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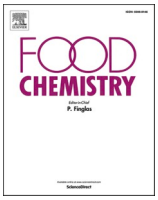

Brewers spent grain protein hydrolysate as a functional ingredient for muffins: Antioxidant, antidiabetic, and sensory evaluation

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ABSTRACT

This study assessed the fortification of muffins with 2, 4, and 6 % of brewer's spent grain protein hydrolysates to enhance their *in vitro* antioxidant, α-glucosidase, and α-amylase inhibitory activities. In addition, oxidative stability, hardness, color and sensory properties of fortified muffins were investigated. The fortification of muffin formulations with 6 % hydrolysates increased antioxidant activity six times higher than that of the control sample. As the hydrolysate increased to 6 %, the α-amylase and α-glucosidase inhibition also increased to 88 and 40 %, respectively. The 6 % fortified muffins exhibited lower peroxide and thiobarbituric acid values during a 14 day storage than the control muffins, while higher hydrolysate levels darkened the color and softened the texture. Sensory evaluation indicated that muffins with 2% hydrolysates achieved similar overall acceptance as the control. It can be concluded that brewer's spent grain hydrolysate is suitable for functional bakery products.

1. Introduction

Health is an inevitable requirement for promoting comprehensive human development and a basic condition for economic and social development (Hu et al., 2021). In recent years, the demand for functional foods with health benefits has notably risen, particularly those incorporating natural and nature-derived components like antioxidants, omega-3 oils, bioactive peptides, and vitamins (Abedinia et al., 2021; Zhang et al., 2022). Dietary proteins are crucial macronutrients for the human body, serving as critical components in food that provide unique functionality and enhance the quality of related products (Zhang, Cheng, Wang, & Fu, 2021). Protein hydrolysates, which are produced through controlled hydrolysis using proteolytic enzymes, are important ingredients in the production of functional food products (Wang et al., 2022; Liu, Zhang, Li, Zhu, & Jiang, 2023). These hydrolysates possess biological activity that can have beneficial effects on human health, such as antioxidant, antidiabetic, antihypertensive, antimicrobial, and immunomodulatory effects (Zheng et al., 2022; Liu et al., 2023). Several studies have shown that plant and grain protein hydrolysates can be incorporated into a range of food products. For instance, pasta supplemented with amaranth protein hydrolysate and bread fortified with maize germ protein hydrolysate (Karimi, Gavlighi, Sarteshnizi, & Udenigwe, 2021). In addition, it has been reported that protein hydrolysates can function as a physical barrier between the oil and oxygen and/or prooxidants to protect the oil from oxidation in the food system (Rahmani-Manglano et al., 2020).

Brewers' spent grain (BSG) is a residual material generated during beer production that contains protein levels ranging from 15 to 30 % (Naibaho et al., 2022). BSG, which accounts for approximately 85 % of all by-products produced during brewing, can be obtained throughout the year. Research findings have indicated that brewers spent grain protein hydrolysates (BPH) exhibit a diverse range of biological properties, including antioxidant and antidiabetic activities (Abeynayake, Zhang, Yang, & Chen, 2022). Regarding synthetic antioxidants, although they possess strong antioxidant activity compared to natural antioxidants, there has been growing concern over the possible carcinogenic effects of synthetic antioxidants in food products (Gulcin, 2020). Our earlier research study explored the technological, functional, and bioactive qualities of protein hydrolysates sourced from BSG (Bazsefidpar, Ahmadi Gavlighi, Ghandehari Yazdi, & Jafari, 2023). Our

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results revealed that the produced hydrolysates exhibited potent antioxidant and antidiabetic effects. These findings suggest that the protein hydrolysates obtained from BSG protein possess significant possibilities for application as functional food components. As a result, fortification of muffin with BPH can improve their physicochemical, textural, oxidative stability, and sensory properties. To the best of our knowledge, this is the first study on fortifying muffins with BPH. In this regard BPH was produced using Flavourzme as a protease enzyme. Then, the effect of BPH addition on the physicochemical, textural, oxidative stability, and sensory properties of muffins was evaluated. Additionally, the antioxidant activity and alpha-amylase and alpha-glucosidase inhibitory properties of the fortified muffins were studied.

2. Material and methods

2.1. Plant material

The BSG used in this study was obtained from a non-alcoholic malt beverage company (Behnoush Iran, Iran, Tehran) and had a moisture content of 85 %. To conduct subsequent analyses, the BSG was dried in a hot air oven at 50 °C and then stored in bags inside a refrigerator set to 4 °C.

