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RESEARCH ARTICLE

Assessment of antioxidant activity of barley (*Hordeum vulgare* L.) grass powder processed through different drying processes

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Abstract

The young part of barley plant (*Hordeum vulgare* L.) known as barley grass (BG) that has been considered to be of particular use to humans as a good nutritional source of vitamins, minerals, chlorophyll, phenols and many bioactive compounds in addition to its different health benefits. The objective of the study was to determine the antioxidant properties of BG powder prepared by different drying processes. Three drying methods with different time-temperature combinations [hot air oven drying (50 °C, 6 hr and 60 °C, 5 hr), microwave drying (600 W, 15 min and 800 W, 10 min) and vacuum oven drying (50 °C, 15 lb, 7 hr)] were utilised in the study for the preparation of BG powder. For the estimation of antioxidant properties of BG powder samples, anti-radical activity (%) (2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique), total phenol content (mg gallic acid equivalent/gram dry weight method) and chlorophyll content (Arnon-1949 method) were measured. After quantitative analysis of antioxidant content of prepared BG powder, the results illustrated that microwave drying at 600 W for 15 min shows the best results in terms of retention of chlorophyll (595.67 mg/100 g), total phenolic content (43.56 mg gallic acid equivalent (GAE)/g) and antioxidant activity (66.37 %) in comparison with other drying methods like hot air oven drying and vacuum oven drying. The findings of the study support the development of BG powder using commonly available drying methods such as hot air oven, microwave oven and vacuum oven. Evaluation of the powders indicated appreciable levels of chlorophyll, phenolic compounds and notable antioxidant (antiradical) activity. These results suggest that barley grass powder can serve as a nutrient-dense food ingredient and may contribute to enhancing the nutritional quality of the daily diet.

Keywords: antioxidant activity; barley grass powder; drying methods; microwave drying; phenolic compounds

Introduction

The global population is on the rise, leading to increased hunger and malnutrition in certain regions, while the nutritional quality of some widely eaten foods is diminishing due to depleted soils. Consequently, people will need to increasingly rely on alternative food sources or nutritional supplements to obtain the essential vitamins, minerals, chlorophyll and enzymes necessary for maintaining good health and boosting immunity against diseases.

In various cultures around the globe, grasses are recognised for their regenerative and health-protective benefits for both animals and humans. Consequently, cultivating and preserving grasses, along with other dietary supplements and food products, could provide the global population with high levels of protein, enzymes, minerals and trace minerals that are easily absorbed by the human digestive system. Additionally, the cultivation and preservation of plant materials with specific nutrient profiles are used to supply individuals with particular nutrient combinations that enhance their ability to resist and recover from diseases, as well as to quickly heal skin conditions and wounds.

In general, young parts of plants are known for having higher levels of certain vitamins, provitamins, antioxidants and other bioactive compounds. Barley grass (BG) (*Hordeum vulgare* L.) is rich in calcium, copper, iron, magnesium, potassium, zinc, β-carotene, folate, pantothenic acid, vitamins B1, B2, B6, C and E, as well as superoxide dismutase, catalase and chlorophyll (1). Nonetheless, the nutrient levels in all barley types are influenced by factors such as the location of cultivation, soil quality, average rainfall and the method of harvesting (2).

Barley grass consists of the young green leaves and stems during the vegetative growth phase, ranging from the seedling stage at 10 days post-sprouting (barley sprout) to the elongation phase (barley green), which marks the peak of its nutritional value before barley enters its reproductive cycle (3–5). In addition to being a popular, green-coloured beverage (5), BG is utilised for its preventive benefits against chronic diseases, particularly those affecting circulation, as well as for its anti-cancer properties, obesity reduction, anti-diabetic effects, anti-arthritis benefits, cholesterol reduction, antioxidant properties and anti-inflammatory effects (6).

Barley grass has traditionally been consumed as a juice. However, in today's fast-paced world, many people lack the time, space, resources and expertise to cultivate BG. Additionally, transporting fresh BG over long distances is not cost-effective due to its short shelf life. In this situation, it is very easy and expedient to use BG in powder format. This powdered version retains all the nutrients of BG as well as having a much larger amount of dietary fibre, being more cost-effective and longer shelf life from BG juice. Barley grass powder has the same essence; even children can include it in their daily routine and improve their intake of vitamins, minerals, antioxidants that tend to shield the body from diseases of degeneration.

