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From Barley (*Hordeum vulgare* L.) to Superfood: Fermentation's Impact on Nutritional Profile and Food Safety

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ABSTRACT

Background: Currently, new agri-food technologies have unveiled the nutritional value of barley. Our study focuses on the nutritional enhancement of barley (*Hordeum vulgare* L.). This study compares the nutritional characteristics of fermented and unfermented barley from Tiaret, Algeria. The analysis focuses on various physicochemical parameters, mineral compositionand heavy metals.

Methods: The methods applied included the determination of dry matter (AOAC 9001.1), lipids (AACC 86-38), proteins (AOAC 950.09), gluten (AACC 38-10.01) and fibers (AACC 32-01.01). Acidity and pH were measured according to the current AACC standards (02.02.02, 02.52.01). Phenolic compounds were extracted using Kondakova's methodand antioxidant activity was evaluated by DPPH. X-ray fluorescence spectrometry and atomic absorption spectrophotometry were used to determine mineral compounds and heavy metals.

Result: Results revealed the significant differences between fermented and unfermented barley. Fermented barley showed higher water content (9.68% vs 6.34%), less protein (9.06% vs 10.76%), more lipids (1.91% vs 1.55%) and fewer total fibers (6.08% vs 8.06%). Fermented barley had higher acidity and lower pH. It is richer in polyphenols but lower in flavonoids. Mineral composition also varies, with generally higher levels in unfermented barley, except for calcium and chlorine. Both types of barley showed low concentrations of heavy metals.This study highlights the nutritional differences between fermented and unfermented barley, offering interesting perspectives for their use in the agri-food industry. The distinct profiles of these barley types suggest potential for targeted applications in food processing and product development, considering their specific nutritional attributes and functional properties, taking into account the consumer's consumption habits.

Key words: Atomic absorption spectrophotometry, Bioindicator, Fermented barley, Unfermented barley, X-ray fluorescence spectrometry (XRF).

INTRODUCTION

Barley is a cereal grain belonging to the Poaceae family. It has been cultivated for thousands of years in many parts of the world for its edible grain, which can be used to make various food products, such as bread, beer, whiskey and breakfast cereals. Barley is also used for the production of livestock feed, as well as for soil improvement and environmental protection. It is rich in fiber, vitamins and minerals and its protein content is comparable to that of wheat (USDA, 2019).

Barley is one of the first cereals domesticated by humansand its cultivation dates back over 10,000 years. It originated in the Middle East, where it was cultivated by ancient Sumerian and Egyptian civilizations. Over time, barley spread throughout the world and became an important cereal crop in many countries (Fuller, 2003).

According to Ben Mbarek and Boubaker (2017), it could be used primarily for livestock feed. Barley is rich in nutrients such as fiber, protein, B vitamins and minerals and is considered an important source of complex carbohydrates, thus falling into the family of long sugars. The nutritional properties of barley can help reduce the risk of chronic diseases such as cardiovascular disease and diabetes (FAO, 2018).

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This research focuses on comparing the nutritional value of fermented and non-fermented barley through a series of physicochemical evaluations. The experiment aims to analyze the content of various biological compounds in both barley types. These compounds include water, organic and mineral matter, pH, secondary metabolites like total polyphenols and total flavonoids and the antioxidant capacity of these phenolic compounds. Finally, the analysis will determine the presence of certain heavy metals in both fermented and non-fermented barley samples.

MATERIALS AND METHODS

The entirety of the work was conducted at the Laboratory of Food Technology and Nutrition at the University of Abdelhamid Ibn Badis in Mostaganem, Algeria, over a period extending from 2021 to 2024.

Origin and characteristics of fermented barley samples

The fermented barley samples used in this study were meticulously collected from a traditional underground silo known as Matmoura. The matmoura, also known as matmora or matmour, is a traditional method of grain storage used primarily in North Africa, particularly in Algeria, Morocco and Tunisia. This technique was developed to preserve grains for long periods, especially in regions where modern storage facilities were not available. (Benkheira, 2018), situated in the Tiaret wilaya locality. These barley grains, bearing a distinct grayish-yellow hue, exude a strong smell of alcohol resulting of fermentation, a testament to their extended storage within the silo's depths.