2.2. Chemical

Rat intestinal acetone powder, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 4-nitrophenyl β-D-glucopyranoside (pNPG), porcine pancreatic α-amylase (Cat no. A3176), and rat intestinal α-glucosidase (Cat no. I1630) were purchased from Sigma-Aldrich Company (St, Louis, MO, USA). Flavourzyme ≥ 500 U/g (declared enzyme activity 500 LAPU/g) from *Aspergillus oryzae* (Cat no. P6110) was obtained from Novozymes Company (Bagsværd, Denmark). PAHBAH (4-hydroxybenzohydrazide) and soluble starch ACS reagent were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

2.3. Proximate composition of BSG

The fat, moisture, and ash contents of the BSG were determined using the AOAC (2000) methods and reported on a dry weight basis. Protein content (% N × 6.25) was determined by Kjeldahl method (AOAC 2000). Values reported are means of triplicates.

2.4. Production of the BSG protein hydrolysate

The BSG protein was extracted by conducting an alkaline extraction procedure based on the method described by Bazsefidpar et al. (2023). In summary, a blend of dry brewer's spent grain and water was homogenized using an IKA 18 digital Ultra-Turrax from Germany.The pH of the mixture was adjusted to 11.5 by adding 2N NaOH. Subsequently, the mixture was heated, leading to the separation of a protein-rich extract. The protein was then precipitated by adjusting the pH of the solution to 4 using 2N HCl. The resulting brewers' spent grain protein concentrate (BPC) was isolated through centrifugation, freeze-dried, and stored at 4 °C for further analysis. Then, the resulting BPC was hydrolyzed using the method outlined by He, Girgih, Malomo, Ju, and Aluko (2013) with the use of Flavourzyme enzyme. The pH of the 5 % w/v BPC suspension was adjusted and heated to the temperature specified by the enzyme (50 °C at pH = 7), and the enzyme was added in a ratio of 1:20 based on the protein content of the BPC. The final enzyme activity was 100 U/g. The hydrolysis process was conducted using the pH-stat method for 2 h, and the enzyme was inactivated by heating in a water bath (Memmert, WNB22, Germany) at 95 °C for 15 min. Finally, the sample was centrifuged (Sigma, 3-30K) at 10,000 × g for 15 min, and the supernatant was freeze-dried (Christ, Alpha1-2 LDplus) to produce BSG protein hydrolysate (BPH).

2.5. Amino acid composition

As outlined by Liu et al. (2012), the analysis of amino acid profiles was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC) with an Agilent 1100 HPLC system by Agilent Ltd. In the initial step, the samples were hydrolyzed within glass tubes for a duration of 12 h, utilizing 6 M HCl at a temperature of 120 °C. The resulting digests were filtered through a 0.22 μm pore size filter. The separation procedure was conducted using a Zorbax analytical column (C18×250 mm; Agilent) at a temperature of 40 °C, with UV detector spectra monitored at 338 nm. The elution process was carried out using two mobile phases: sodium acetate/triethylamine/tetrahydrofuran (400:0.10:2, v/v/v) adjusted to pH 7.1 with acetic acid, and sodium acetate/methanol/acetonitrile (1.5:2.5:2.5, v/v/v) also adjusted to pH 7.1. The elution occurred at a flow rate of 1 ml/min. An external standard was used in the form of a solution containing 17 amino acids.

2.6. Preparation of muffin

The muffins were prepared following the method of Cermeño et al. (2021) with slight modifications. The formulation included 65 g of wheat flour, 37.5 g of sucrose, 3 g of baking powder, 0.61 g of salt, 1 small egg, 20 mL of corn oil, and 30 mL of water. Initially, the BPH was ground for 1 min and sieved to remove any large particles before being added to the dry ingredients. To prepare the functional muffins, BPH was substituted for wheat flour at varying levels of 2, 4, and 6 % (w/w). Wet ingredients, including water, corn oil, and wipped egg were mixed with the dry ingredients for 2 min using a Braun Hand Blender (Braun, Waiblingen, Germany). Four different muffin batters were prepared, including a control without BPH and batters with BPH at 2, 4, and 6 % (w/w). The muffin mixture was poured into muffin cups and subsequently baked in a preheated oven for 15 minutes. Following the baking process, the muffins were allowed to cool down and then placed into plastic bags, which were kept at room temperature for a duration of 14 days.