This study was aimed at preparing the BG powders using various heat processing techniques including hot air oven drying, microwave drying and vacuum oven drying and comparing the impact of these methods on the bioactive components and antioxidant properties of the powders.

Materials and Methods

Procurement of raw material

The barley seeds used in the experiments were obtained from a local market in Jaipur, Rajasthan, India. The seeds were acquired from a commercial source and deemed food-grade; but the specific cultivar/variety was not known. The fresh barley greens were cultivated indoors in mud pots. Barley grass consists of young green leaves and stems during the vegetative growth phase, starting from the seedling stage 10 days after sprouting (barley sprout) and continuing to the elongation stage (barley green), which marks the nutritional peak before the barley enters its reproductive cycle.

Experimental site

The investigation was carried out in the Public Health Nutrition Lab, Poornima University, Jaipur, India.

Method of preparation of barley grass powder

The freshly harvested BG leaves were gathered for a process of washing, sorting, cutting and grading to eliminate microorganisms and dirt. Subsequently, the leaves were dried using various methods: hot air oven drying at 50 °C for 6 hr and 60 °C for 5 hr, microwave drying at 600 W for 15 min and 800 W for 10 min and vacuum oven drying at 50 °C with 15 lb pressure for 7 hr (7). The dried leaves were then ground into a powder and stored for further analysis (Fig. 1).

Chemical analysis

The antioxidant activity of BG powder was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (8). Briefly, 0.1 g of dried sample was extracted with 10 mL of methanol and centrifuged to obtain a clear extract. A 0.1 mM DPPH solution was prepared in methanol. An aliquot of 1 mL from the extract and mixed with 3 mL of DPPH solution and then kept at dark, room temperature for 30 min. Absorbance was measured at 517 nm using a blank. The values were expressed as percentage (%) inhibition of DPPH radicals.

DPPH radical scavenging activities were mainly performed to compare the samples dried by various treatments in the present study. Knowledge on absolute comparison was not the scope of this work (most often, comparison data is expressed as percentage inhibition with a standard antioxidant e.g. ascorbic acid or trolox), but to compare the relative changes in antioxidant activity due to processing conditions. Thus, percentage inhibition values were deemed adequate to compare the efficacy of the selected drying techniques.

Determination of total phenolic content was performed using the Folin-Ciocalteu method (9). 0.1 g of sample was homogenised with 10 mL of 80 % (v/v) methanol and filtered for extraction. After that, 0.5 mL of such extract was then combined with 2.5 mL of 10 % Folin-Ciocalteu reagent followed by adding 2 mL of the sodium carbonate (7.5 %) solution. The mixture was maintained at room temperature for 30 min and the absorbance measured at 765 nm. The results were given as milligram GAE (gallic acid equivalents)/gram dry weight.

The estimation of chlorophyll content was carried out using standard spectrophotometric method (10). To extract the phenolic compounds, 0.1 g of dried sample was mixed with 10 mL of 80 % acetone and centrifuged. The supernatant was measured at 645 and 663 nm to determine chlorophyll content using standard equations.

Storage

The prepared barley grass powder samples were stored in airtight containers under refrigerated conditions (4 ± 1 °C) and protected from light until further analysis.

Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). All experiments were conducted in triplicate and results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed using the F-test to determine significant differences among sample means, with significance considered at $p < 0.05$.

Results

Average chlorophyll content (mg/100 g) of barley grass powder

The data regarding average chlorophyll (mg/100 g) content of the BG powder, prepared by different drying process presented in Table 1. According to the data, microwave drying of BG leaves (600 W/15 min) shows the maximum retention of the chlorophyll (595.67 mg/100 g) in the BG powder followed by other drying methods like microwave drying (800 W/10 min) (548.33 mg/100 g), hot air oven drying (60 °C/5 hr) (508.00 mg/100 g), vacuum oven drying (492.00 mg/100 g) and hot air oven drying (50 °C/6 hr) (440.33 mg/100 g).

The ANOVA table indicates that the calculated F-value (382.99) exceeds the critical F-value (3.83) at the 5 % significance level. ANOVA revealed significant differences ($p < 0.05$) among drying methods for chlorophyll content. The experimental results reveal that chlorophyll content varies depending on the drying method employed in the preparation of BG powder, with the highest concentration observed in the microwave drying method (600W, 15 min), yielding 595.67 mg/100 g.