Method of analysis

Evaluation of biochemical composition in fermented and unfermented barley

Determination of water content (44.01.01 AACC)

Water content is determined by dehydration. Samples of 5g are placed in porcelain crucibles and left to dehydrate for 24 hours in an oven at 105°C. After cooling the containers in a desiccator (an instrument used to determine the relative humidity, which can be expressed as a percentage or in grams) for 45 minutes, the remaining dry matter is then weighed by difference with the initial massand the amount of water evaporated is thus deduced. Expression of water content in dry matter (MS):

$$MS\ (\%) = \frac{\text{Dry matter mass (g)}}{\text{Total samples mass (g)}} \times 100$$

Determination of protein content (ISO 5983 2:2009)

The Kjeldahl method, a well-established technique, is employed to determine the protein content in fermented barley. This method involves a three-step process: digestion or mineralization to break down proteins into ammonium sulfate, distillation of ammonia to isolate it and titration of ammonia to quantify its amount. The protein content is then calculated by multiplying the total nitrogen percentage by a protein conversion factor specific to barley. This method provides valuable information about the nutritional profile of fermented barley, enabling assessment of its potential benefits.

Calculation of protein content using Kjeldahl method

The percentage of protein in a sample is determined by multiplying the percentage of total nitrogen by a specific "F" factor for the type of food being analyzed. This factor accounts for the average nitrogen content of proteins in that food.

F factor for barley: According to FAO and WHO (1973), the F factor for barley is 5.83.

Calculation formula:

$$\%\ \text{Protein} = \%\ \text{Nitrogen} \times \text{F factor (5.83)}$$

Where,

% Nitrogen: Percentage of total nitrogen determined using the Kjeldahl method.

Calculation of Nitrogen Content:

$$\%\ \text{Nitrogen} = 0.0014 \times (V1 - V0) \times 100 / m$$

Where:

% Nitrogen: Percentage of total nitrogen.

V0: Volume in milliliters of sulfuric acid solution used for the blank test.

V1: Volume in milliliters of sulfuric acid solution used for the determination.

m: Mass in grams of the test sample.

Gluten content determination

The NA/735/1990 reference method, established by the Association of Official Analytical Chemists (AOAC), provides a standardized procedure for determining the gluten content in cereal flours. This method relies on the unique properties of gluten, a protein complex found in wheat, rye and barley, to isolate and quantify it. The method involves initial washing, gluten washing, gluten collection and drying and weighing. Observations during extraction, such as appearance and plasticity, provide insights into gluten quality. Accurate gluten content determination is crucial for food labeling, regulatory compliance, quality control, research and clinical applications.

Lipid content determination

The Soxhlet method, outlined in the Official Journal of the European Communities (OJEC) NL. 279/17 and complying with standards NF: V03-713/1984 and ISO: 7302/1982, determines the lipid content of solid foods. This gravimetric method involves weighing the initial sample and the extracted fat at the end of the process.

A 15 g sample of solid food were placed in a thimble and continuously extracted with hexane for 6 hours at 60-70°C in a Soxhlet apparatus. After solvent evaporation, the

extracted lipids are weighed, allowing the calculation of lipid content (L%) based on the weights of the empty balloon (P1), the balloon containing the lipids (P2) and the initial sample (P3).

$$L\% = \frac{p_2 - p_1}{p_3} \times 100$$

This reliable and standardized method provides an accurate assessment of food lipid content, valuable for nutritional information, quality controland research in food science and nutrition.

Acidity determination

The acidity determination method (NA.1182/1990) is a standardized technique for measuring the overall acidity of food samples. Aligned with the standards NFV03-712 and ISO7305/1986, this method expresses the acidity in grams of sulfuric acid per 100 grams of dry matter.

The procedure involves several steps

Sample preparation

To ensure homogeneity, approximately 50 grams of the sample are ground and sieved.

Extraction

A weighed portion of the prepared sample (5 grams) is placed in a beaker and mixed thoroughly with 30 ml of ethanol. The beaker is covered and placed on a magnetic stirrer set in ambient temperature of 25°C. Continuous stirring for one hour facilitates the extraction of acids from the sample.