2.7. Extraction of bioactive compounds

To extract the bioactive compounds, the method outlined by Kaur and Kaur (2018) was applied. Firstly, hexane was used to defat the muffins, which were then air-dried at room temperature for one day. Following this, the defatted samples were immersed in methanol and agitated on an orbital shaker (HY-4A laboratory digital orbital shaker, China). The resultant mixture was then subjected to centrifugation, and the supernatant was used for conducting antioxidant and antidiabetic assays.

2.8. Antioxidant properties and lipid oxidation of muffins

2.8.1. DPPH radical scavenging activity

The methodology described by Karigidi, Akintimehin, Akinyemi, Fapetu, and Adetuyi (2022) was used to determine the DPPH radical scavenging activity present in the defatted muffin extract. Initially, the extract of each muffin sample was mixed with a DPPH solution in methanol, which was then stored in a dark environment at a temperature of 25 °C. The samples' ability to scavenge DPPH free radicals was evaluated at 517 nm using a UV-Vis spectrophotometer (Agilent, Cary 60 Uv-Vis, USA). To establish a Trolox standard curve, varying concentrations ranging from 50 - 1100 micromolar in methanol were employed. The DPPH radical scavenging activity was reported in terms of micromoles of Trolox equivalents per gram of muffin.

$$Y = -0.0005X + 0.7094 \ (R^2 = 0.99) \quad (1)$$

2.8.2. Peroxide value

The sodium thiosulfate method was used to determine the peroxide value (PV) of the samples (Mahmoudi, Tavakoilpour, Roozbeh-Nasiraie, & Kalbasi-Ashtari, 2020). PV of each muffin sample was calculated at 7 day intervals during the 14 day storage period and expressed as milli-equivalent peroxide per kilogram. The PV value was calculated using the following equation.

$$PV\ (meq\ /\ kg) = (S \times N)/W \times 1000 \quad (2)$$

In the given equation, 'S' represents the volume of the titrant solution used in milliliters, 'N' represents the normality of the sodium thiosulfate solution (0.01 N), and 'W' represents the weight of the sample in kg.

2.8.3. Thiobarbituric acid reactive substances (TBARs)

The TBA tests for the muffin samples were carried out in accordance with the procedure outlined by Ojagh, Rezaei, Razavi, and Hosseini (2010). Each sample's absorbance (As) was measured at 530 nm after being prepared with a butanol solution and compared to a blank or water sample (Ab). The TBA value was determined for each muffin sample every 7 days over a 14 day storage period. The TBA value was calculated for each muffin sample using the provided equation in mg MDA eq/kg .

$$TBA = ((A_s - A_b) \times 50)/200 \quad (3)$$

2.9. Texture analysis

The texture profile of the muffins was evaluated using the Brookfield CT 3 Texture Analyzer, as described by Goswami, Gupta, Mridula, Sharma, and Tyagi (2015). A TA4/1000 probe (cylinder, 38.1 mm D, and 20 mm L) and a 100 N load cell were attached to the CT 3 Texture Analyzer. The muffin crust was removed, and 2×2×2 cm cubes of the crumb were cut for analysis. The muffin samples underwent a double compression test with a 50 % target deformation, 2 mm/s pre-test speed, 1 mm/s post-test speed, and 6 g trigger load. The texture was assessed by measuring the crumb's hardness.

2.10. Color analysis

The color values (L*, a*, and b*) of the muffin crumb and crust were measured using a colorimeter (Color Flex, Hunter Lab Inc. USA). The L* value reflects the level of whiteness (100) or blackness (0) of a color. The a* value indicates red (positive value) or green (negative value), while the b* value represents the amount of yellow (positive value) or blue (negative value). In addition, the difference in color between the control samples and those containing BPH was determined using the formula below:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + [(\Delta b)^2]^{1/2} \quad (4)$$

The following interpretation was given for the values: if ΔE is less than 1, then the human eye cannot easily perceive any differences in color; if ΔE is between 1 and 3, then the differences in color are not noticeable to the human eye; and if ΔE is greater than 3, then the human eye can clearly distinguish the differences in color (Jarpa-Parra et al., 2017)

2.11. Rat α-glucosidase and α-amylase assays

The inhibition of rat α-glucosidase and α-amylase enzymes was carried out using the methods described by Mirab, Gavlighi, Sarteshnizi, Azizi, and Udenigwe (2020).

