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RESEARCH ARTICLE

Effect of Different Processing Methods on Composition, Biological Activities and Permeability of Barley (*Hordeum vulgare* L.): An Indigenous Cereal

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Abstract

Barley (*Hordeum vulgare* L.) is a cereal rich in soluble dietary fibres, and antioxidant and bioactive compounds. *Ayurveda* reported its use as a therapeutic food. Being a rich source of functional ingredients, it has the potential to be incorporated for nutritional enrichment. Still, there are certain limitations to its use. The present study first time analyses the effect of different processing methods recommended in *Ayurveda* on variation in its composition and permeability. The hulled barley was processed namely dry roasting, roasting with cow ghee and roasting after overnight soaking in *Triphala* decoction. Further evaluated for proximate composition, *in vitro* anti-glycation and permeability of soluble fibres. A variation in proximate composition was seen in all processed forms. Soluble fibre content was maximum in hulled barley. The β-glucan of hulled barley was 39.41 mg/g, which was increased up to 62.22 mg/g in dry roasted barley. All processed forms exhibited inhibition of glycation, which was maximum with *Triphala*-soaked barley. Processing improved the permeability of soluble fibres, which was maximum with dry roasted form (54%). Dry-roasted barley showed improvement in almost all analyzed parameters. Hence, it can be explored further to facilitate the use of barley as a dietary supplement.

Keywords: *Ayurveda*, Advanced Glycation End Products, β-glucan, Functional Food

Abbreviations: **ABTS:** (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), **BSA:** Bovine Serum Albumin, **DNPH:** 2,4-Dinitrophenylhydrazine, **HCL:** Hydrochloric Acid

1. Introduction

Functional foods along with sources of nutrients provide physiological benefits like health-promoting or disease-preventing properties. The various cereals that are rich in vital components along with calorific value such as proteins, vitamins, antioxidants etc., are gaining more attention because they provide valuable effects on health and decrease the risk of various diseases[1]. Barley (*Hordeum vulgare* L.) is one such cereal mainly cultivated for its use by the malting and brewing industries. Barley has a good amount of soluble dietary fibres, mainly mixed linkage 1→3, 1→4)-β-D-glucan commonly known as β-glucan. It is considered a potential prebiotic, as resistant starch which selectively promotes the growth of beneficial intestinal microorganisms[2]. β-glucan can form viscous solutions and help in decreased intestinal transport and delayed gastric emptying[3]. Recently, this cereal has been accepted as an ingredient of various functional foods and processed products because of its high fibre, mineral and antioxidant contents[3].

The medicinal value of barley has also been well-reported. It is known to maintain blood Low-density lipoprotein-cholestero, increase satiety thereby decreasing energy intake, reduce post-prandial glycemic responses and improve digestive functions[4]. Several studies have examined the potential of diets

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and dietary components as a first-line intervention in the prevention and management of various metabolic diseases[5]. *Ayurveda* has also mentioned its use in dietary and medicinal preparations owing to its health benefits[6]. There are a few *in vitro, in vivo*, and clinical studies reported on barley which have demonstrated its anti-hyperglycaemic and anti-obesity activities[7,8].

Despite being an indigenous crop along with the proven effects in conditions like obesity and diabetes, barley has been a highly neglected cereal. Less than 6% of the total production is used as food due to its certain limitations including its public perception, low functional gluten, poor palatability etc[9]. It is not as commonly consumed as its relative counterparts like oats. Though few products of barley like bread and pasta are available in the market[10], there has been limited success of these products due to poor baking quality[11]. The bioavailability of many drug/food ingredients can be compromised due to their poor solubility and/or low permeability in the GI tract[12]. There are few reports regarding skin cell response after cereal β-glucans exposure[13], intestinal permeability in healthy adults etc[14]. Thus, to incorporate barley into staple food products, there is a need to address the issues hindering its consumption.

