranged from 45 to 1690cP, with cross-linked starch having the lowest and hydroxypropylated starch having the highest. A high breakdown viscosity is closely linked to a higher swelling ability of the starch granules and a decrease in the shear resistance of the starch paste during the heating process. As a result, the lower the breakdown, the better the thermal stability and shear resistance of the starch paste (Chan *et al*., 2010). Kuakpetoon and Wang, (2006) [17] also reported a decrease in the breakdown viscosity of corn starch. When compared to native starch, the setback (SB) viscosity in all chemically modified starch samples was reduced. Lower setback viscosities indicated a lower tendency of the starch granules to retrograde (Sandhu *et al*., 2008) [29].

5. Starch gel hardness

A texture analyzer is used to measure the hardness of starch gel (Yu *et al*., 2016) [36]. An increase in gel hardness in starch mainly results from the retrogradation which occurs in amylose and amylopectin components (Karim *et al*., 2008) [16], and the inhibition of amylose chain interactions reducing the formation of junction zones results in a decrease in starch gel hardness (Liu *et al*., 1999) [22]. Significant differences ($p < 0.05$) in the gel hardness of native and chemically modified barley starch samples were observed. Gel hardness showed a decreasing trend after modification in comparison to the native starch. Gel hardness in native and chemically treated pigeon pea starch samples varied from 119.11-195.00 g. The modification affects the hardness value of gels and the lowest hardness value (119.11 g) was observed for hydroxypropylated while the native starch gel (195.00 g) had the highest value. Cross-linking decreased the amount of amylose leaching resulting in a decrease in the amount of amylose in the continuous network and thus, forming a weak gel structure (Gunaratne and Corke, 2007). These results are also comparable with the lower cold paste viscosity values of cross-linked starch. A similar result for the reduction in gel hardness for hydroxypropylated starch was also observed by Liu *et al*. (1999) and they stated that the hydroxypropyl groups suppress the starch chain association and thus the solidification of starch gel can be effectively retarded by using hydroxypropylation.

Conclusion

This study concludes that chemical modifications can significantly alter the physicochemical and functional properties of barley starch, such as amylose content, swelling power, solubility, color evaluation, pasting properties, and gel hardness. The modified starches had lower swelling volume, swelling power, solubility, and gel hardness compared to the native starch. The findings suggest that chemical modifications offer a promising approach to improve the undesirable properties of barley starch for various food and industrial applications.

Table 3: Swelling power, Solubility, and gel hardness of native and modified barley starches

Samples	Swelling power (%)				Solubility (%)				Gel hardness (g)
	55°C	65°C	75°C	85°C	55°C	65°C	75°C	85°C	
Native	1.60±0.06^{a}	1.82±0.05^{a}	3.63±0.09^{a}	5.89±0.19^{a}	3.76±0.10^{b}	5.88±0.09^{a}	6.04±0.10^{a}	7.38±0.05^{a}	195.00±6.96^{d}
Oxidized	1.89±0.09^{b}	3.25±0.04^{c}	5.74±0.21^{c}	8.54±0.08^{c}	3.71±0.04^{a}	6.12±0.09^{c}	6.44±0.09^{c}	7.61±0.08^{b}	177.26±6.16^{c}
Cross-linked	1.95±0.05^{c}	2.51±0.03^{b}	4.02±0.09^{b}	6.29±0.31^{b}	3.78±0.34^{c}	5.95±0.06^{b}	6.35±0.11^{b}	16.11±0.09^{d}	166.62±5.85^{b}
Hydroxypropylated	2.06±0.04^{d}	4.23±0.10^{d}	8.86±0.24^{d}	14.63±0.12^{d}	5.91±0.05^{d}	7.70±0.03^{d}	10.04±0.22^{d}	16.00±0.14^{c}	119.11±10.00^{a}

Table 4: Pasting characteristics of native and modified barley starches

Samples	PV (cp)	HPV (cp)	BD (cp)	CPV (cp)	SB (cp)	Pasting Temp (°C)	Peak Time (Min)
Native	3350±11.13^{d}	2160±12.12^{d}	1195±16.55^{c}	4380±10.06^{d}	2230±20.20^{c}	91.32±0.02^{d}	5.87±0.00^{b}
Oxidized	2920±20.51^{b}	1771±20.25^{c}	1143±20.13^{b}	3553±10.21^{c}	1754±15.63^{b}	87.31±0.02^{b}	6.03±0.05^{c}
Cross-linked	1098±50.08^{a}	1055±12.50^{a}	45±12.08^{a}	1425±18.02^{a}	365±16.24^{a}	89.62±0.03^{c}	6.21±0.00^{d}
Hydroxypropylated	2963±30.98^{c}	1321±39.50^{b}	1690±26.83^{d}	3535±24.04^{b}	2238±29.73^{c}	73.47±0.01^{a}	4.86±0.02^{a}

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