Average anti-radical activity (percentage) of barley grass powder

The data associated with average anti-radical activity (percentage) content of the BG powder, prepared by different drying process presented in Table 2. According to the data, microwave drying of BG leaves (600 W/15 min) shows the maximum anti-radical activity

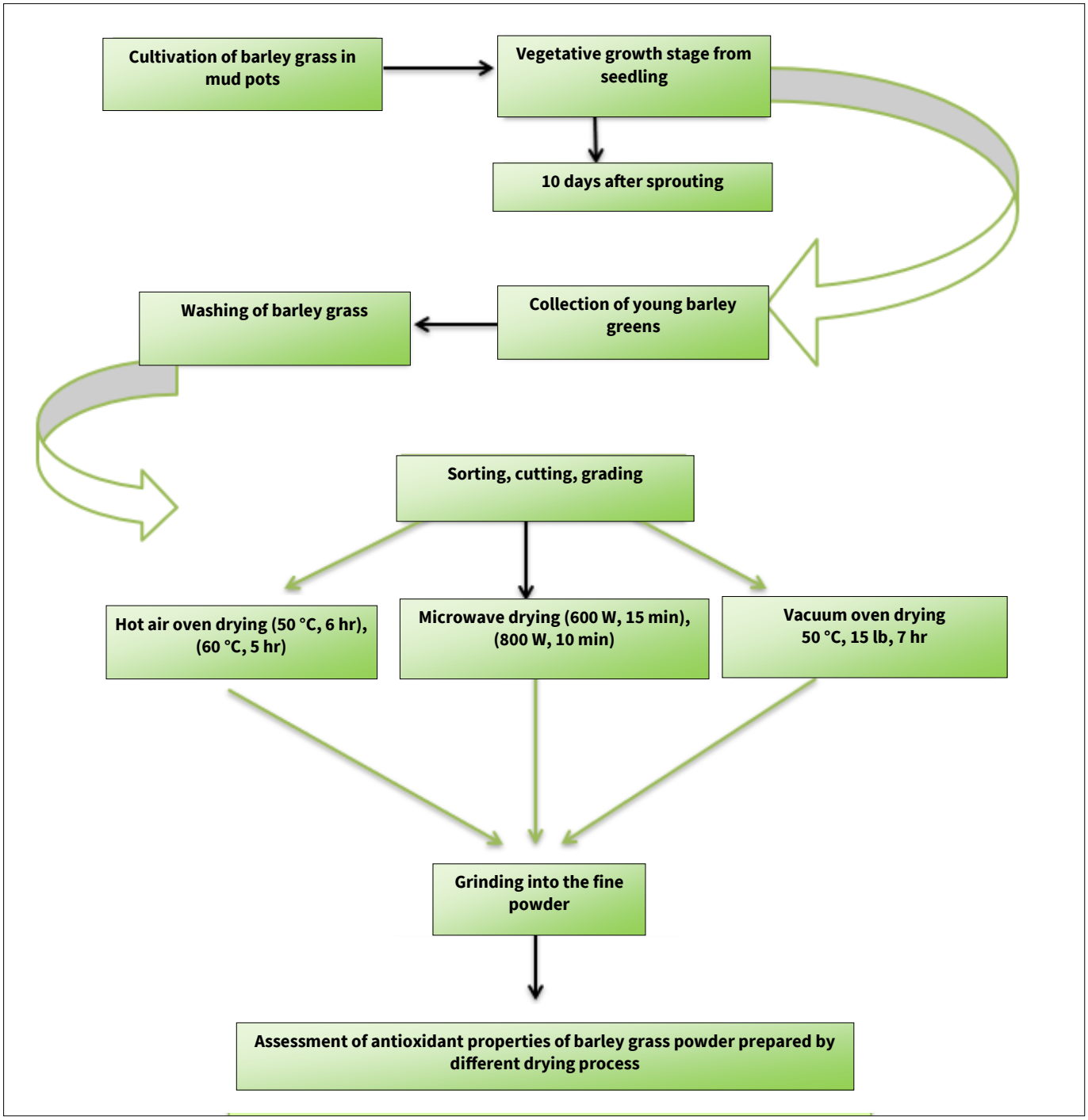


Fig. 1. Process of preparing barley grass powder with different drying methods.