Centrifugation

After stirring, the mixture is allowed to cool to room temperature. It is then transferred to a centrifuge tube and centrifuged at high speed for two minutes. The clear supernatant containing the extracted acids is decanted into a clean beaker, while the settled solids are discarded.

Titration

A 20 ml aliquot of the clear supernatant is pipetted into a titration beaker. A few drops of phenolphthalein indicator solution are added to the titration beaker. A burette is filled with 0.05 N sodium hydroxide solution. The supernatant is titrated by slowly adding the sodium hydroxide solution from the burette while stirring continuously. The color change of the indicator solution is monitoredand the endpoint is reached when a persistent pale pink color is observed.

Calculation of acidity

The volume (V) of sodium hydroxide solution used in the titration is recorded. The acidity (A) in grams of sulfuric acid per 100 grams of dry matter is calculated using the following formula:

$$A = (V \times N \times 0.049 \times 100) / (\text{Sample weight} \times \% \text{ dry matter})$$

Where,

A: Acidity in g/100 g dry matter.

V: Volume of sodium hydroxide solution used in ml.

N: Normality of sodium hydroxide solution (0.05 N).

0.049: A Conversion factor from sodium hydroxide to sulfuric acid.

Sample weight: Weight of the sample used in grams.

% dry matter: Percentage of dry matter in the sample.

Hydrogen potential determination

The experimental procedure adheres to the standard NF V 05-108, 1970. The pH meter was calibrated using sterile distilled water and then the electrode was immersed in an aqueous solution of ground barley. The solution was prepared by adding 5 g of ground barley to 50 ml of distilled water, stirring for 5-7 minutes, filtration should be carried out to ensure the accuracy of the pH measurement.

Determination of secondary metabolites of phenolic compounds

The objective of this experiment was to perform an extraction of secondary metabolites of phenolic compounds, particularly considering total polyphenols and total flavonoids.

Barley extract preparation methods

The phenolic compound extraction method employed in this study was based on the procedure described by Kondakova (2009). Seven grams of ground plant material from each sample of fermented and non-fermented barley were weighed and placed into separate test tubes. Twenty-eight milliliters of 90% ethanol (v/v) were added to each tubeand the contents were thoroughly mixed using a vortex mixer. The covered tubes were then immersed in a water bath maintained at 60°C for 20 minutes, with vortexing performed twice during the incubation period. The two-phase mixture (liquid and solid) in each tube was separated by centrifugation at 2000 rpm for 15 minutes. The liquid supernatant was decanted and the solid residue was resuspended in 14 ml of the same solvent for a second extraction step. The second supernatant was combined with the first and the resulting extract was transferred to a rotary evaporator for solvent removal at 40°C for 15 minutes. The obtained aqueous extract was stored at -20°C until further analysis (Kondakova, 2009).

Total polyphenols

According to Hmid (2013) , 300 µl of each extract or dilution is placed in a test tube in the presence of 1.5 ml of freshly prepared Folin-Ciocalteau reagent (10 times diluted). After vigorous shaking and an 8-minute rest at 22°C, 1 ml of a 7.5% sodium carbonate (Na_2CO_3) aqueous solution is added. After 30 minutes of incubation at room temperature in the dark, the absorbance is measured using a Perkin Elmer Lambda 25 UV/Vis spectrophotometer at a wavelength of 760 nm. A blank is prepared under the same conditions, replacing the extract with the ethanol-water mixture. The calibration curve is prepared using gallic acid at different concentrations of 10, 50, 100, 400 and 800 mg/l. The total polyphenol content is expressed as mg gallic

acid equivalent/g of dry matter (DM). All measurements are repeated three times.

$$\text{Total polyphenols} = a.\ f/C$$

a: Concentration of polyphenols (µg gallic acid equivalent/ mg extract) determined from the calibration curve. This value is obtained by measuring the absorbance of the sample at 760 nm and comparing it to the absorbance of known concentrations of gallic acid.

f: Dilution factor (×22). This factor accounts for the dilution of the extract during the assay procedure. In this case, the extract is diluted by a factor of 22, so the dilution factor is 22.

C: Concentration of the extract. This value represents the amount of extract used in the assay, typically expressed in grams per milliliter (g/mL).