Interestingly, *Ayurveda* has described various processing methods for barley like pearling and milling, dry roasting, roasting with ghee, fermenting, soaking in herbal decoctions followed by drying and milling etc.,[15-19] before using it as a dietary ingredient. The objectives of these processing methods appear to convert into barley in a palatable form without affecting its therapeutic efficacy. There are a few studies on processes like hulling, pearling, germination, frying, steam soaking and malting and their effects on the physicochemical properties of barley[20,21]. However, there are hardly any studies to demonstrate the effect of these processes on the therapeutic potential of barley.

The present study was planned to process barley employing different methods described in *Ayurveda* and further study the variation in their physicochemical properties, proximate composition, antioxidant and anti-glycation activity and permeability of soluble fibres isolated from the different processed forms.

2. Materials and Methods

2.1 Sample Procurement and Preparation

Barley was procured from a local source in Pune (Maharashtra) and authenticated. The processing was done by one of the authors (Jyoti Shirodkar) in her pharmacy as per methods described in classical texts of *Ayurveda*[15-19]. Dry roasted barley was prepared by roasting hulled barley at 180-200 ºC for 25 mins till it turned golden brown. Another processing method involved roasting hulled barley (100gm) at 180-200 ºC with 7 ml of cow ghee till it became golden brown after around 35-40 mins. The last decoction. For this, hulled barley was soaked in a decoction of *Triphala* (a combination of 3 fruits viz. *Terminalia chebula, Phyllanthus emblica* and *Terminalia belerica* mixed in equal quantities) till it got completely submerged. After soaking for 24h excess decoction was removed and hulled barley was shed dry. *Triphala* decoction was prepared by boiling *Triphala* powder (1 part) along with water (8 parts) on a medium flame. The boiling was continued till the water was reduced to 1/4th quantity. All processed forms were then subjected to milling to form flour. The appearance of the processed samples was recorded and they were stored in air-tight containers at room temperature till further analysis. Four samples used were labelled as Hulled barley (Control) (SI), Dry roasted barley (SII), Roasted with cow ghee (SIII) and dried after overnight soaking in *Triphala* decoction (SIV).

2.2 Physicochemical Analysis

All four samples were tested for moisture and ash content as per standard pharmacopoeia methods[22].

2.3 Proximate Composition Analysis

As a part of this analysis, crude fat (using ether extraction method), total carbohydrate (using phenol sulphuric acid) and protein content by Kjeldahl method[23] were estimated in all samples. Further, total soluble and insoluble fibre per cent was determined by the standard AOAC method.

2.4 Extraction and Estimation of β-Glucan

Two gm flour from each sample was mixed with 20 ml of 1M NaOH and was kept at room temperature for 30 min followed by neutralization with 1M HCl. The β-glucan

was precipitated from the supernatant using 80% ethanol and the precipitate was separated by centrifugation. The obtained pellet was dried and re-dissolved in Phosphate Buffer Saline (PBS). A colorimetric Congo red dye method[22] was used with slight modifications to determine the concentration of β-glucan. Purified β-1, 3-D-glucan over a range of 10-150 µg was used as a standard to calculate the concentration in the study samples.

2.5 Estimation of Biological Activities

All four samples were used for the estimation of polyphenol content, and antioxidant and anti-glycation activities *in vitro*. For this analysis, an aqueous extract of barley flour was prepared.

2.6 Preparation of Extracts

Five grams of barley flour was taken in 50 ml sterile distilled water and was kept on a shaker for 3h followed by centrifugation at 3000 rpm[24]. Clear supernatant (aqueous extract) was stored at 4°C until further use. The extract thus prepared was used as a stock solution (1g/ml), from which different concentrations were prepared.

2.7 Total Phenolic Content

It was measured using the equivalent of Gallic acid by the Folin-Ciocalteu method. The assay was performed in triplicate and the results are expressed as mg/g of dry mass of Gallic Acid Equivalents (GAE).