Table 1. Average chlorophyll content of barley grass powder obtained by different drying procedures

Drying variations	N	Mean	SD
HAO (50 °C/6 hr)	3	440.33	2.52
HAO (60 °C/5 hr)	3	508.00	5.29
MW (600 W/15 min)	3	595.67	6.66
MW (800 W/10 min)	3	548.33	3.51
VAC (1)	3	492.00	4.00

ANOVA skeleton

Source of variation	SS	DF	MS	Fcal	Ftab	Result
Treatment	41261.73	4	10315.43	382.99	3.83	S
Replication	2.53	2	1.27	0.05	4.46	NS
Error	215.47	8	26.93			
Total	41479.73	14				

Values are expressed as mean ± SD (n = 3). SS: sum of squares; DF: degrees of freedom; MS: mean square; Fcal: Calculated F-value; Ftab: tabulated F-value; HAO: hot air oven; MW: microwave; VAC: vacuum drying; S: significant; NS: non-significant.

Table 2. Average anti-radical activity (percentage) of barley grass powder obtained by different drying methods

Drying variations	N	Mean	SD
HAO (50 °C /6 hr)	3	**56.24**	**1.02**
HAO (60 °C /5 hr)	3	**57.44**	**0.56**
MW (600 W/ 15 min)	3	**66.37**	**0.68**
MW (800 W/ 10 min)	3	**59.14**	**1.12**
VAC (1)	3	**62.22**	**1.25**

ANOVA skeleton

Source of variation	SS	DF	MS	Fcal	Ftab	Result
Treatment	199.75	4	49.94	95.34	3.83	S
Replication	5.04	2	2.52	4.81	4.46	S
Error	4.19	8	0.52			
Total	208.99	14				

Values are expressed as mean ± SD (n = 3). SS: sum of squares; DF: degrees of freedom; MS: mean square; Fcal: Calculated F-value; Ftab: tabulated F-value; HAO: hot air oven; MW: microwave; VAC: vacuum drying; S: significant; NS: non-significant.

(percentage) (66.37 %) in the BG powder followed by other drying methods like vacuum oven drying (62.22 %), microwave drying (800 W/10 min) (59.14 %), hot air oven drying (60 °C/5 hr) (57.44 %) and hot air oven drying (50 °C/6 hr) (56.24 %).

The ANOVA table shows that the calculated value of F (8096.26) was higher than the table value F (3.83) at 5 % probability level. ANOVA revealed significant differences ($p < 0.05$) among drying methods for percent anti-radical activity. The experimental analysis indicates that the percent anti-radical activity varies with the type of drying method used in the preparation of BG powder and present study show maximum amount 66.37 % found in microwave drying (600 W, 15 min).

Average total phenol content of barley grass powder obtained by different drying procedures

The data relevant to average total phenolic content (mg GAE/100 g) of the BG powder, prepared by different drying process presented in Table 3. According to the data, microwave drying of BG leaves (600 W/15 min) shows the maximum phenolic activity (43.56 mg GAE/100 g) in the BG powder followed by other drying methods like microwave drying (800 W/10 min) (42.75 mg GAE/100 g), vacuum oven drying (39.39 mg GAE/100 g), hot air oven drying (60 °C /5 hr) (38.61 mg GAE/100 g) and hot air oven drying (50 °C /6 hr) (36.44 mg GAE/100 g).

The ANOVA table shows that the calculated value of F (20.13) was higher than the table value F (3.83) at 5 % probability level. ANOVA revealed significant differences ($p < 0.05$) among drying methods for total phenolic content. The experimental analysis indicates that the amount of total phenol varies with the type of drying method used in the preparation of BG powder and present study show maximum amount 43.56 % found in microwave drying (600 W, 15 min).

Discussion

Dehydration is one of the most efficient post-harvest processes for the procurement of the nutritional, functional and sensory aspects of the plant-based materials like leaves, roots, barks stem etc. In the present analysis, significant differences were analysed in the retention of chlorophyll, total phenolic activity and percent anti-radical activity of the prepared BG powder with the utilisation of different drying process. The results illustrated that microwave drying at 600 W for 15 min shows the best results in terms of retention of chlorophyll (595.67 mg/100 g), total phenolic content (43.56 mg GAE/g) and antioxidant activity (66.37 %) in comparison with other drying methods like hot air oven drying and vacuum oven drying.