Total flavonoid assay

Total flavonoid quantification will be performed using a colorimetric method. One milliliter of 20-fold diluted ethanolic extract will be mixed with 1 ml of a fresh aluminum chloride ($AlCl_3$) solution (2%). After 10 minutes of incubation at room temperature (Djeridane *et al*., 2006), the absorbance of the preparation will be measured at 430 nm using an extract blank as a reference. A calibration curve prepared with quercetin at different concentrations (50, 100, 200, 400 and 800 µg/ml) under the same conditions as the samples will be used for flavonoid quantification. Flavonoid contents will be expressed in mg quercetin equivalent/g dry matter (DM). All manipulations will be repeated three times.

$$\text{Flavonoid} = a.\ f\ /\ C$$

a: Total polyphenol concentration (µg gallic acid equivalent/ mg extract) determined from the calibration curve.

f: Dilution factor (×22).

C: Extract concentration.

***In vitro* evaluation of antioxidant activity of extracts**

The objective of this experiment is to evaluate the antioxidant activity using the DPPH free radical scavenging method. The anti-radical activities of *Hordeum vulgare* L. extracts are measured according to the method described by (Kirby and Schmidt, 1997). This assay is based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical with a deep purple color, after its reduction by hydrogen from an antioxidant contained in the extract, this radical becomes pale yellow.

Prepare a series of test tubes, clearly labeling each one for its corresponding extract dilution. Using a pipette, carefully add 500 µl of each extract dilution to its designated test tube. Prepare a separate control tube by adding 500 µl of 90% methanol. This tube will serve as the baseline for comparison. To all test tubes, including the control, add 500 µl of freshly prepared diluted DPPH solution using a pipette. Ensure all tubes receive the same volume. To prevent the assay from being affected by light, cover the tubes with aluminum foil or place them in a light-proof box. Incubate all the tubes at room temperature for 30 minutes. This allows the extract's antioxidant compounds to interact with and reduce the DPPH radicals. After incubation, turn on the spectrophotometer and set the wavelength to 517 nm. This is the specific wavelength at which DPPH absorbance is measured.

The DPPH inhibition percentage is calculated using the following formula:

DPPH Inhibition (%) =

$$\frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Where:

Abs control: Absorbance of the control tube, which contains 500 µl of 90% methanol and 500 µl of DPPH solution. This serves as a reference to measure the absorbance of DPPH in the absence of any antioxidant interference.

Abs sample: Absorbance of the sample tube, which contains 500 µl of the extract dilution and 500 µl of DPPH solution. This value reflects the absorbance of DPPH after interacting with the antioxidant compounds present in the extract.

Determination of mineral matter by X-ray fluorescence (XRF) spectrometry

X-ray fluorescence spectrometry is conducted using a portable X-ray fluorescence spectrometer (PXRF), specifically the Bruker Tracer III-SD (Serial No. T3S2731; Bruker AXS Microanalysis GmbH, Germany).

The Bruker Tracer III-SD PXRF spectrometer is a portable instrument designed for field-based XRF analysis. It employs a radioisotope excitation source, typically a sealed radioactive isotope of Am-241, to generate the high-energy X-rays required for XRF analysis. The emitted X-rays from the sample are detected by a silicon drift detector (SDD), which converts the X-ray energy into electrical signals. These signals are then processed and analyzed to determine the elemental composition of the sample.

Determination of heavy metals

The aim of this research is to confirm whether or not this species of fermented and unfermented barley concentrates various pollutants such as heavy metals, in witch case they can be considered as bioindicator species for heavy metals pollution.

A sample of 1 g was mineralized using a mixture of 10 ml of sulfuric acid (H_2SO_4) and 5 ml of nitric acid (HNO_3) for 60 minutes at 250°C using an automatic Kjeldahl digestion unit VELP (DKL series, 8). The resulting solutions were made up to 50 ml with distilled water and then filtered through Whatmann filter paper to remove impurities. The filtrates were transferred into glass tubes previously rinsed with a 5% HNO_3 solution and finally stored at room temperature for further analysis (Shaltout *et al*., 2015).

Elemental analysis

According to Shaltout *et al*., (2015), the concentrations of lead (Pb), mercury (Hg) and arsenic (As) were determined in milligrams of metal using a Shimadzu AA-7000 atomic absorption spectrophotometer (AAS). The levels were recorded from the digital scale of the AAS and calculated using the following equation:

$$C = R \times (D / W)$$

Where,
C: Element concentrations ($mg.kg^{-1}$).
R: AAS digital scale reading.
D: Dilution of the prepared sample.
W: Weight of the sample.

