PILSEN, RED ALE AND STOUT BREWER'S SPENT GRAIN: POLYPHENOLS, ANTIOXIDANT AND ANTIHYPERTENSIVE ACTIVITIES, AND PHYSICOCHEMICAL PROPERTIES

FLÁVIO FONCECA VERAS*
GABRIELA DIERSMANN AZEVEDO**
VOLTAIRE SANT ANNA***
ADRIANO BRANDELLI****

O objetivo deste trabalho foi avaliar a concentração de compostos fenólicos, a capacidade antioxidante e anti-hipertensiva, e as características físico-químicas de bagaço de cervejas Pilsen, Red Ale e Stout. Polifenóis totais, ácidos fenólicos e flavonóis totais foram medidos espectrofotometricamente, enquanto a atividade antioxidante foi avaliada por ABTS, DPPH e poder quelante de ferro. Os resíduos de cerveja foram avaliados quanto às capacidades de retenção de água e óleo, pH e cor. Os resultados indicaram que os subprodutos estudados apresentaram uma alta concentração de polifenóis totais, flavonóis e ácidos fenólicos. A avaliação do potencial antioxidante revelou até 60,9% de eliminação de radicais ABTS, 12,3% de radicais DPPH e 12,4% de poder quelante de ferro. Além disso, observou-se inibição de 64,6% da enzima conversora de angiotensina-I, sugerindo capacidade anti-hipertensiva. Os resultados da capacidade de absorção de água e óleo indicaram que os pós do resíduo apresentaram valores semelhantes a farinhas de cereais. Os valores do CIELab mostraram que o bagaço Pilsen apresentou cor amarelo claro, enquanto o Red Ale e Stout demonstrou cor escura. O pó de bagaço de cerveja apresentou valores de pH inferiores a 5,0, indicando condições ácidas, as quais podem contribuir para a estabilidade bacteriana em armazenamento de médio a longo prazo do resíduo seco. O presente trabalho mostrou que os bagaços de cerveja secos podem ser uma fonte alternativa de compostos funcionais, representando uma maneira sustentável de gerenciamento de resíduos industriais.

PALAVRAS-CHAVE: RESÍDUO DE CERVEJA; POLIFENÓIS; GESTÃO DE RESÍDUOS; COMPOSTOS FUNCIONAIS

*UERGS-Encantado, Rua Alegrete 821, ZIP-code 95960-000 Encantado, Brazil; telefone: (51) 981405159; e-mail: nutricao@educteutonia.com.br

**UERGS-Encantado, Rua Alegrete 821, ZIP-code 95960-000 Encantado, Brazil; telefone: (51) 37513376; Lattes ID: http://lattes.cnpq.br/4566525016636030

***UERGS-Encantado, Rua Alegrete 821, ZIP-code 95960-000 Encantado, Brazil; telefone: (51) 37513376; e-mail: voltaire-santanna@uergs.edu.br

****UFRGS, Av. Bento Gonçalves 9500, ZIP-code 91501970 - Porto Alegre, Brasil; telefone: (51) 33086249 Lattes ID: http://lattes.cnpq.br/2645681829598421

*****UFRGS, Av. Bento Gonçalves, 9500 laboratorio 218, ZIP-code 91501970; telefone: (51) 33086249 Lattes ID: http://lattes.cnpq.br/8126172449861016

1. INTRODUCTION

Upcycling economy is a huge trend worldwide, mainly for food industries since they generate great amounts of organic solid residues, which management is a big challenge. However, they may represent a great source of functional ingredients for the human diet or as a source of interesting compounds in pharmaceutical applications (BARBOSA-PEREIRA et al., 2014; LYNCH et al., 2016).

Brewers' spent grain (BSG) is the organic solid part of the brewery process, obtained after malt-controlled cooking conditions and filtration of the wort. The production of 100 L of beer generates around 20 kg of this residue, which represents 85% of the industrial brewing residue and is currently disposed to animal feeding. However, several studies indicate that, after proper processing, BSG may be a source of commercial high-value products (XIROS; CHRISTAKOPOULOS, 2012; KTENIOUDAKI et al., 2015; LYNCH et al., 2016). Mussato, Dragone and Roberto (2006) observed that BSG is an interesting source of proteins and fibers since up to 25% of its composition corresponds to proteins. BSG also showed considerable quantity of vitamins such as folic acid, niacin, biotin, thiamine, choline, riboflavin. Additionally, it presents tocopherols which are vitamin E precursors (IKRAM et al., 2017). Regarding the polyphenol content, BSG is a phenolic acid-rich source, mainly composed by catechins, epicatechins, gallic acid, *p*-coumaric acid and ferulic acid (MUSSATO; DRAGONE; ROBERTO, 2006; IKRAM et al., 2017).