2.12. Consumer sensory evaluation

The method described by Goswami et al. (2015) was used to carry out a sensory analysis of the muffin samples. A consumer panel, comprising 51 individuals (25 men and 26 women), was randomly selected from the potential customers within the target markets. The chosen participants, aged between 25 and 50, were employees of the Zarmacaron Industrial Company in Alborz, Iran. This panel was engaged to assess the sensory attributes of the muffin samples, encompassing factors such as texture, flavor and odor, color, general appearance, and overall acceptance.The study employed a 9-point hedonic scale with a range of 1 to 9, where 1 indicated dislike extremely, 2 dislike very much, 3 dislike moderately, 4 dislike slightly, and 5 neither like nor dislike, 6 like slightly, 7 like moderately, 8 like very much, and 9 like extremely. Prior to testing and between samples, the panelists were directed to rinse their mouths with water.

2.13. Statistical analysis

The experiments were conducted three times, and the results were expressed as the mean value ± standard deviation (SD). Minitab 16 (Minitab Inc., State College, PA, USA) software was employed for statistical analysis. One-way analysis of variance (ANOVA) and Tukey test were used to compare the variations among samples ($p < 0.05$). The graphs were created using Excel 2013 software.

3. Results and discussion

3.1. Proximate composition of BSG and BPC

The proximate composition of both the BSG and BPC samples is presented in Table 1S. According to Table 1S, BSG contained 21.59 % protein, 7.48 % fat, 3.77 % ash, and, accounting for the difference, 67.16 % carbohydrates. BSG proteins were enriched in the BPC fraction, which comprised 64.54 % proteins, 2.20 % fat, 2.12 % ash, and the remaining 29.14 % accounted for carbohydrates (on a dry basis). The protein content of BPH was 71.25 %.

3.2. In vitro Antioxidant properties and lipid peroxidation of muffins

3.2.1. Free radical scavenging capacity (DPPH)

As depicted in Fig 1, the inclusion of BPH concentrate in the formulations led to a significant enhancement in the antioxidant activity of the muffins. With increasing of BPH concentrate in muffin formulations from 2 to 6 %, the radical scavenging activity notably rose to 238.85 μmol TE/mg which was ~ six times higher than the control sample. This result agrees with Karimi et al. (2021), who found that adding protein hydrolysate from maize germ to bread increased its antioxidant activity compared to the control bread. The type of amino acid present in

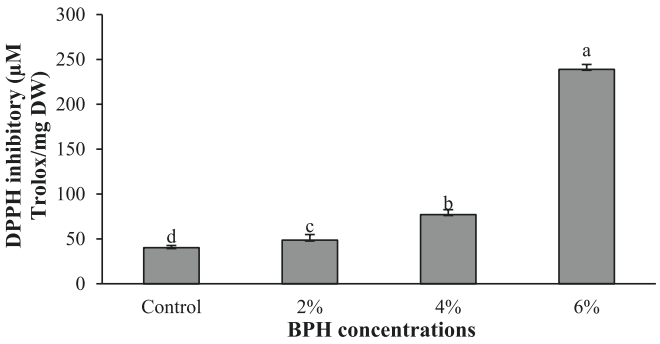

Fig. 1. Effect of brewers' spent grain protein hydrolysate (BPH) on the antioxidant activity of muffin samples. Data are means ± SD of three replicates. Values with different letters are significantly different ($p < 0.05$).

hydrolysates could also influence antioxidant activity. The amino acid composition of BPH is described in Table 2S. Hydrophobic amino acids were observed to be potent antioxidants, probably because of their ability to stabilize free radicals by donating protons (Nourmohammadi, SadeghiMahoonak, Alami, & Ghorbani, 2017). This result is explained by the fact that Flavourzyme, as an endoprotease enzyme, has a preference for the production of hydrophobic amino acids, which may interact with the active site of these enzymes and provide an inhibitory effect (Sinthusamran, Benjakul, Kijroongrojana, & Prodpran, 2019). Moreover, the existence of aromatic amino acids such as tyrosine and phenylalanine in protein fractions with high radical scavenging properties contributes to the antioxidant activity of BPH concentrate. The donation of protons to electron-deficient species while maintaining stability via resonance structures gives aromatic amino acids the ability to scavenge free radicals (Table 2S). On the other hand, the control sample had some antioxidant activity. This suggests that Maillard reaction products, which are formed during food processing and storage, could function as natural antioxidants to prevent or slow down lipid oxidation in food (Mohan, Udechukwu, Rajendran, & Udenigwe, 2015).