RESULTS AND DISCUSSION

Evaluation of biochemical composition in fermented and unfermented barley

Fig 1 summarizes the content levels of fermented barley, with a moisture content of 9.86%, protein content of 9.06%, gluten content of zero, fat content of 1.91%, total fiber content of 6.80% and free acidity of 0.14%. It also presents data for unfermented barley, with a moisture content of 6.34%, protein content of 10.76%, gluten content of zero, fat content of 1.55%, total fiber content of 8.06% and free acidity of 0.0577.

Table 1 compiles the pH results, which are approximately 6.48 for fermented barley and 6.88 for unfermented barley.

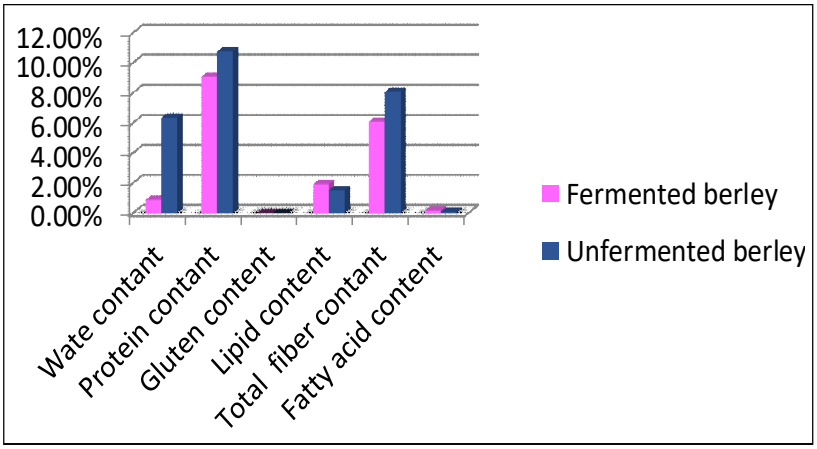

Fig 1: Comparison of nutrient content in fermented and unfermented barley.

Table 1: Comparative analysis of nutritional composition, secondary metabolites, mineral contentand heavy metals in fermented and unfermented barley.

Category	Parameter	Fermented barley	Unfermented barley
Nutritional composition	Water content	9.86%	6.34%
	Protein content	9.06%	10.76%
	Gluten content	0%	0%
	Lipid content	1.91%	1.55%
	Total fiber conten	6.09%	8.06%
	Fatty acid acidity	0.14%	0.06%
Secondary metabolites	Total polyphenols (mg GAE/g)	46.99 ± 0.57	14.15 ± 0.33
	Flavonoids (mg EQ/g)	1.93 ± 0.21	3.11 ± 0.14
Antioxidant activity (DPPH)	Inhibition (IC50, mg/ml)	47.654 ± 0.33	42.416 ± 0.70
Minerals (XRF Spectrometry)	Magnesium (Mg)	519 ppm	841 ppm
	Iron (Fe)	0.12%	0.76%
	Calcium (Ca)	0.35%	0.34%
	Phosphorus (P)	0.37%	0.63%
	Chlorine (Cl)	0.64%	0.52%
	Potassium (K)	1.59%	2.09%
Heavy metals (AAS Spectrometry)	Mercury (Hg)	0.048 mg/kg	0.049 mg/kg
	Lead (Pb)	0.048 mg/kg	0.048 mg/kg
	Arsenic (As)	0.13 mg/kg	0.13 mg/kg

The water content of cereal grains naturally fluctuates depending on ambient humidity, generally reaching equilibrium between 13 and 15% (Feillet, 2002). Our study revealed a protein content of 9.06% in fermented barley and 10.76% in unfermented barley, aligning with standards established by Colas (1991) recommending a protein content between 10 and 13% points out that grain protein content is influenced by various factors, including variety, nitrogen fertilization, ripening conditions, soil and climatic characteristicsand agronomic practices Colas (1991).

