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EXTRAÇÃO, CARACTERIZAÇÃO E APLICAÇÃO DAS PROTEÍNAS DO TRUB: UM SUBPRODUTO ORIUNDO DE CERVEJARIA

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Tese apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutora em Ciência de Alimentos.

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Tese apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Doutor em Ciência de Alimentos.

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Maringá – 2022

Orientadora

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BIOGRAFIA

Bianka Rocha Saraiva nasceu em 21 de fevereiro de 1994 na cidade de Luiziânia, São Paulo. Filha de Francisco Saraiva Deolindo e Maria Lucia Fernandes Rocha Saraiva. Concluiu o Ensino Médio (2011) na Escola de Ensino Médio e Educação Profissional em Nível Técnico São Francisco de Assis, o curso de graduação em Engenharia de Alimentos (2017) pela Universidade Estadual de Maringá (UEM) e o mestrado em Ciência de Alimentos (2019) pela mesma Universidade. Ingressou no doutorado do Programa de Pós-Graduação em Ciência de Alimentos em março de 2019 com defesa da tese em fevereiro de 2022. Desde 2015 é membro do Grupo de Pesquisa em Alimentos Funcionais da UEM. Tem experiência na área de Ciência e Tecnologia de Alimentos, atuando principalmente nos temas de aproveitamento de subprodutos agroindustriais, alimentos funcionais, extração de proteínas, produtos lácteos, cárneos e de panificação.

Dedico

Aos meus pais, Francisco e Maria Lucia, que não mediram esforços para que eu pudesse ir atrás dos meus sonhos.

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APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos:

1. Bianka Rocha Saraiva., Fernando Antônio Anjo, Ana Carolina Pelaes Vital, Paula Toshimi Matumoto-Pintro. Soluble protein isolate from brewing by-product (trub) using the Box-Behnken design. Journal of Food Processing and Preservation.

2. Bianka Rocha Saraiva, Júlia Castilho Zancheta, Mariana Sversut Gibin, Fernando Antônio Anjo, Anderson Lazzari, Evandro Ribeiro Machado Filho, Francielle Sato, Paula Toshimi Matumoto-Pintro. Brewing by-product valorization: trub debittered for nutritional and quality improvement of pasta. International Journal of Gastronomy and Food Science.

GENERAL ABSTRACT

INTRODUCTION. The search for alternative sources of nutrients has been driven by increase in world demand for food, resulting from an estimated population increase of up to 50% in 2050. Increase in demand compromises food sustainability, as resources for food production are limited. Alternative for obtaining nutrients is agricultural and agro-industrial by-products recycling. Among them, spent brewery grain, residual yeast and hot trub are generated from beer production, which increased by about 40% from 1998 to 2020. Hot trub is a precipitate formed during wort boiling, its composition is rich in nutrients, such as proteins and carbohydrates, and can be reused as an alternative source of these compounds for food. During trub formation, proteins can adsorb bitter compounds (from hops), imparting a bitter taste to by-product, which has limited its use in large quantities. Technologies development is necessary to reduce these limiting bitter compounds content for trub use as ingredient for foods nutritional enrichment and to enable compounds of interest extraction, such as proteins and phenolic compounds, enabling using trub new ways.

AIMS. This work had general objective of developing and optimizing processes to increase possibilities of trub using. As specific objectives, to determine optimal conditions for extracting proteins from trub to produce a protein isolate of vegetable origin; and to determine optimal conditions for extracting bitter compounds from trub, using it for nutritional enrichment of pasta, a product that has been used as vehicle for adding new food ingredients.