During malt production, the utilization of different barley germination conditions, kilning and roasting temperature induces changes in the profile of antioxidant compounds. A significant decrease of bound phenolics takes place with a corresponding increase of soluble esterified fraction, summed to thermal degradation and formation of Maillard reaction compounds (DVOŘÁKOVÁ et al., 2008; ČECHOVSKÁ et al., 2012; MOREIRA et al., 2013). Some studies have been performed evaluating single BSG, such as Pilsen, Melano, Caramel Black, Chocolate, Carared (DVOŘÁKOVÁ et al., 2008; ČECHOVSKÁ et al., 2012; MOREIRA et al., 2013), although the residue of beer production is a combination of several malts. Additionally, comparative studies on the concentration of bioactive compounds, and properties such as color, water hydration and pH of the byproduct powder are little reported. These properties are important since they may impact the processing characteristics considering the utilization of BSG as a functional ingredient in products such as bread, pasta, cookie manufactures.

Thus, the objective of the present work is to evaluate polyphenolics content, the antioxidant and anti-hypertensive capacity, color, water holding capacity and pH of the brewer's spent grain from Pilsen, Red Ale and Stout beers.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Spent grain from Pilsen, Red Ale and Stout beers were obtained in the laboratory to control all previous processing steps. For Pilsen beer, 2.3 kg of Pilsen malt was used; for Red Ale beer, the combination malt used was: 1.7 kg of Pilsen, 0.2 kg of Melano 80, 0.2 kg of Cararred 20, 0.2 kg of Cara1 20; for the Stout beer, the malt combination used was: 1.8 kg of Pilsen, 0.1 kg of Cara1 20, 0.2 kg of black, 0.2 kg of pale ale. Malts were purchased in local specialized (Porto Alegre, RS, Brazil). All beer production was performed by mixing the malts with 7 L of mineral water for 42-45ºC for 15 min, then 52-55ºC for 25 min and finally for 62-65ºC for 15 min. After, the system was heated up to 82-85ºC for 5 min, when the washing process was performed. After 30 min of washing and wort filtration, it was added to the malt 8 L of 80ºC mineral water and washed for 30 min. BSG were dried in a static air oven at 60ºC (LARRAURI et al., 1997) up to moisture content of 12% (wet basis), milled in an industrial blender (model LI-2N, Skymsen, Brazil) and sieved to 5 mm particle size. Byproduct

powders were stored in sterile dark polyethylene bags at -18ºC until utilization.

2.2. POLYPHENOLS, ANTIOXIDANT AND ANTI-HYPERTENSIVE ACTIVITIES

Extraction of polyphenols and compounds with antioxidant activity from dried samples was performed like suggested by Sant'Anna et al. (2012). Briefly, 1 g of dried spent grain was kept in 50 mL of ethanol: water (50:50, v/v) for 30 min at 60ºC. Folin-Ciocalteau reagent (2 M) and gallic acid (99% of purity) were obtained from Vetec Química Fina (Duque de Caxias, RJ, Brazil). All other chemicals were purchased from Neon (Suzano, SP, Brazil).

Total phenolic content was determined by diluting 40 µL of extract in 3.2 mL of distilled water and 200 µL of the Folin-Ciocalteau reagent and maintained in the dark for 5 min. Then 600 µL of a saturated solution of sodium carbonate was added and allowed to react for 1.5 h in the dark, when absorbance was measured at 765 nm (SINGLETON; ROSSI, 1965) by UV-1600 spectrophotometer (Pró-Análise, Brazil) and constrained to a calibration curve of gallic acid. Results were expressed as mg gallic acid equivalent per 100 gram of dry bagasse weight (mg GAE 100g-1). The total flavonols and phenolic acids were determined following the procedure described by Mazza et al. (1999). Briefly, hydrochloric acid and ethanol solutions were added to the extracts and the absorbance at 360 and 320 nm was measured for total flavonoids and phenolic acids, and the results were expressed as mg quercetin equivalent (mgQE) and mg caffeic acid equivalent (mgCAE) per 100g bagasse dry for TF and PA, respectively.