Even ours amples were empty of gluten, other authors like Godon (1994) proposes that bacteria can degrade gluten into volatile gases, mainly in the form of ammonia. Additionally, Godon (1982) suggests that the action of free fatty acids, released by lipases, can damage protein structure, thus contributing to the reduction of gluten content. Sharma and Kothari (2017) discussed the impact of malting and fermentation on barley's nutritional profile. They highlighted that during fermentation, various enzymes are activated or produced by microorganisms. These enzymes, including proteases and lipases, can break down proteins and lipids, potentially affecting gluten structure and content.

Determination of secondary metabolites

Determination of total polyphenol content

Table 1 shows the total polyphenol (TPP) content expressed in mg gallic acid equivalent per gram of plant material (mg GAE/g). The polyphenol content of the fermented barley extract is equal to 46.99±0.57 mg EAG/g, while that of the non-fermented barley extract is around 14.15±0.33 mg EAG/g. The polyphenols in the fermented barley extract are clearly higher than those in the unfermented barley extract.

The polyphenol content of unfermented barley was 14.15 ± 0.33 mg GAE/g, consistent with results reported by Khalaf (2014). In contrast, fermented barley exhibited a significantly higher concentration of 46.99 ± 0.57 mg GAE/g. This substantial increase suggests a major interest in the antioxidant activity of fermented barley

Phenolic compounds are secondary metabolites involved in numerous physiological processes (Merouane, 2013). Their role as natural antioxidants is associated with the prevention and treatment of various diseases (Chen *et al*., 2004).

Determination of total flavonoid content

Table 1 shows the flavonoid content of fermented and unfermented barley extracts, expressed in mg of Quercetin equivalent per g of sample. The flavonoid content of the unfermented barley extract, evaluated at 3.11±0.14 mg EQ/g, was found to be higher than that of the fermented barley, equal to 1.93±0.21mg EQ/g.

Regarding the initial flavonoid content in unfermented barley, our results align with the work of Zhang *et al*. (2020), who reported concentrations ranging from 2.8 to 3.5 mg QE/g in different barley varieties. This concordance validates the reliability of our initial measurements and provides a solid foundation for analyzing the effects of fermentation.

The observed decrease in flavonoids during fermentation can be explained by several biochemical mechanisms documented in recent literature. Liu *et al*. (2021) demonstrated that this reduction is primarily due to the action of microbial β-glucosidases, which hydrolyze glycosylated flavonoids into their corresponding aglycone forms. While these aglycones are potentially more bioavailable, they are generally less stable and may undergo further degradation.

However, as highlighted by Chen *et al*. (2023), the quantitative decrease in total flavonoids should not be interpreted as a simple loss of nutritional value. Indeed, the fermentation process can lead to the formation of compounds that are more easily assimilated by the organism, thus improving their bioavailability. This transformation may even generate specific bioactive metabolites with interesting biological properties.

Antioxidant activity determination for fermented and unfermented barley

Fig 2 depicts the inhibition percentage for fermented barley, showing a linear curve derived from the equation y = 1.425X +3.568 with a coefficient of determination or correlation R^2 equal to 0.996.

Fig 3 is characterized by a linear curve of the form y = 0.721X + 5.161 with a coefficient of determination or

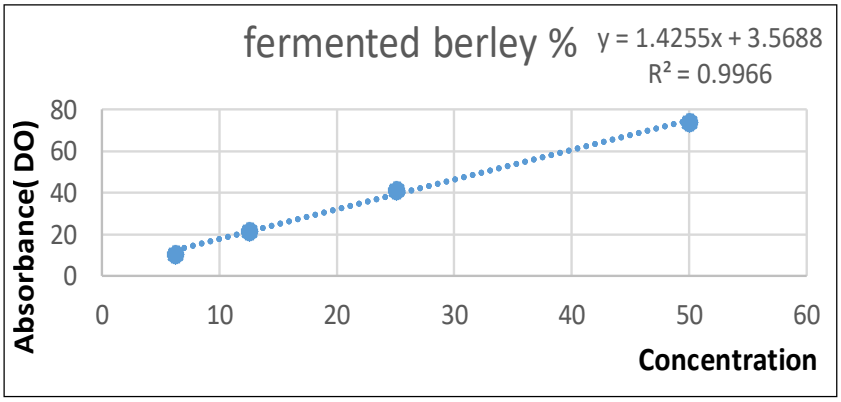

Fig 2: Inhibition percentage for fermented barley.