3.2.2. Oxidative stability

The peroxide (PV) and thiobarbituric acid reactive substances (TBARs) values are typically employed to identify primary and secondary forms of oxidation products (Manju, Jose, Gopal, Ravishankar, & Lalitha, 2007). The changes in the PV and TBARs values of muffins during storage with the addition of BPH to the formulation are presented in Table 1. The PV exhibited an increase, reaching its peak on the seventh day, after which it decreased during the remaining storage period. On the other hand, the TBARs value showed a significant increase during the 14 day storage period. The PV and TBARs values of the BPH muffins were lower than the control muffin at the end of the storage period ($p < 0.05$). A similar result was reported by Peng, Kong, Xia, and Liu (2010), who showed that an increase in protein concentration, along with peptides, resulted in a decline in both PV and TBARs values. These results can be attributed to the fact that enzyme hydrolysis causes a breakdown in the protein structure, resulting in the creation of active and available amino acid residues that can effectively prevent the oxidation of unsaturated fatty acids (Kong & Xiong, 2006). Moreover, polypeptides can act as physical barriers by surrounding lipid droplets and forming a membrane, which can prevent droplets from undergoing oxidation (Kong & Xiong, 2006). Endoproteases such as Flavourzyme are known to selectively produce hydrophobic amino acids, which have a higher affinity for binding with oil (Bazsefidpar et al., 2023).

3.3. Textural analysis

The texture analysis revealed that as BPH addition increased, muffin hardness reduced for all samples (Table 2). The results showed that the addition of BPH to the muffin formulation reduced the hardness induced by staling. This is consistent with previous study by Karimi et al. (2021) who also observed a reduction in hardness with the addition of hydrolysates to bread formulation. This phenomenon is attributed to the interaction between hydrophilic groups in the hydrolysates and starch molecules for water molecules during the gelatinization process. This competition leads to incomplete gelatinization, resulting in a softer texture, as explained by Karimi et al. (2021).

Table 1
Effect of brewers' spent grain protein hydrolysate (BPH) on the peroxide (PV) and thiobarbituric acid reactive substances (TBARs) values of muffin samples.

Treatments	Day 1	Day 7	Day 14
Peroxide value (meq O_2/kg)			
Control (0 %)	0.02±0.01 ^{Ac}	2.17±0.08 ^{Aa}	1.89±0.04 ^{Ab}
2 %	0.01±0.02 ^{Bc}	1.42±0.07 ^{Ba}	0.82±0.04 ^{Bb}
4 %	0.01±0.02 ^{Bc}	0.98±0.12 ^{Ca}	0.65±0.05 ^{Cb}
6 %	0.01±0.01 ^{Bc}	0.75±0.02 ^{Da}	0.42±0.05 ^{Db}
TBARs value(mg MDA eq/kg)			
Control (0 %)	0.02±0.12 ^{Ac}	0.10±0.06 ^{Ab}	2.04±0.03 ^{Aa}
2 %	0.02±0.13 ^{Ac}	0.66±0.06 ^{Bb}	0.98±0.07 ^{Ba}
4 %	0.01±0.06 ^{Bc}	0.29±0.02 ^{Cb}	0.74±0.07 ^{Ca}
6 %	0.01±0.03 ^{Bc}	0.18±0.03 ^{Db}	0.32±0.02 ^{Da}

Data are means ± SD of three replicates. $^{A-D}$ significance difference in the rows ($p < 0.05$). $^{a-c}$ significance difference in the columns ($p < 0.05$).

Table 2
Effect of brewers' spent grain protein hydroly hydrolysate sae (BPH) on the hardness of muffin samples.

Treatments	Day 1	Day 7	Day 14
Hardness (N)			
Control (0 %)	53.00± 0.01^{Ac}	65.00± 0.12^{Ab}	88.00± 0.04^{Aa}
2 %	32.00± 0.05^{Bc}	36.00± 0.10^{Bb}	46.00± 0.07^{Ba}
4 %	21.00± 0.01^{Cc}	31.00± 0.20^{Cb}	37.00± 0.05^{Ca}
6 %	19.00± 0.01^{Dc}	25.00± 0.03^{Db}	32.00± 0.04^{Da}

Data are means ± SD of three replicates.
$^{A-D}$ significance difference in the rows ($p < 0.05$). $^{a-c}$ significance difference in the columns.