MATERIAL AND METHODS. Different variables were studied to determine optimal conditions for extracting proteins and bitter compounds from trub in water. For protein isolate production, independent variables studied at different levels were pH (11, 12 and 13), concentration (2.5, 5.0 and 7.5 g/L) and extraction time (30, 60 and 90 min). For bitter compounds extraction, variables were pH (7, 10 and 13), extraction steps (1, 2 and 3) and extraction time (20, 40 and 60 min). Optimal extraction conditions were determined using response surface methodology of Box-Behnken and desirability function. Protein isolate was obtained after precipitation of extracted proteins and centrifugation steps, and precipitate was lyophilized to carry out solubility, phenolic compounds and antioxidant activity analyses. The reduced bitterness trub obtained under optimal extraction conditions was lyophilized and evaluated for technological properties, phenolic compounds, antioxidant activity, proximate composition, color, and protein conformation and differences in relation to trub before processes were also evaluated. Reduced bitterness trub was used in pasta nutritional enrichment in place of wheat flour at 5, 7.5 and 10%. Proximate composition, color, quality properties, texture and scanning electron microscopy were evaluated in pasta with and without addition (control) of reduced bitterness trub.

RESULTS AND DISCUSSION. Optimal conditions for trub proteins extraction were pH 12.31, 5.37 g/L concentration and 51.78 min extraction time at 80 °C, obtaining more than 80% extraction efficiency. Protein isolate obtained showed 94.56% of water-soluble proteins in a wide pH range (6-12), and phenolic compounds content and antioxidant activity indicate its use in functional foods as protein antioxidants. Optimal conditions for bitter compounds extracting from trub were pH 10 and two extraction steps of 40 min at 90°C. The extraction process used reduced phenolic compounds content, which are

associated with trub bitterness, with a consequent reduction in the antioxidant activity. Protein conformation was also altered, which may be related to solubility reduced of reduced bitterness trub in relation to trub. After bitter compounds extraction process, trub protein content was concentrated to 45.72% and fiber content to 9.27%, becoming a potential ingredient for nutritional enrichment. Use of reduced-bitter trub in pasta resulted in a protein enrichment of up to 33.51% in relation to control, also increasing fiber content and reducing total carbohydrate content. Scanning electron microscopy images showed that starch was more incorporated into control pasta protein matrix when compared to enriched pasta matrix, which is related to increase in cooking time and greater water absorption of pasta with trub addition, but did not influence pasta mass loss. Regarding texture, the trub addition increased cohesiveness, elasticity and reduced its adhesiveness and hardness.

CONCLUSIONS. Optimal regions for extraction processes of proteins and bitter compounds from trub were successfully obtained using response surface methodology. Protein isolate of plant origin produced in optimal conditions determined showed solubility in water in a wide pH range (6-12), antioxidant capacity and bioactive compounds. Reduced bitterness trub produced under optimal extraction conditions presented high process yield and had its nutritional content concentrated, providing pasta enriched preparation with proteins and fibers and reduced total carbohydrate content. Pasta with reduced bitterness trub addition presented a soft and cohesive texture, greater water absorption without altering the cooking loss. Trub, after different processes, can be used to nutritionally enrich foods and as a protein ingredient of plant origin, adding value to by-product and increasing opportunities for its application in food.

Key words: By-products, protein isolate, bitter compounds, Box-Behnken design, process optimization, vegetable protein.

RESUMO GERAL

INTRODUÇÃO. A busca por fontes alternativas de nutrientes tem sido impulsionada pelo aumento da demanda mundial de alimentos, resultante do aumento populacional estimado em até 50% em 2050. O aumento da demanda compromete a sustentabilidade alimentar, pois os recursos para a produção de alimentos são limitados. Uma alternativa para a obtenção de nutrientes é a reciclagem de subprodutos agrícolas e agroindustriais. Entre eles, o grão gasto da cervejaria, o fermento residual e o trub quente são oriundos da produção de cerveja, que aumentou cerca de 40% de 1998 a 2020. O trub quente é um precipitado formado durante a fervura do mosto, sua composição é rica em nutrientes, como proteínas e carboidratos, podendo ser reutilizado como fonte alternativa desses compostos para alimentação humana. Durante a formação do trub as proteínas podem adsorver compostos amargos (provenientes do lúpulo), conferindo sabor amargo ao subproduto, o que tem limitado sua utilização em grande quantidade. O desenvolvimento de tecnologias é necessário para reduzir o teor desses compostos amargos limitantes para uso do trub como ingrediente para enriquecimento nutricional de alimentos e para possibiliar a extração de compostos de interesse, como proteínas e compostos fenólicos, possibilitando novas formas de utilização do trub.