Antioxidant analyses were performed by the determination of 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging activity (RE et al., 1999), which involves the generation of ABTS radical chromophore by the oxidation of ABTS with potassium persulfate. The ABTS radical cation was produced by reacting 7 mmol L-1 ABTS stock solution with 140 mmol L-1 potassium persulfate, and allowing the mixture to stand in the dark for 16 h at room temperature before use. For the assay, the ABTS•+ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. An aliquot of 30 µL of extract was mixed with 1 mL of $ABTS^{\bullet+}$ solution and an absorbance (734 nm) reading was taken after 6 min. Distilled water, instead of a sample, was used as a control. The results were expressed as: scavenging activity (%) = $[1 - (A/A_0)] \times 100$, where A is the absorbance of the test and A_0 is the absorbance of the control.

The 1,1-diphenyl-2-picrilhidrazil (DPPH) antioxidant activity was evaluated according to Brand-Williams, Cuvelier and Berset (1995). In the dark, aliquots of 0.1 mL sample were transferred to test tubes with 3.9 mL radical DPPH (60 µmol L-1 DPPH solution, diluted in methyl alcohol). After 45 min, the scavenging activity was measured spectrophotometrically by the decrease in absorbance at 517 nm. Likewise, the same proportions (0.1 mL distilled water and 3.9 mL DPPH radical) were used as a control, using methyl alcohol as blank. The results were expressed as scavenging activity (%) = $[1-(A/A0)]\times100$, where *A* is the absorbance of the test and *A*0 is the absorbance of the blank.

The chelating capacity of Fe2+ was measured using the method described by Chang, Wu and Chiang (2007), which involves reaction of the extract with FeSO4 (Fe2+) and ferrozine, following by absorbance measure at 562 nm. The results were expressed as chelating capacity (%) compared to the control (distilled water).

Antihypertensive activity was evaluated by the Angiotensin-Converting Enzyme (ACE) procedure (CUSHMAN; CHEUNG, 1971), with slight modifications. Aliquots of 20 µL of the extracts were added to 200 µL of buffered substrate solution (5 mmol L-1 hippuryl-histidyl-leucine in 50 mmol L-1 HEPES-HCl buffer containing 300 mmol L-1 NaCl, pH 8.3). The reaction started by adding 40 µL of angiotensin I-converting enzyme (0.1 U mL-1) to the described system and maintained at 37ºC. Reaction was stopped after 30min with addition of 150 µL of 1 mol L-1 HCL. Then, the hippuric acid release was extracted with 1 mL of ethyl acetate, and the organic phase was transferred to a glass tube heat evaporated. The residue was dissolved with 800 mL of distilled water and measured at 228 nm. Residue extract inhibitory activity was expressed as percentage according to the Equation 1.

Where, A is the absorbance without sample, B is the absorbance without the enzyme and C is the absorbance with the sample and the enzyme.

2.5 WATER AND OIL HOLDING CAPACITY

Water and oil holding capacity was determined according to Rosell, Santos and Collar (2009) with few modifications. Briefly, 0.5 g (±0.1 mg) of dried residue powder were suspended in 10 mL distilled water or commercial soy oil and let them to hydrate overnight. The system was centrifuged and the supernatant weighted. Liquid absorption capacity was calculated by the difference of liquid added and not absorbed. Moisture was discounted and results expressed as grams of water/oil absorbed per gram of dried sample.

2.6 PH

To determine the samples pH, 1 g of the residue was diluted in 7.5 mL boiled and cooled distilled water for 1 h gently shaking every 15 minutes. The system was filtered on Whatman paper No. 1 and the aqueous extract was submitted to pH analysis (Universal, model MT-610) (AOAC, 1999).

2.8 COLOR

CIELAB parameters were determined using D-65 diffused illumination of a Minolta Chroma CR-400 Colorimeter. The instrument was calibrated with a standard white plate. The measured parameters were L^* (lightness), a^* (redness), and b^* (yellowness).

2.9 STATISTICAL ANALYSIS

Obtained values were compared using Tukey's test by Microsoft Excel 2000 (MapInfo Corporation, Troy, NY, USA), and differences were considered statistically significant when $p<0.05$. Graphical plots were performed using Microsoft Excel 2000 (MapInfo Corporation, Troy, NY, USA).