The shorter drying time and quick internal heating is associated with the less enzymatic degradation and oxidation of the chlorophyll pigments which can be connected with the highest retention of the chlorophyll in the microwave dried samples of the BG powder (11). While chlorophyll breakdown into pheophytin may be increased due to the longer exposure to heat in the hot air oven and vacuum oven dried samples of the BG powder, which resulted into the poor retention of the chlorophyll in the samples. Similar studies were also reported in previous researches in which they assessed that the retention of chlorophyll (23.33 mg/100 g), total phenolic content (25.20 mg GAE/g) and percent anti-radical activity (58.29 %) was on higher side in the wheat grass powder samples prepared by microwave drying while retention of nutrients was not efficient in the conventional drying methods like hot air oven drying and vacuum oven drying (7). Another study evaluated the efficiency of shade drying, oven drying and freeze drying in the preparation of wheatgrass powder (12). The findings indicated that oven drying resulted in better retention of polyphenols, such as tannins

Table 3. Average total phenol content (mg G.A/gm) of barley grass powder obtained by different drying procedures

Drying variations	N	Mean	SD
HAO (50 °C /6 hr)	3	36.44	**0.69**
HAO (60 °C /5 hr)	3	38.61	**1.05**
MW (600 W/ 15 min)	3	43.56	**0.89**
MW (800 W/ 10 min)	3	42.75	**1.03**
VAC (1)	3	39.39	**0.70**

ANOVA skeleton

Source of variation	SS	DF	MSS	Fcal	Ftab	Result
Treatment	98.92	4	24.73	20.13	3.83	S
Replication	0.03	2	0.02	0.02	4.46	NS
Error	9.83	8	1.23			
Total	108.78	14				

Values are expressed as mean ± SD (n = 3). SS: sum of squares; DF: degrees of freedom; MS: mean square; Fcal: Calculated F-value; Ftab: tabulated F-value; HAO: hot air oven; MW: microwave; VAC: vacuum drying; S: significant; NS: non-significant.

(8.18 mg/100 g), as well as micronutrients like iron (19.65 mg) and zinc (8.35 mg), compared to the other drying methods examined. The present study elaborates the efficiency of microwave drying in the retention of thermolabile components like chlorophyll in plant materials like BG.

In the present study, percent anti-radical activity of the BG was determined by DPPH radical scavenging assay and the results showed the similar pattern as microwave dried samples have the maximum retention. The ample presence of bioactive phenolic compounds in the BG powder is responsible for the antioxidant activity and because of shorter drying period, microwave drying reduces the oxidative damage and the high preservation of these bioactive phenolic compounds and shows the high percent anti radical activity (13). Many researchers analysed that the phenolic compounds are particularly susceptible to heat and longer exposure to heat may leads to polymerisation or breakdown follows by reduces percent anti radical activity (14, 15). The results of the present study coincide with the results of one previous study, that showed that phenolic retention and antioxidant capacity of leafy vegetables was significantly associated with drying temperature and duration (16).

Similarly, the microwave dried samples of BG powder show the highest value in context with total phenolic content (43.56 mg GAE/g) in comparison to those dried in hot air oven and vacuum oven. A quick drying process like microwave drying helps in the retention of heat sensitive phenolic content and restricts oxidation of phenolic content (17–19). Microwave drying likely improves retention because it inactivates the polyphenol oxidase enzyme. In slower drying processes, this enzyme breaks down beneficial phenolic compounds. This finding aligns with earlier research on mint leaves, which also demonstrated that microwave drying preserves more antioxidants and phenolics than conventional methods.

Overall, the statistical interpretation (ANOVA table) of the presented data indicated significant differences ($p < 0.05$) across the drying treatments for all studied parameters. The present data illustrated that the quality feature of the BG powder significantly associated with the type of the drying method and associate time-temperature combination. The study demonstrated that microwave drying, particularly at 600 W for 15 min is most feasible drying method for the retention of nutritional and phytochemical integrity and development of high-quality BG powder for its application as functional food.

Among all the selected drying methods, vacuum oven drying consistently demonstrated the second-best performance, particularly in terms of anti-radical activity. This may be attributed to the reduced oxygen environment, which helps limit oxidative degradation of bioactive compounds. However, its slightly lower efficacy compared to microwave drying could be due to longer exposure times and comparatively lower heat transfer efficiency, which may lead to gradual losses of heat-sensitive antioxidants.