3.4. Color characteristics

Table 3 represented the effect of adding BPH on the color characteristics of muffin crust and crumb. Compared to the control muffin, the fortified muffin had significantly different crust and crumb colors ($p < 0.05$). The results in Table 3 showed that the control muffins had higher L* values for both crust and crumb color, whereas the a* and b* values were lower compared to muffins substituted with BPH ($p < 0.05$). Singh et al. (2016) observed a brownish color in biscuits with the addition of shrimp protein hydrolysate, which can be attributed to the occurrence of the Maillard reaction between the hydrolysate and reducing sugars. This reaction is affected by peptides and hydrolysates with a high number of free amino groups and has a significant impact on flavor and color development. The final stage of the Maillard reaction results in the condensation of carbonyl compounds and amines, leading to melanoidin formation and color development. Additionally, the calculated ΔE values in this study revealed a noticeable color difference that can be easily observed by the human eye, indicating a significant improvement in muffin color with increasing BPH concentration in the formulation (Table 3).

3.5. In vitro Antidiabetic property of supplemented muffins

The enzymes α-amylase and α-glucosidase play a key role in breaking down starch into glucose for absorption after a meal, making them a target for diabetes management. According to the Fig 2, the inhibition of α-amylase and α-glucosidase in muffins was significantly improved with increasing BPH concentrate in the muffin formulations. As the BPH

Table 3
Effect of brewers' spent grain protein hydrolysate (BPH) on the colour properties of crust and crumb of muffin samples.

Treatments	L*	a*	b*	ΔE
Crust				
Control (0 %)	68.85±0.01^{a}	10.56±0.02^{d}	40.49±0.04^{d}	
2 %	53.19±0.04^{b}	19.59±0.12^{c}	41.10±0.30^{c}	18.08
4%	50.56±0.14^{c}	22.28±0.02^{b}	48.93±0.03^{b}	23.30
6 %	49.44±0.07^{d}	23.90±0.05^{a}	53.41±0.06^{a}	26.86
Crumb				
Control (0 %)	78.43±0.02^{a}	4.52±0.07^{d}	31.69±0.05^{c}	
2 %	73.5±0.01^{b}	7.17±0.04^{c}	31.67±0.05^{c}	6.07
4 %	72.88±0.06^{c}	8.07±0.02^{b}	34.14±0.02^{b}	6.61
6 %	68.9±0.08^{d}	8.58±0.01^{a}	36.32±0.04^{a}	11.33

Data are means ± SD of three replicates. Values with different lowercase letters in the same columns are significantly different ($p < 0.05$).

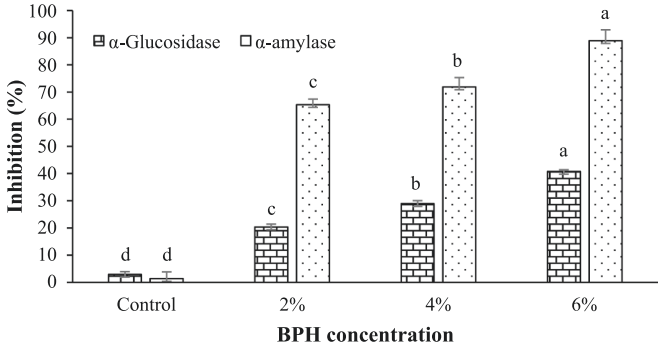

Fig. 2. Effect of brewers' spent grain protein hydrolysate (BPH) on the α-amylase and α-glucosidase inhibitory properties of Muffin samples. Data are means ± SD of three replicates. Values with different letters are significantly different ($p < 0.05$).

concentrate increased up to 6 %, the α-amylase and α-glucosidase inhibition also enhanced up to 88.87 % and 40.81 %, respectively. The inhibitory activity of peptides may be influenced by the composition of the amino acid residues. The inhibitory peptides are thought to interact more with the active site of α-amylase and α-glucosidase via hydrogen bonds and electrostatic interactions. Therefore, the presence of amino acids with hydroxyl groups (serine, threonine and tyrosine) or basic amino acids (lysine and arginine) at the amino end of the peptides could play a critical role in alpha-glucosidase inhibition (Table2) (Karimi, Azizi, & Ahmadi Gavlighi, 2020). Similar results have been reported in previous studies by Bazsefidpar et al. (2023), who also observed the inhibitory activities of BPHs towards α-amylase and α-glucosidase. Furthermore, Karimi et al. (2021) reported that the addition of maize germ protein hydrolysate to bread increased the inhibitory potential against α-amylase activity. This could be due to the interaction or binding of the hydrolysate to the enzyme's active site, interrupting the enzyme-substrate interaction, or binding to the allosteric site in the enzyme structure, such as calcium and chloride ion sites, resulting in an unstable conformation that could disrupt α-amylase activity.