3 RESULTS AND DISCUSSION

3.1 POLYPHENOLICS AND COMPOUNDS WITH ANTIOXIDANT AND ANTIHYPERTENSIVE ACTIVITY

The total phenolic content, flavonols, phenolic acids, antioxidant and antihypertensive activity of the dried residues are presented in Table 1. In the present work, BSG used were those after washing process and before must fermentation, thus the beer residues studied were the combination of different malts.

TABLE 1. CONCENTRATION OF POLYPHENOLICS AND COMPOUNDS WITH ANTIOXIDANT AND ANTIHYPERTENSIVE CAPACITY FROM PILSEN, RED ALE AND STOUT BAGASSE.

	Pilsen	Red Ale	Stout
Total Polyphenols (mg GAE 100g-1)	3140.3±223.8a	24792±99.7b	2351.6±417.0b
Flavonols (mg QE 100g-1)	7111.40±22.3a	7072.9±52.6a	7124.0±198.7a
Phenolic Acids (mg CAE 100g-1)	1296.33±128.9a	926.8±65.7b	782.1±51.3c
ABTS (%)	60.9±1.3a	45.1±2.0b	47.1±2.4b
DPPH (%)	10.1±0.2a	9.4±1.0a	12.3±2.1a
Chelating	2.2±0.1b	12.4±0.6a	11.7±1.4a
Antihypertensive (%)	64.6±1.5a	31.7±3.1b	30.9±1.5b

[a,b,c] different superscript letters among columns indicate significant differences (p<0.05)

The results show that the byproduct from the Pilsen beer process presented about 3140 mg GAE 100g^{-1} (d.b.) of total polyphenols which was higher (p<0.05) than those observed in Red Ale and Stout residues. There was no significant difference (p>0.05) between total polyphenolics from Red Ale and Stout bagasse. Similar results were observed for phenolic acids: the dry matter from Pilsen beer presented higher (p<0.05) concentration (1296 mg CAE 100g^{-1} d.b.) than those from Red Ale and Stout. Red Ale residue presented higher (p<0.05) concentration of phenolic acids then the Stout bagasse. The concentration of total flavonols was not affected significantly (p>0.05) among the BSG evaluated. Birsan et al. (2019) observed that ferulic acid was the most predominant phenolic acid comprising over of 50% of the total polyphenols followed by p-coumaric acid in light, dark and mixed BSG. Moreira et al. (2013) observed that malt bagasse from Pilsen, Melano, Melano 80 and Carared presented higher amounts of phenolic compounds when compared to chocolate and black bagasse (19.5 and 16.2 mg GAE g^{-1} d.b. for light and dark types, respectively). Samaras et al. (2005) found that catechin and ferulic acid were the most abundant phenolic compounds in killed and roasted malts, and that ferulic acids were highly sensitive to degradation while roast treatment.

Results from antioxidant activity showed that residue from Pilsen beer production presented the capacity to scavenge 60.9% of ABTS radicals which was higher (p<0.05) than those compounds extracted from Red Ale and Stout (45.1% and 47.1%, respectively). Fărcaş et al. (2015) observed that the antioxidant activity in BSG is directly proportional to the darkening of the malt color, mainly due to compounds degradation during roasting temperature higher than 150ºC. Meanwhile, the compounds with the ability to scavenge DPPH free radicals were not different (p>0.05) among the samples (Table 1). In roasted malts, Maillard reaction products were responsible for the majority of the antioxidant activity (SAMARAS et al., 2005). On the other hand, chelating power from Stout and Red Ale bagasse extracts presented higher (p<0.05) values than from Pilsen residue. The superior reducing power of the darker caramel malts was partly due to the presence of a Maillard-derived 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one (DDMP), which was responsible for 21-55% of their electrochemical capacity (ČECHOVSKÁ et al., 2012). McCarthy et al. (2012) observed that phenolic extract from BSG presented DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells, which was related to high concentrations of polyphenols and to iron chelation capacity.

ACE inhibition is currently used for hypertension treatment, since it is a tool to control

over activation of renin angiotensin aldosterone system (RAAS), helping to control increased blood pressure (HAMMOUD et al., 2007). Extract from Pilsen spent grain presented antihypertensive activity of 64.6%, meanwhile compounds from Red Ale and Stout presented the capacity to inhibit 31.7% and 30.9% of this enzyme associated with control of hypertension. These results are higher than the 0.33 μM solution of quercitin from apple peel extract, which presented about 11% (BALASURIVA; RUPASINGHE, 2012). The results suggest that this ability may be related to the concentration of polyphenolics and phenolic acids in the bagasse matrix. The presence of hydroxyl groups and the B ring in quercetin seems to play an important role in ACE inhibitory activity of polyphenols (BALASURIVA; RUPASINGHE, 2012).