OBJETIVOS. Este trabalho teve como objetivo geral desenvolver e otimizar processos para aumentar as possibilidades de utilização do trub. Como objetivos específicos determinar as condições ótimas de extração das proteínas do trub para produção de um isolado proteico de origem vegetal, e determinar as condições ótimas de extração dos compostos amargos do trub, possibilitando sua utilização no enriquecimento nutricional de macarrão, produto que vem sendo utilizado como veículo para adição de novos ingredientes alimentícios.

MATERIAL E MÉTODOS. Diferentes variáveis foram estudadas para determinar as melhores condições de extração das proteínas e dos compostos amargos do trub em água. Para a produção do isolado proteico as variáveis independentes estudadas em diferentes níveis foram pH (11, 12 e 13), concentração (2,5, 5,0 e 7,5 g/L) e tempo de extração (30, 60 e 90 min). Para a extração dos compostos amargos as variáveis foram pH (7, 10 e 13), etapas de extração (1, 2 e 3) e tempo de extração (20, 40 e 60 min). As condições ótimas de extração foram determinadas com o auxílio da metodologia de superfície de resposta de Box-Behnken e da função desejabilidade. O isolado proteico foi obtido após as etapas precipitação das proteínas extraídas e de centrifugação, sendo o precipitado liofilizado para realização das análises de solubilidade, compostos fenólicos e atividade antioxidante. O trub de amargor reduzido obtido nas condições ótimas de extração foi liofilizado e avaliado quanto à propriedades tecnológicas, compostos fenólicos, atividade antioxidante, composição centesimal, cor, e conformação proteica e as diferenças em relação ao trub antes do processo também foi avaliada. O trub de amargor reduzido foi utilizado no enriquecimento nutricional de macarrão em substituição a farinha de trigo em 5, 7,5 e 10%. A composição centesimal, cor, propriedades de qualidade, textura e a microscopia eletrônica de varredura foram avaliados no macarrão com e sem adição (controle) do trub de amargor reduzido.

RESULTADOS E DISCUSSÃO. As condições ótimas para a extração das proteínas do trub foram pH 12,31, concentração de 5,37 g/L e tempo de extração de 51,78 min

a 80 °C, obtendo mais de 80% de eficiência de extração. O isolado proteico obtido apresentou 94,56% de proteínas solúveis em água em uma ampla faixa de pH (6-12), e o teor de compostos fenólicos e atividade antioxidante indicam seu uso em alimentos funcionais como antioxidantes proteicos. As condições ótimas para extração dos compostos amargos do trub foram pH 10 e duas etapas de extração de 40 min a 90°C. O processo de extração utilizado reduziu o teor de compostos fenólicos, que estão associoados ao amargor do trub, com consequente redução da atividade antioxidante. A conformação proteica também foi alterada, o que pode ser relacionado a redução da solubilidade do trub de amargor reduzido em relação ao trub antes do processo. Após a extração dos compostos amargos o teor de proteínas do trub foi concentrado para 45,72%, e o de fibras para 9,27%, se tornando um ingrediente potencial para enriquecimento nutricional. A utilização de trub de amargor reduzido ao macarrão resultou no enriquecimento proteico da massa em até 33,51% em relação ao controle, aumentando também o teor de fibras e reduzindo o de carboidratos totais. As imagens de microscopia eletronica de varredura mostraram que o amido foi mais incorporado na matriz proteica da massa controle quando comparado à matriz da massa enriquecida, o que está relacionado com o aumento do tempo de cozimento e maior absorção de água da massa com adição de trub, mas esta adição não influenciou na perda de massa do macarrão. Em relação a textura, a adição de trub aumentou a coesividade, a elasticidade e reduziu sua adesividade e a dureza.