The findings of the study are found in accordance with some previous research that microwave drying method offers a high retention of the nutrients and bio-active compound as well as high energy efficiency (20, 21). Thus, by selecting ideal and suitable drying conditions for plant materials is vital for keeping the nutritional, antioxidant and therapeutic potential of BG, which is extensively used for its antioxidant, detoxifying and health-promoting characteristics (1, 22).

The results provide evident guidance from an industrial perspective to selecting the appropriate drying methods for producing barley grass powder. Microwave drying technology is widely accepted due to its fast process and higher content of antioxidants and polyphenols, hence it's suitable for large-scale production of value-added food products (23, 24). Both air drying and osmotic dehydration have the disadvantage of causing oxidative degradation during processing (1) and vacuum oven drying, which may be used as an alternative in this case can impact throughput because of a longer duration for desorption (25). This information can help manufacturers find a trade-off between product quality and operational efficiency.

Although there are already studies reporting effects of drying techniques on retention of bioactive compounds in plant materials, this is the novel effort, exclusively addressed to BGpowder under controlled drying technologies. In contrast to previous works that have compared drying methods in a general way, our study establishes an optimal protocol (600 W for 15 min) to maximise the retention of antioxidants, concurrently measuring chlorophyll and phenolic content and antioxidant activity. From this integrated analysis of these parameters, it is possible to gain a better knowledge on the preservation quality of BG. In addition, this research adds practical relevance by suggesting a practical time-and-energy efficient drying method that can be easily transposed into functional food and nutraceutical applications. Therefore, this work adds to a growing body of evidence while providing more specificity by identifying a clearly defined process condition in which the quality of the final product may be enhanced.

Conclusion

This study shows that the antioxidant capacity of barley grass (BG) powder is significantly affected by the drying method. The microwave drying at 600 W, for 15 min out of the three evaluated methods (hot air oven drying, microwave drying and vacuum oven drying) was the most effective in preserving antioxidant activity. This process, in turn, enabled better retention of chlorophyll, total phenolics and total antioxidant capacity possibly due to the quick internal heating mechanism and lower processing time than for other conventional methods that therefore minimise enzymatic degradation and oxidative loss of bioactive compounds.

The present findings have important practical implications for food processing and nutraceutical industries, as drying optimisation can improve the functional quality of BGpowder while increasing its health-promoting potential. Overall, this study supports microwave drying of fruits and vegetables as a favourable technology for generating high-quality antioxidant-rich vegan powders. In addition, such research may also be helpful for developing functional foods, dietary supplements and value-added products that can address oxidative stress-related disorders.

Further studies could be oriented towards scaling-up the optimal drying conditions for industrial applications, determining the stability of bioactive compounds during storage and investigating the bioavailability of active phytochemicals and their *in vivo* efficacy. In addition, study on its adaptation to other matrices of green plants may have a broader application in the functional food industry.

Limitations of the study

The present study provides valuable data on the effect of different drying conditions on antioxidant activity of powder from grass samples of *Hordeum vulgare* L., but some limitations should be discussed. Few drying methods have been investigated in controlled experimental settings that are drastically different compared to typical industrial-process conditions. Moreover, these parameters were focused more on the total antioxidant activity and few selected phytochemical parameters in this study; thus, individual bioactive compound characterisation goes beyond the scope of this work. Nonetheless, we also did not take an in-depth investigation of those categories associated with the conditions under which raw materials are collected, such as growth stage and environmental/soil aspects. However, such factors should not have detracted from the comparison of drying methods carried out in this work.

Future recommendations

The current results may be extended in the future by further investigating various synergistic drying technologies for quality enhancement of BG powder. Increased analytical techniques, such as chromatographic methods, can also be used to select specific bioactive compounds profiling. Further studies of pilot- or industrial -scale conditions would lead to the verification of the practical feasibility of the obtained results. Finally, assessment of storage stability, optimal packaging conditions and other product development opportunities may encourage greater incorporation of BG powder into functional foods.

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Authors' contributions

PS contributed to conceptualisation and methodology, investigation, data curation, resources and writing, review and editing of the manuscript. SV and AKSP contributed to investigation, supervision, validation and writing, review and editing. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

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