It is important to point out that phenolic compounds are mainly located in the pericarp, and thus the solvent molecules must penetrate the cell walls of the plant tissue before reaching the solutes and thus the extraction method imply changes in polyphenol extraction yield (GUIDO; MOREIRA, 2017; ZUORRO et al., 2019).

3.2 PH, WATER AND OIL HOLDING CAPACITY AND COLOR

The average pH value and water and oil absorption capacity of the dried samples of the brewery residue is shown in Figure 1. Beer bagasse was below 5.0, which was not affected (p>0.05) by the combination of the different malts to produce the spent grains studied. Larsson and Sanderberg (1995) observed similar pH values for malted oats, indicating greater microbial stability in the matured samples due to the acidic pH of the samples. Ivanova et al. (2017) observed that Pilsener and Carafa BSG presented respectively pH of 5.64 and 4.67 and related the acid conditions to the presence of high content of phenolic acids and amino acids. Acid conditions may imply a barrier to bacterial growth and help on dried BSG shelf-life, although this condition may propitiate a good fungal growth environment, which is important for dried foods.

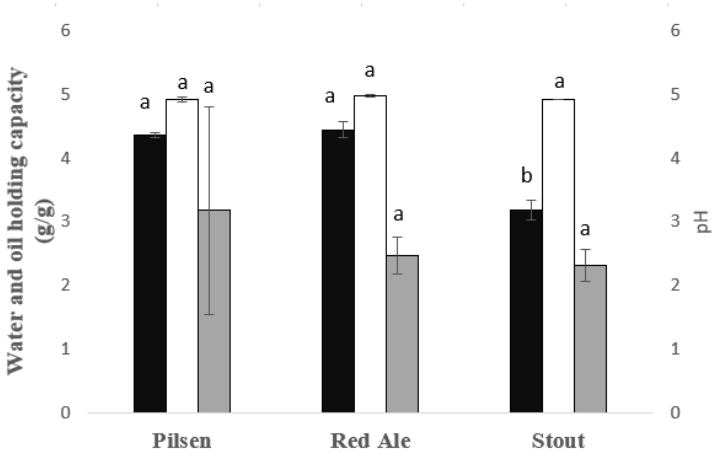

Figure 1. Values of pH (white bars), oil (grey bars) and water (black bars) holding capacity of different dried brewer's spent grain. [a,b] Different superscripts indicate statistical differences (p<0.05).

The absorption capacity of water was greater than that of oil (Figure 1). This is possibly due to the composition of this residue, rich in monosaccharides and polysaccharides and their derivatives (SANTANA; OLIVEIRA, 2005). Oil absorption capacity presented no significant difference among the samples studied (p>0.05), meanwhile water absorption capacity of Pilsen bagasse presented higher

values (p<0.05) than Red Ale and Stout spent grains. Rosell, Santos and Collar (2009) and Reis et al. (2007) observed that commercial fiber from apple, bamboo, oats and vegetable flours presented water retention capacity with values similar to those found for BSG in the present work.

Water retention capacity depends on the fiber's processing form as well as its physicochemical structure (ROSELL; SANTOS; COLLAR, 2009; COLLAR; SANTOS; ROSELL, 2007) and high temperatures may alter the physical properties of the fiber matrix, breaking the pores and increasing the density, which consequently alters the hydration properties (SANTANA; OLIVEIRA, 2005). According to Sousa and Correia (2010), a greater water absorption capacity by ingested fiber leads to a higher volume of food cake, increases the viscosity of the solutions in the gastrointestinal tract, a greater sensation of satiety, delaying the gastric emptying of meals rich in carbohydrates, thus reducing the glycemic response.

The results of the color analysis, according to the CIELab methodology, are shown in Figure 2.