CONCLUSÕES. As regiões ótimas dos processos de extração das proteínas e dos compostos amargos do trub foram obtidas com sucesso através da metodologia de superfície de resposta. O isolado proteico de origem vegetal produzido nas condições ótimas determinadas apresentou solubilidade em água em ampla faixa de pH (6-12), capacidade antioxidante e compostos bioativos. O trub de amargor reduzido produzido nas condições ótimas de extração apresentou alto rendimento de processo e teve seu teor nutricional concentrado, proporcionando a elaboração de um macarrão enriquecido com proteínas e fibras e redução do teor de carboidratos totais. O macarrão com adição de trub reduzido de amargor apresentou textura macia e coesa, maior absorção de água sem alterar a perda por cozimento. O trub, após diferentes processos, pode ser usado para enriquecer nutricionalmente os alimentos e como ingrediente proteico de origem vegetal, agregando valor ao subproduto e aumentando as oportunidades para sua aplicação em alimentos.

Palavras chaves: Subprodutos, isolado proteico, compostos amargos, Box-Behnken *design*, otimização de processos, proteína vegetal.

ARTICLE 1

Soluble protein isolate from brewing by-product (trub) using the Box-Behnken design

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ORIGINAL ARTICLE

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Soluble protein isolate from brewing by-product (trub) using the Box-Behnken design

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Abstract

Brewing by-products, as trub, can be exploited as new nutrients sources, especially proteins. The objective of this study was to optimize the protein extraction from trub using the Box-Behnken design and response surface methodology to obtain a protein isolate and verify its antioxidants properties and phenolic compounds. Optimal extraction condition was at a pH of 12.31, concentration of 5.37 g/L, with 51.78 min of extraction at 80°C (fixed temperature). Under these conditions, a protein isolate from trub was obtained (94.56% protein; 19.89% yield), its presented high water solubility (63.09 to 93.22%) over a wide pH range (6 to 12), high free radical scavenging capacity (63.03 and 186.07 TEAC µmol/100 g for DPPH and ABTS, respectively) and 35.36 mg gallic acid equivalents (GAE)/g of phenolic compounds. These results brings new information about a vegetable protein from a by-product enabling its extraction and applicability in different food matrices.

Novelty impact statement

Alkaline solution was efficient to extract the proteins from the trub, a beer production by-product that has been little reused. Vegetable protein isolate showed phenolic compounds, antioxidant activity and solubility in water over a wide pH range; and can be used as new protein source.

1 | INTRODUCTION

Novel sources of vegetable proteins are under study in the quest for global food and nutritional security (El Bilali et al., 2019), to address the fact that in 2050, world demand for food may increase by up to 50% due to population growth (Food and Agriculture Organization of the United Nations [FAO], 2017). Vegetable proteins are expected to show rapid market growth, as they can meet various consumer demands involving religious issues, dietary constraints, and restrictions related to products of animal origin (Hadnadev et al., 2018).

Nonconventional legumes, such as alfalfa, have been studied as new protein sources due to greater resistance to adversities, such as pests and diseases (Bhat & Karim, 2009), besides their high protein content, these plants have bioactive constituents, such as polyphenols (Sahni et al., 2020). Besides that, food processing by-products have also been studied as new protein sources, its reuse adds value to something that would be discarded or only destined to animal feed. By-products from oil extraction (Gerzhova et al., 2016; Hadidi et al., 2021; Hadnadev et al., 2018), and beer production have recently been researched as protein source (Saraiva, Anjo, et al., 2019; Saraiva, Agustinho, et al., 2019).

Beer production generates three different by-products, the brewer's spent grain, residual yeast, and hot trub. Among them, brewer's spent grain is generated after the mashing stage, and due its composition rich in nutrients, mainly fibers and proteins (Mussatto & Roberto, 2005), has been used in many researches, and it is also widely used in animal feed (Amoah et al., 2017; De Souza et al., 2016; Faccenda et al., 2017; Radzik-Rant et al., 2018), and human food, such as bakery products (Cappa & Alamprese, 2017; Combest & Warren, 2018; Fărcaş et al., 2017; Ktenioudaki et al., 2015; Lynch

et al., 2016) and meat products (Choi et al., 2014; Kim et al., 2013; Nagy et al., 2017; Saraiva, Agustinho, et al., 2019). In addition, it has been used for compounds extraction, such as polysaccharides (Ahmad et al., 2009; Benito-Román et al., 2013; Coelho et al., 2014; Du et al., 2014) and phenolic compounds (McCarthy et al., 2013; Meneses et al., 2013; Mussatto et al., 2007; Faulds et al., 2002).