2.8 ABTS [2, 2 azino-bis (3-ethylbenzothiazoline-6-sulphonic acid] Scavenging Activity

ABTS radical cations (ABTS+) were produced by reacting its solution (7mM) with 2.45mM potassium persulphate in equal proportion. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. The solution was then diluted with deionized water to obtain an absorbance of 0.7 units at 734nm. Fresh ABTS+ solution was prepared for each assay. Sixty microliter of sample extract was added to 2940µl of ABTS+ solution and the mixture was incubated for 6 minutes in dark condition. Trolox was used as standard. The absorbance was read and percentage inhibition was calculated using the formula: Percent (%) inhibition = [(A1-A0)/A1] X 100, where, A1 = Absorbance of the control, A0 = Absorbance of a sample.

2.9 Antiglycation Activity

Albumin glycation was performed according to the previously reported method by Tupe *et al.*[25] Glycated samples were prepared by incubating 1 ml of Bovine Serum Albumin (BSA; 10 mg/ml), 1 ml of fructose (250mM) in 1 ml of potassium phosphate buffer (200 mM, pH 7.4 containing 0.02 % sodium azide) along with aqueous extracts (1 ml of stock solution) and incubated at 37 °C for 4 days. Glycation control (BSA + fructose) was maintained under similar conditions. Before incubation, all the solutions were filtered through 0·22µm membrane filters in sterile plastic-capped vials. After the incubation period, it was ensured that the samples were free of microbiological contamination. The unbound fructose from the samples and test control was removed by dialysis against the phosphate buffer (200mM, pH 7.4) and dialysates were subsequently stored at 4°C till further analysis.

The anti-glycation potential of all samples was determined by estimation of the intermediates of the glycation process viz. fructosamine, protein carbonyls and protein amyloid from dialysates. A Thiobarbituric Acid (TBA) assay was used for the estimation of fructosamine. Protein carbonyl groups were estimated using DNPH while protein amyloid was estimated using congo red. Further, their per cent inhibition was calculated using the formula: Inhibitory activity (%) = [(A0- A1)/A0] × 100, where A1 is absorbance of Glycation control, A0 is the absorbance of the sample. All reactions were carried out in triplicates.

2.10 Estimation of Permeability

To determine the permeability of samples, the Franz diffusion cell system was used. It is a known and widely used methodology to evaluate *in vitro* drug permeation. The Franz diffusion cell consists of a donor chamber and a receptor chamber separated by a membrane. Before being subjected to this system, the samples (2gm barley flour in 20 ml of 1M NaOH) were digested *in vitro*. In brief, the sample extract was cooled on ice before acidification with HCL and pepsin. It was then incubated in an amber-coloured bottle at 37°C in a water bath shaking at 95 rpm for 1 hr. Further, the pH of the partially digested extract was raised to 5.3 by adding sodium bicarbonate followed by pancreatin. The sample was incubated again in the shaking water bath using the

same condition mentioned above for 2 hr to complete the digestion process. The sample (barley flour) thus digested was introduced in the donor compartment of Franz cell and was kept for 2h at room temperature. The phosphate buffer solution was used as a medium in the receptor compartment, from which soluble and insoluble fibre content was estimated after the assay. The fibre content of the plain buffer and the digested sample (before being subjected to permeability testing) was also estimated. The quantification of fibre content was performed using the method mentioned in the case of proximate analysis. Per cent Permeability was calculated as; = (Soluble fibre % in elute)/ (soluble fibre % in digested sample) x 100.

2.11 Data Analysis

The data are presented as Mean ± standard deviation. All the groups were compared with control (SI). One-way Analysis of Variance (ANOVA) was performed using Graphpad Prism version 5. Tukey's multiple comparison tests were used to do inter-sample comparisons. Statistical significance was considered at $p<0.05$.