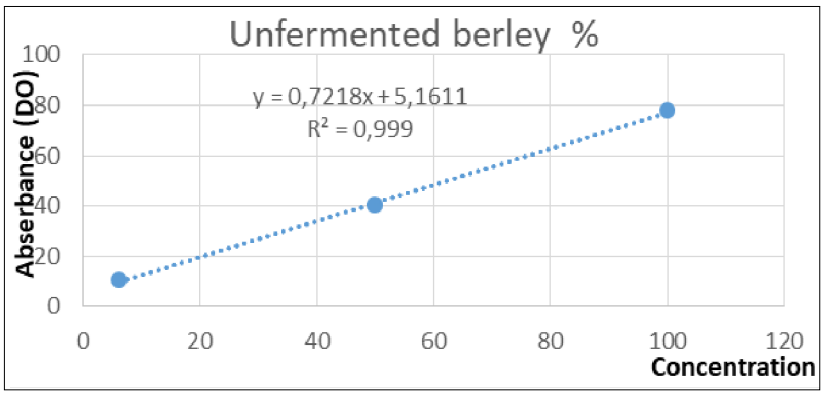

Fig 3: Inhibition percentage for unfermented barley.

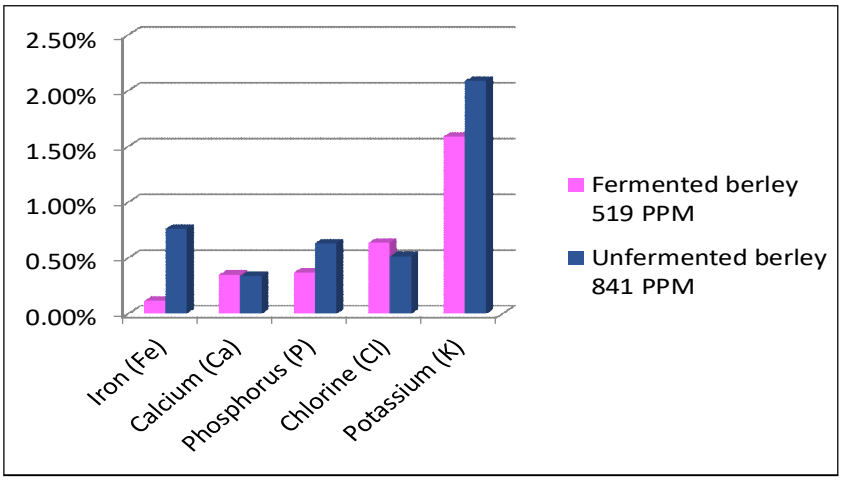

Fig 4: Mineral concentration results obtained by X-ray fluorescence spectrometry(FRX).

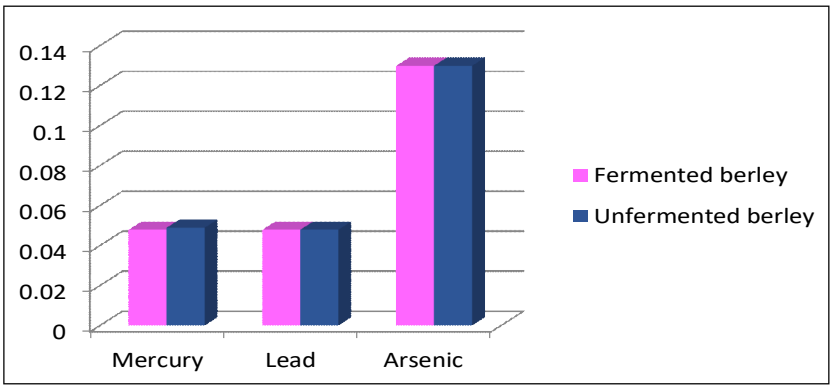

Fig 5: Heavy metal content in fermented and unfermented barley.

correlation R^2 equal to 0.999, expressing the inhibition percentage for unfermented barley.

It is deduced that Fig 4 and 5 illustrate the effectiveness of extracts from fermented and unfermented barley in scavenging the DPPH radical, with very significant coefficients of determination or correlation.