Residual yeast is obtained after fermentation process, is also rich in nutrients such as proteins and carbohydrates, and its use has been explored mainly in animal feed (Levic et al., 2010; Øverland et al., 2013; Stone, 2006), but also as food additive in bakery products (Coldea et al., 2017; Martins et al., 2015), sauces (Melo et al., 2015; Worrasinchai et al., 2006) and meat products (Pancrazio et al., 2016).

Hot trub, generated after wort boil, is composed mainly by coagulated proteins, polyphenol-proteins complex, carbohydrates, lipids, and bitter flavor compounds (Santos Mathias et al., 2015; Saraiva, Anjo, et al., 2019). Recently, trub phenolic compounds and flavonoids were used to evaluate its antimicrobial and antioxidant capacity (Costa et al., 2021). Although trub has a high nutritional value, its use for human consumption has been little explored due to the bitter flavor. However, processes that reduce its bitterness can extend its use in different products, such as demonstrated by Saraiva, Anjo, et al. (2019) and then applied to the processed trub in ice cream, improving the product's technological properties (Saraiva et al., 2020). Trub after extraction of bitter compounds presented high protein content (70.34%), but low water solubility, thus more research is needed to obtain a protein ingredient with better technological properties to improve the trub use in human food (Saraiva et al., 2020).

Alkaline extraction and isoelectric precipitation by pH are one of the most common techniques for the production of edible protein, are relatively simple, can be performed inexpensively (Mechmeche et al., 2017), and have been recognized as viable methods for vegetable proteins extraction (Shen et al., 2008). Extraction variables effects, such as pH, time, temperature, and the solid-to-solvent ratio, can be assessed through optimization processes involving experimental protocols, such as Box-Behnken design and response surface graphics, which allows to determine the optimum regions of processes employed. Therefore, this study aimed to determine the optimal conditions for proteins extraction from trub using the Box-Behnken design and response surface methodology, to obtain a protein isolate and verify its antioxidant capacity and phenolic compounds content.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

Trub (moist) was obtained from a traditional Pilsner-type brewery from the Industrial Norte Paranaense de Bebidas (INBEB; Londrina, PR, Brazil), shortly after the boiling stage of the wort. This by-product was dried at 55°C (24 hr) with air circulation, ground, standardized at 60 mesh, and stored at 8°C before analysis. Bradford reagent, Folin–Ciocalteu reagent, gallic acid, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylcromane-2-carboxylic acid (Trolox), trichloroacetic acid, phosphate buffer, potassium persulphate, and sodium carbonate were from Sigma–Aldrich (USA). Potassium ferricyanide, aluminium chloride, ferric chloride, HCl, methyl alcohol, and ethyl alcohol were of analytical grade.

2.2 | Protein extraction method

Trub powder (24.09% ± 0.22 protein and 6.01% ± 0.02 moisture, on a dry basis) was extracted in distilled water using combinations of the independent variables: pH, concentration, and extraction time. Temperature was not used as a variable due to preliminary results and was fixed at 80°C for all runs. For protein extraction, trub powder and deionized water were subjected to heating (80°C) with agitation under predetermined conditions (Table 1). After this step, they were centrifuged (MPW-351R, Poland) at 1696×*g* for 10 min at 25°C, filtered (quantitative paper, weight 80 g m^2) and the supernatant obtained was denominated as protein extract (PE).