3. Results

The appearance of all four samples was observed (Figure 1). Hulled barley was slightly yellow coloured while after roasting the colour of barley turned to light brown (SII and SIII). The SIV appeared dark yellow.

3.1 Effect of Processing on Physico-Chemical Properties

The effect of processing methods on physicochemical analysis is given in Table 1:A. Moisture content was found maximum in SI (11.42%), i.e., in control. A significant ($p<0.001$) reduction was observed in SII (9.68%) and SIII (9.09%). Per cent, ash content was found significantly ($p<0.001$) higher in SIV (6.9%) while it was found significantly ($p<0.05$) lower in SI (3.3%) as compared to other samples.

3.2 Effect of Processing on Proximate Analysis

In proximate analysis (Table 1: B), the total carbohydrate content was found maximum in SI (64.07%) and minimum in SIV (51.60%). The total carbohydrates in SIII ($p<0.01$) and SIV ($p<0.001$) showed a statistically significant decrease. The maximum percentage of fat content was observed in SIII (8.547%), which was significantly higher ($p<0.001$) as compared to SI (4.99%). A decrease in fat content was seen in the other 2 processed forms, which was non-significant. The amount of protein was increased in SIII (21.36%) and SIV (54.06%) as compared to control (17.16%). Total soluble fibre content was significantly lowered ($p<0.001$) in all processed forms. Insoluble dietary fibre was found to be significantly more ($p<0.001$) in SIV (26.75%). Total β -glucan content was found maximum in SII (62.2 mg/g of barley) and minimum in SIII (36.61 mg/g of barley). The increase in the amount of total β-glucan in SII was statistically significant ($p<0.05$).

In the case of total phenols, significantly ($p<0.001$) high phenol content was found in SIV (34.77 mg/g of GAE). The phenol content of the other 2 processed samples was similar to that of the control. All processed forms of barley showed comparable radical scavenging activity (Table 1 C).

Figure 1. Appearance of different processed samples.

3.3 Effect of Processing on Anti-Glycation Activity

To determine the anti-glycation potential of all processed forms of barley, estimation of fructosamine, protein carbonyl and protein amyloid was carried out. Irrespective of the intermediate of the glycation process, all of the processed forms showed inhibitory activity. The per cent inhibition of fructosamine was found similar in all the samples. The per cent inhibition of protein carbonyls was found minimal in SI (34.74%) and maximum in SII (82.28%). The other two processed forms, i.e., SIII (43.97%) ($p<0.01$) and SIV (60.29 %) ($p<0.001$) showed statistically significant inhibition (Table 1: D). Protein amyloid inhibition was found maximum and statistically significant in SIV (69.83%) ($p<0.001$) and minimum in the the other two samples. Conclusively, anti-glycation activity was shown by all processed forms.

3.4 Effect of Processing on Permeability

The permeability of the soluble fibre estimated was maximum in SII and minimum in SIII, insoluble dietary fibre was found negligible i.e., less than 0.1 % (Table 1:E).

4. Discussion

The present study was carried out to explore the effect of different processing methods on parameters which restrict the use of barley in a regular diet such as poor palatability and bioavailability. We employed 3 different processing methods described in *Ayurveda* such as dry roasting, roasting with ghee and overnight soaking in *Triphala* decoction and drying. The raw and processed samples were subjected to physicochemical, proximate analysis, antioxidant and antiglycation activity and permeability study. We observed that all these methods