Table 1 shows the two inhibitory concentrations (IC50) obtained from extracts of fermented and unfermented barley respectively. The inhibitory concentration of fermented barley was found to be 47.654±0.33 (mg/ml), while that of unfermented barley was 42.416± 0.70 (mg/ml).

The IC50, inversely related to a compound's antioxidant capacity, was used to evaluate antioxidant activity. A lower IC50 value indicates higher antioxidant activity (Prakash *et al*., 2007).

Determination of mineral matter by X-ray fluorescence spectrometry (FRX)

The FRX spectrometer was used to highlight the results of the various mineral assays carried out on fermented and unfermented barley, as shown in Fig 4. The concentration of magnesium (Mg), iron (Fe), phosphorus (P) and potassium (K) in unfermented barley was higher than in fermented barley. On the other hand, calcium (Ca) and chlorine (Cl) levels are virtually identical.

Mineral contents in fermented and unfermented barley were similar, not exceeding 0.3% for either type. These results are consistent with previous research (Svihus and Gullord, 2002; Ben Salem *et al*., 2005). Barley provides essential minerals such as magnesium, phosphorusand calcium, as well as smaller amounts of iron, zinc, copper, manganeseand selenium (Atkinson, 2008).

Determination of heavy metals

Fig 5 presents the results for heavy metals in fermented barley: lead at 0.048 mg/kg, mercury at 0.048 mg/kg and arsenic at 0.13 mg/kg. The results for heavy metal concentrations in unfermented barley were nearly identical, with mercury at 0.049 mg/kg, lead at 0.048 mg/kg and arsenic at 0.13 mg/kg.

The results indicate similar levels of heavy metals in both fermented and unfermented barley. This similarity suggests that the fermentation process does not have a significant impact on the concentration of these contaminants.

The lead concentration of 0.048 mg/kg in both samples is well below the maximum limit of 0.2 mg/kg set by the European Commission Regulation (EC) No 1881/2006 for cereals (European Commission, 2006). This compliance is crucial, as barley is an important cereal crop with various food and feed applications (Sharma *et al*., 2019).

The mercury concentration (0.048 mg/kg in fermented barley and 0.049 mg/kg in unfermented barley) is also below the limit of 0.1 mg/kg established for foodstuffs by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) (FAO/WHO, 2011). These low levels are particularly important given barley's increasing use in functional foods and nutraceuticals (Sharma and Kothari, 2017).

For arsenic, the concentration of 0.13 mg/kg in both samples is higher than that of lead and mercury. Although there is no specific regulatory limit for total arsenic in cereals in the European Union, this value is below the 0.2 mg/kg limit proposed by some researchers for inorganic arsenic in rice (Meharg *et al*., 2009). However, it's worth noting that barley, especially when malted, can accumulate higher levels of certain heavy metals compared to other cereals (Kosová *et al*., 2020).

CONCLUSION

This study aimed to comparatively evaluate fermented and non-fermented barley using various physicochemical analysis methods. Biochemical analyses revealed the presence of key biomolecules, including water, proteins, lipids, total fibe and fatty acids. Notably, gluten was not detected in either sample. The pH of both fermented and unfermented barley fell within a near-neutral range. The antioxidant activity of polyphenols was significantly elevated in fermented barley compared to unfermented barley. Conversely, unfermented barley displayed greater antioxidant activity associated with flavonoids. The lower IC50 value observed in unfermented barley extracts suggests a stronger free radical scavenging capacity compared to fermented barley extracts. Mineral analysis revealed similar compositions in both fermented and unfermented barley, with slight variations. Importantly, heavy metal levels (lead, mercury and arsenic) in fermented barley were within acceptable limits, excluding it as a bioindicator for these contaminants. Based on these findings, both fermented and unfermented barley could be valuable additions to human diets. The absence of gluten suggests potential benefits for individuals with gluten sensitivity or celiac disease. Additionally, their antioxidant properties warrant further investigation in the context of gut health. However, the potential prebiotic effects of these barleys require further research to confirm their contribution to gastrointestinal microbiota health.

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Informed consent

Not applicable as this study did not involve human subjects or clinical trials

Conflict of interest

The authors declare that there are no known conflicts of interest associated with this publication

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