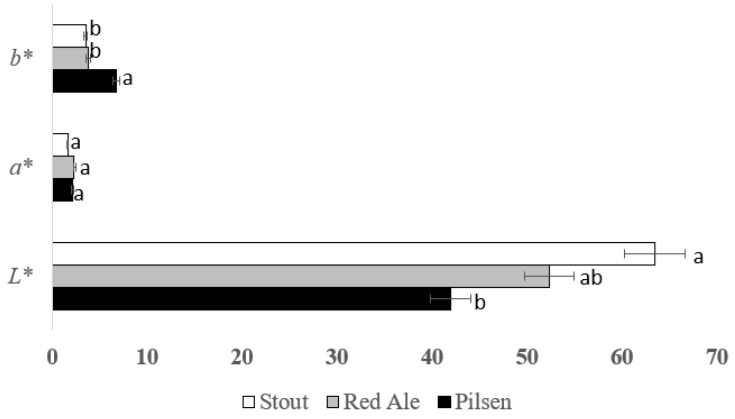

Figure 2. CIELab parameters for Pilsen, Red Ale and Stout spent grains. [a,b] Different superscripts indicate statistical differences (p<0.05).

L*-values lower than 50 indicate that samples present a dark color. Results show that, as expected, Stout and Red Ale bagasse present dark color and Pilsen byproduct a lightness color. Dried spent from Stout beer presented L*-value of 63.4, which was not significantly (p>0.05) different from those from Red Ale grains although it was higher (p<0.05) than the color parameter from the Pilsen residue (L* = 42). Carotenoids are important pigments in barleys (PAZNOCHT, et al., 2018), thus spent grains from Pilsen beer presented higher (p<0.05) b*-value (6.8). Positive b*-values are related to the yellowness of the samples, thus, possibly, the decrease on the roasted samples' parameter due to carotenoid degradation. The a*-values, related to material reddish color, was not different among the dried residues (p>0.05). Noscente et al. (2019) observed that pasta added of BSG had their yellow index enhanced, which corroborate that addition of brewery residue to food formulation may significantly impact on sensory characteristics of products. This information is important for industrial quality control parameters and must be considered.

4 CONCLUSIONS

The results of the present work showed that residues from Pilsen, Red Ale and Stout beer production have an important concentration of polyphenols and compounds with antioxidant and anti-hypertensive capacities. Heat treatment used for the production of caramelized and toasted malts seems to impact negatively on these bioactive compounds. Results of water and absorption

capacity indicated that residue powders present similar values to cereal flours. The brewery's spent grain powder presents acid pH which may contribute to bacterial stability for medium-long term storage of the dried residues. Thus, although more studies are necessary, powder from Pilsen, Red Ale and Stout brewery's spent grain has great potential to be used as a functional ingredient in the food industry.

REFERENCES

1. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS**. Official methods of analysis of the Association of the Analytical Chemistry**. 15. ed. Arlington, 1990.

2. BALASURIVA, N.; RUPASINGHE, H.P. Antihypertensive properties of flavonoid-rich apple peel extract. **Food Chemistry**, v.135, n.4, p.2320-2325, 2012. https://doi.org/10.1016/j.foodchem.2012.07.023.

3. BARBOSA-PEREIRA, L.; BILBAO, A.; VILCHES, P.; ANGULO, I.; LLUIS, J.; FITÉ, B.; PASEIRO-LOSADA, P.; CRUZ, J.M. Brewery waste as a potential source of phenolic compounds: Optimization of the extraction process and evaluation of antioxidant and antimicrobial activities. **Food Chemistry**, v.145, p.191–197, 2014. https://doi.org/10.1016/j.foodchem.2013.08.033

4. BRAND-WILLIAMS, W.; CUVELIER, M.; BERSET, C.: Use of a free radical method to evaluate antioxidant activity. **LWT-Food Science and Technology**, v.28, p.25–30, 1995. https://doi.org/10.1016/S0023-6438(95)80008-5

5. BIRSAN, R.I.; WILDE, P.; WALDRON, K.W.; RAI, D.K. Recovery of polyphenols from brewery's spent grain. **Antioxidants,** v.8, p.380-392, 2019. https://doi.org/10.3390/antiox8090380

6. CHANG, C.-Y.; WU, K.-C.; CHIANG, S.-H.: Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. **Food Chemistry**, v.100, p.1537-1543, 2007. https://doi.org/10.1016/j.foodchem.2005.12.019

7. ČECHOVSKÁ, L.; KONEČNÝ, M.; VELÍŠEK, J.; CEJPE, K. Effect of Maillard Reaction on Reducing Power of Malts and Beers. **Czech Journal of Food Science**, v.30, n.6, p.548-556, 2012. https://doi.org/10.17221/288/2012-CJFS