Table 1. Effect of processing on physicochemical properties

Sr. no.	Attributes (%)	SI	SII	SIII	SIV
A	**Physicochemical analysis**				
1	Moisture	11.42 ± 0.26	9.68 ± 0.35***	9.09 ± 0.18***	10.68 ± 0.26
2	Ash	3.3 ± 0.28	3.8 ± 0.05*	3.7 ± 0.14*	6.9 ± 0.28***
B	**Proximate analysis**				
1	Carbohydrate	64.07±2.35	59.72±1.00	54.11±2.96**	51.60±2.58***
2	Fat	4.99±0.63	4.43±0.84	8.55±0.33***	4.03±0.54
3	Protein$	17.16	16.98	21.36	54.06
4	Total Soluble fibre	7.07±0.02	4.61±0.04***	6.01±0.05***	6.22±0.03***
5	Total Insoluble fibre	16.74±0.06	19.39±0.02***	23.89±0.16***	26.75±0.06***
5	β- glucan (mg/g of barley)	39.41±0.93	62.22±0.79*	36.61±0.68	49.50±0.39
C	**Anti-oxidant activity**				
1	Total Phenolic Content (mg/g of GAE)	13±1.5	12.17±2.47	10.17±0.76	34.77±0.75***
2	ABTS assay (%)	22.95±1.20	21.35±0.49*	20±0.71***	19.35±0.21***
D	**Per cent inhibition of intermediates of glycation products**				
1	Fructosamine	11.02 ± 1.1	7.08 ± 2.2	11.81± 4.4	14.96 ± 6.6
2	Protein Carbonyl	34.74 ± 2.76	82.28 ± 1.13***	43.97 ± 3.3**	60.29±1.13***
3	Protein amyloids	39.66 ± 3.44	43.97 ±6.03	43.10 ± 1.72	69.83 ± 0.86***
E	**Per cent permeability**				
1	Soluble fibre (%)	42	54	28	32

*p<0.05, **p<0.01, ***p<0.001as compared to SI

$ Single set of data

changed the physicochemical properties and proximate composition of hulled barley with improvement in its biological activities. Interestingly, the sample processed only with roasting demonstrated increased permeability as compared to the raw (unprocessed) form.

Barley, although indigenous to India, has not been much utilized in food items. Considering the potential health benefits of incorporating barley into functional foods, improvement in its palatability and permeability become crucial. *Ayurveda* has already recognized its health benefits and therefore has mentioned various processing methods to overcome its limitations. Though there are various studies on different processing methods which has evaluated different properties after processing such as starch content, glycemic index, viscosity, purification and characterization of β-glucan[26] processing methods mentioned in *Ayurveda* like roasted with ghee, soaking with *Triphala* decoction and its therapeutic activities after processing are not explored in detailed.

The moisture content of cereals is essentially an important factor affecting their shelf life, the lower the moisture content higher the shelf life. As expected and also reported earlier, the heating process reduced the moisture content as compared to the control. The sample soaked in *Triphala* decoction was found to have moisture content similar to that of the control may be due to overnight soaking in the decoction (aqueous medium). This sample also showed maximum per cent ash content probably through the absorption of minerals found in *Triphala*. Dry roasted and cow ghee roasted samples were found to have ash content comparable to control, which indicates that roasting has minimal effect on this parameter. A similar trend was observed by Agume *et al.,* when they soaked and roasted soybean flour[27].

Whole grain barley is reported to contain about 78-83 % of carbohydrate.[20] In our study, a control sample was found to have carbohydrate content (64.07%) by the reported value[28]. Processing resulted in a reduction of total carbohydrates in the processed samples. The reduction/loss of carbohydrates might be the result of the solubility of carbohydrates in water during soaking and the Maillard reaction which occurs between amino acids, amines, aldehydes and the carbonyl group of reducing sugars at high temperatures during roasting.

Heat treatment may affect dietary fibre in different ways. An increased temperature leads to a breakage of weak bonds between polysaccharide chains. These changes are important for the effectiveness of barley because of its low glycemic index which could be further enhanced by lowering the amount of total carbohydrates.

In the case[29] of fat content, ghee-roasted barley showed a statistically significant increase due to the addition of fats from ghee. None of the other processing methods have affected the amount of fat content in barley. The amount of protein in barley is reported to range in between 7-25%[30], we have found similar content in the control sample (17.16%). Barley seeds soaked in *Triphala* decoction have shown a nearly three-fold increase in the amount of protein. This increase may be due to the presence of nitrogen-containing compounds in *Triphala*[30].