3.6. Sensory analysis of muffins

The sensory characteristics of the muffin samples were evaluated using a 9-point hedonic scale, and the results are presented in Table 4. The muffins without BPH and with 2 % BPH received the highest scores for all sensory attributes assessed, including texture, color, flavor and odor, general appearance, and overall acceptance among fortified samples. Conversely, the muffins with 6 % BPH received the lowest scores. This can be explained by the disruption of disulfide bonds among gluten proteins due to the addition of protein, leading to reduced trapped air within the gluten network and a deficiency of gluten-forming proteins (Prieto-Vázquez del Mercado, Mojica, & Morales-Hernández, 2022). In terms of taste and smell, the presence of low molecular weight hydrophobic amino acids at increased levels of BPH could be the primary cause of muffin bitterness (Kriisa et al., 2022). The interaction between ingredients during baking, possibly due to intensified Maillard browning reactions and higher BPH fortification, resulted in darker muffins with a darker crust and crumb at higher BPH levels (El Sohaimy, Brennan, Darwish, & Brennan, 2021). However, all samples, including both the control and those containing BPH, attained scores higher than 6.5 for all the sensory attributes evaluated. The fortified muffin with 2 % had no significant effect on sensory properties. These findings are consistent with previous studies that investigated the sensory properties of baked goods fortified with protein hydrolysates, such as whey and casein hydrolysates and Pacific white shrimp hydrolysates (Gani et al., 2015; Sinthusamran et al., 2019). However, the reduced sensory evaluations observed in baked goods fortified with protein hydrolysates may be attributed to the presence of bitter peptides, which can cause a bitter aftertaste.

Table 4
Effect of brewers' spent grain protein hydrolysate (BPH) on the sensory attributes of the muffin samples.

Treatments	Texture	Flavor and odor	Color	General appearance	Overall acceptance
Control (0 %)	8.70 ±1.22^{a}	8.60 ±1.18^{a}	8.40 ±1.33^{a}	8.75±1.32^{a}	8.45±1.22^{a}
2 %	8.62 ±1.06^{a}	8.33 ±1.23^{a}	8.12 ±1.45^{a}	8.11±1.24^{a}	8.22±1.38^{a}
4 %	7.60 ±1.02^{b}	7.20 ±1.24 ^{b}	7.10 ±1.28^{b}	7.10±1.04^{b}	7.10±1.32^{b}
6 %	6.53 ±1.23^{c}	6.68 ±1.45^{c}	6.50 ±1.19^{c}	6.54±1.20^{c}	6.62±1.26^{c}

Data are means ± SD of three replicates. Values with different letters are significantly different ($p < 0.05$)

4. Conclusion

In this study, BPH was added to muffins as a natural ingredient with potential antioxidant and antidiabetic properties. Results showed that the addition of BPH up to 6 % significantly improved the radical scavenging activity of the fortified muffins by ~ six times compared to the control. Muffins fortified with the highest concentration (6 %) exhibited lower PV and TBARs values throughout storage compared to the control, suggesting enhanced oxidative stability. In addition, as the concentration of BPH rose to 6%, the inhibition of α-amylase and α-glucosidase also showed notable improvements, reaching 88.87 % and 40.81 %, respectively. The muffins without BPH and with 2% BPH received the highest scores for all sensory attributes assessed, including texture, color, flavor, odor, general appearance, and overall acceptance among fortified samples, whereas the muffins with 6 % BPH received the lowest scores. Overall, BPH has the potential to be used as an added value ingredient in the bakery industry to enhance the functional properties of baked goods.

CRediT authorship contribution statement

Nooshin Bazsefidpar: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Amir Pouya Ghandehari Yazdi:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization. **Amin Karimi:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Matin Yahyavi:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Mahdi Amini:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Hassan Ahmadi Gavlighi:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Jesus Simal-Gandara:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137565.

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