8. COLLAR, C.; SANTOS, E.; ROSELL, C.M. Assessment of the rheological profile of fibre-enriched bread doughs by response surface methodology. **Journal of Food Engineering**, v.78, n.3, p.820–826, 2007. https://doi.org/10.1016/j.jfoodeng.2005.11.026

9. CUSHMAN, D.W.; CHEUNG, H.S. Spectrophotometric Assay and Properties of the Angiotensin-Converting Enzyme of Rabbit Lung. **Biochemical Pharmacology**, v.20, p.1637-1648, 1971. https://doi.org/10.1016/0006-2952(71)90292-9

10. DVOŘÁKOVÁ M.; GUIDO L.F.; DOSTÁLEK P.; SKULILOVÁ Z.; MOREIRA M.M.; BARROS A.A. Antioxidant properties of free, soluble ester and insoluble-bound phenolic compounds in different barley varieties and corresponding malts. **Journal of the Institute of Brewing**, v.114, p.27–33, 2008. Doi: https://doi.org/10.1002/j.2050-0416.2008.tb00302.x

11. FĂRCAŞ A.C.; SOCACI, S.A.; DULF, F.V.; TOFANĂ, M.; MUDURA, E.; DIACONEASA, Z. Volatile profile, fatty acids composition and total phenolics content of brewers' spent grain by-product with potential use in the development of new functional foods. **Journal of Cereal Science**, v.64, p.34-42, 2015. https://doi.org/10.1016/j.jcs.2015.04.003

12. GUIDO, L.F.; MOREIRA, M.M. Techniques for Extraction of Brewer's Spent Grain Polyphenols: a Review. **Food and Bioprocess Technology**, v.10, n.7, p.1192-1209, 2017. https://doi.org/ 10.1007/s11947-017-1913-4

13. HAMMOUD, R. A.; VACCARI, C.S.; NAGAMIA, S.H.; KHAN, B.V. Regulation of the reninangiotensin system in coronary atherosclerosis: a review of the literature. **Vascular Health Risk Management**, v.3, p.937-945, 2007. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2350139/

14. IKRAM, S.; HUANG, L.Y.; ZHANG, H.; WANG, J.; YIN, M. Composition and Nutrient Value Proposition of Brewers Spent Grain. **Journal of Food Science**, v.82, n.10, p.2232-2242, 2017. https://doi.org/10.1111/1750-3841.13794

15. IVANOVA, K.; DENKOVA, R.; KOSTOV, G.; PETROVA, T.; BAKALOV, I.; RUSCOVA, M.; PENOV, N. Extrusion of brewers' spent grains and application in the production of functional food. Characteristics of spent grains and optimization of extrusion. **Journal of the Institute of Brewery**, v.123, n.4, p.544-552, 2017. https://doi.org/10.1002/jib.448

16. KTENIOUDAKI, A.; ALVAREZ-JUBETE, L.; SMYTH, T. J.; KILCAWLEY, K.; RAI, D. K.; GALLAGHER, E. Application of bioprocessing techniques (sourdough fermentation and technological aids) for brewer's spent grain breads. **Food Research International,** v.73, p.107–116, 2015. https://doi.org/10.1016/j.foodres.2015.03.008

17. MUSSATTO, S.I.; DRAGONE, G.; ROBERTO, I.C. Brewer's spent grain: generation, characteristics and potential applications. **Journal of Cereal Science**, v.**43**, p.1–14, 2006. https://doi.org/10.1016/j.jcs.2005.06.001

18. LARRAURI, J.A.; RUPÉREZ, P.; BORROTO, B.; SAURA-CALIXTO, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. **Journal of Agricultural and Food Chemistry**, v.45, p.1390-1393, 1997. https://doi.org/10.1021/jf960282f

19. LARSSON, M.; SANDBERG, A.-S. Malting of oats in a pilot-plant process. Effects of heat treatment, storage and soaking conditions on phytate reduction. **Journal of Cereal Science**, v.21, n.1, p.87-95, 1995. https://doi.org/10.1016/S0733-5210(95)80012-3

20. LYNCH, K.M.; STEFFEN, E.J.; ARENDT, E.K. Brewers' spent grain: a review with an emphasis on food and health. **Journal of the Institute of Brewing,** v.122, p.553–568, 2016. https://doi.org/10.1002/jib.363