Barley is known to be a rich source of dietary fibre, including both soluble fibres ranging from 3-20% and insoluble dietary fibre about 11-14%. Processing by heat changes the ratio of soluble to insoluble fibre[31]. Similar results have been obtained in our study in the case of dry-roasted and ghee-roasted samples. We also noticed an increase in insoluble dietary fibre content after processing. The increase in insoluble dietary fibre content may be a result of increased resistant starch content/ formation of protein-fibre complex/Maillard reaction products. The starch content increases because of heating and gets quantified as insoluble dietary fibre as suggested and reported by Thed and Phillips[32] (1995) in a study on processed potato products.

Barley β-glucan is reported to range between 3-10%. The processing of barley has been reported to affect its β-glucan content which is reflected in its physicochemical characteristics and ultimately health effects[33]. Traditional processing of barley such as is known to change either molecular weight (polysaccharide breakdown) or extractability (release from cell wall or aggregation) of total β-glucans or both to improve its content. In our study, dry roasting of barley has significantly increased the amount of total β-glucans but the changes in the amount of β-glucan due to other processing methods were found non-significant. Elevated amounts of β-glucan may be present due to heat inactivation of β-glucanase and its inability to hydrolyse β-glucans[32]. It has been also reported that β-glucan in

the processed form of barley increased from 2.46 to 7.02 % after heating[33,34]. It is reported that the pearling of barley grain at a lower degree showed an insignificant increase in β-glucan content. It has also been reported that the thermal processing of barley impacts β-glucan content differently depending on the time-temperature combination and the nature of heat (dry/wet). Heat led to an increase in soluble β-glucan content due to the conversion of an insoluble part into a soluble part as a function of heat in contrast few studies reported that heat processing decrease the solubility of β-glucan[35].

Barley is known to be a source of phytochemicals especially phenolic compounds that render antioxidant properties to it. Total phenolic content consists of tannins, phenolic acids, coumarins, flavonoids and alkyl resorcinol[36]. We observed an increase in phenolic content after soaking with *Triphala*. This could be due to the tannins present in *Triphala*. However, this increase in phenolic compounds was not reflected in the antioxidant activity as evaluated by the ABTS method. The other 2 samples have neither shown an increase in phenolic compounds nor an increase in antioxidant activity.

We further evaluated the effect of different processed forms of barley on albumin glycation. The glycation reaction involves a series of non-enzymatic reactions between the carbonyl group on reducing sugars and the amino group on proteins, leading to the formation of Advanced Glycation End products (AGEs), involved in the pathogenesis of diabetes and ageing-related complications[25]. Three complementary assays, i.e., inhibition of fructosamine, protein carbonyls and protein amyloids were performed. All studied samples of barley inhibited fructosamine equally, while the roasted barley showed maximum protein carbonyl inhibition. Protein amyloid inhibition was maximum in barley processed with *Triphala*.

One of our objectives was to check the permeability of soluble dietary fibre that can provide an indicative approximate absorbable amount of soluble fibre in the body. The permeability of barley beta-glucan has been earlier reported using cell line ($CaCo_2$) and animal models. In this study, we have used Franz cell diffusion cell for permeability of soluble fibre which is used for drug permeability study. A maximum per cent of soluble β-glucan was observed in a dry roasted form indicating higher permeability of this sample.

Our study has a few limitations as it was restricted to the in *vitro* studies of therapeutic activities as well as permeability. A few aspects such as palatability, acceptability, tolerability and effect on physiological parameters need to be evaluated in clinical settings along with stability testing of the processed forms.

5. Conclusion

To conclude, the processing of barley can help to enhance its biological activity and permeability. Our result has demonstrated that dry roasting of barley, which has shown an overall improvement in almost all studied parameters, can be pursued further to facilitate the use of barley in functional foods.

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