21. MCCARTHY, A.L.; O'CALLAGHAN, Y.C.; CONNOLLY, A.; PIGGOTT, C.O.; FITZGERALD, R.J.; O'BRIEN, N.M. Phenolic extracts of brewers' spent grain (BSG) as functional ingredients – Assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. **Food Chemistry**, v.134, p.641-646, 2012. http://dx.doi.org/10.1016/j.foodchem.2012.02.133

22. MOREIRA, M.M.; MORAIS, S.; CARVALHO, D.O.; BARROS, A.A.; DELERUE-MATOS, C.; GUIDO, L.F. Brewer's spent grain from different types of malt: Evaluation of the antioxidant activity and identification of the major phenolic compounds. **Food Research International**, v.54, p.382-388, 2013. https://doi.org/10.1016/j.foodres.2013.07.023

23. MAZZA, G.; FUKUMOTO, L.; DELAQUIS, P.; GIRARD, B.; EWERT, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. Journal **of Agricultural and Food Chemistry,** v.47, p.4009-4017, 1999. https://doi.org/10.1021/jf990449f

24. NOCENTE, F.; TADDEI, F.; GALASSI, E.; GAZZA, L. Upcycling of brewers' spent grain by production of dry pasta with higher nutritional potential. **LWT – Food Science and Technology,** v. 114, p.108421, 2019. https://doi.org/10.1016/j.lwt.2019.108421

25. PAZNOCHT, L.; KOTÍKOVÁ, Z.; ŠULC, M.; LACHMAN, J.; ORSÁK, M.; ELIÁŠOVÁ, M.; MARTINEK, P. Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains. **Food Chemistry**, v.240, p.670-678, 2018. https://doi.org/10.1016/j.foodchem.2017.07.151

26. RE, R.; PELLEGRINI, N; PROTEGGENTE, A.; PANALA, A.; YANG, M.; RICE-EVANS, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v.26, p.1231–1237, 1999. https://doi.org/10.1016/s0891-5849(98)00315-3

27. REIS, D.S.; NETO, A.C.; FERRAZ, A.V.; FREITAS, S.T. Produção e estabilidade de conservação de farinha de acerola desidratada em diferentes temperaturas. **Brazilian Journal of Food Technology**, v.20, p.1-7, 2007. https://doi.org/10.1590/1981-6723.8315

28. ROSELL, C.M.; SANTOS, E.; COLLAR, C. Physico-chemical properties of commercial fibres from different sources: A comparative approach. **Food Research International**, v.42, p.176–184, 2009. https://doi.org/10.1016/j.foodres.2008.10.003

29. SAMARAS, T.S.; CAMBURN, P.A.; CHANDRA, S.; GORDON, M.H.; AMES, J.M. Antioxidant Properties of Kilned and Roasted Malts. **Journal of Agricultural and Food Chemistry,** v.53, p.8068−8074, 2005. https://doi.org/10.1021/jf051410f

30. SANTANA, A.F.; OLIVEIRA, L.F. Aproveitamento da casca de melancia (*Curcubita citrullus*, shrad) na produção artesanal de doces alternativos. **Alimentos Nutrição Araraquara**, v.6, n.4, p.363-368, 2005. http://serv-bib.fcfar.unesp.br/seer/index.php/alimentos/article/viewFile/496/459

31. SANT'ANNA, V.; BRANDELLI, A.; MARCZAK, L.D.F.; TESSARO, I.C. Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts. Separation and Purification Technology, v.100, p.82-87, 2012. https://doi.org/10.1016/j.seppur.2012.09.004

32. SINGLETON, V.L; ROSSI, J.A.: Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v.20, p.144–158, 1965. https://www.ajevonline.org/content/16/3/144

33. SOUSA, B.A.A.; CORREIA, R.T.P. Biotechnological reuse of fruit residues as a rational strategy for agro-industrial resources. **Journal of Technology Management and Innovation**, v.5, n.2, p.104-112, 2010. http://dx.doi.org/10.4067/S0718-27242010000200010

34. XIROS, C.; CHRISTAKOPOULOS, P. Biotechnological Potential of Brewers Spent Grain and its Recent Applications. **Waste and Biomass Valorization**, v.3, n.2, p.213–232, 2012. https://doi.org/10.1007/s12649-012-9108-8

35. ZUORRO, A.; IANNONE, A.; LAVECCHIA, R. Water–Organic Solvent Extraction of Phenolic Antioxidants from Brewers' Spent Grain. **Processes**, v.7, n.3, p.126-137, 2019. https://doi.org/10.3390/pr7030126