Response variable (protein content), was evaluated in the PE obtained from each run, by mixing the supernatant (50 μl) with Bradford reagent (1.5 ml), and after 10 min of reaction, absorbance was read on a spectrophotometer (Evolution™ 300, Thermo Fisher Scientific, UK) at wavelength of 595 nm. Analytical curve was constructed using a standard of bovine serum albumin (from 0 to 2.0 mg/ml; Bradford, 1976). The Bradford method is based on the formation of a complex between the dye (Brilliant Blue G) and proteins in solution, that causes a shift in the absorption maximum of the dye from 465 to 595 nm (Bradford, 1976). Bradford reagent requires no dilution, is a rapid and simple method for protein detection by spectrophotometry.

2.3 | Experimental design

Response Surface Methodology (RSM) with a Box-Behnken Design were used to determine the optimal experimental conditions for trub protein extraction. The independent variables were used at three different levels: pH (11, 12, and 13), concentration (2.5, 5.0, and 7.5 g/L), and extraction time (30, 60, and 90 min). The variables were defined on the basis of our previous study results (Saraiva, Anjo, et al., 2019) and preliminary tests of variable levels.

Effect of the variables pH (X_1), concentration (X_2), and extraction time (X_3) on the dependent variable protein content extracted was evaluated. As shown in Table 1, the factors chosen for this study were prescribed for high, intermediate, and low levels (+1, 0, −1 successively), coded according to Equation (1):

$$\text{Coded value} = xi = \frac{Xi - Xo}{\Delta X} i = 1, 2, 3 \quad (1)$$

TABLE 1 Conditions of protein extraction process using the Box-Behnken design and the experimental values

	Coded variables			Uncoded variables[a]			Protein content, Y (mg protein/g of trub powder)
Run	x_1	x_2	x_3	X_1	X_2	X_3	Experimental values
1	−1	−1	0	11	2.5	60	119.23
2	1	−1	0	13	2.5	60	138.36
3	−1	1	0	11	7.5	60	96.73
4	1	1	0	13	7.5	60	140.06
5	−1	0	−1	11	5.0	30	110.08
6	1	0	−1	13	5.0	30	165.96
7	−1	0	1	11	5.0	90	103.55
8	1	0	1	13	5.0	90	171.89
9	0	−1	−1	12	2.5	30	185.70
10	0	1	−1	12	7.5	30	159.38
11	0	−1	1	12	2.5	90	156.78
12	0	1	1	12	7.5	90	172.83
13	0	0	0	12	5.0	60	193.83
14	0	0	0	12	5.0	60	192.66
15	0	0	0	12	5.0	60	192.03
16	0	0	0	12	5.0	60	192.38
17	0	0	0	12	5.0	60	193.18

[a]X_1: pH, X_2: concentration (g/L), X_3: extraction time (min).

where x_i is the coded value (dimensionless value) of an independent variable; X_i is the real value of an independent variable; X_0 is the real value of an independent variable at center point; and ΔX is the step change value of an independent variable.

Box-Behnken design matrix has three factors, with 15 runs/experiments, and two replicates in the central points were used to allow for estimation of the pure error sum of squares (Table 1). All experiments were carried out in a randomized order. The response function investigated, Y (mg of protein extracted.g^{-1} of trub powder), was fitted to a second-order polynomial model (Equation 2):

$$Y = \beta o + \sum_{i=1}^{k} \beta iXi + \sum_{i=1}^{k} \beta iiXi^2 + \sum_{i<j=1}^{k} \beta ijXiXj \quad (2)$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction, respectively. Xi and Xj are the factors and k is the number of independent variables (Ghorbannezhad et al., 2016).

The statistical significance of the experimental model was analyzed using analysis of variance (ANOVA). The fit quality of the chosen polynomial model was evaluated from the R squared coefficients.

2.3.1 | Optimal extraction conditions

Variables were optimized with a desirability function, aiming to maximize the extracted protein content. Desirability function optimizes multiple responses simultaneously and can be used in industry to obtained economic advantages, with major efficiency and objectivity in the processes (Bezerra et al., 2008). The response under the predicted optimal condition was validated to compare the observed response with that predicted by the model.

2.4 | Isoelectric point determination

Isoelectric point (IP) (Makeri et al., 2017) and protein solubility (Klompong et al., 2007) were determined with modifications in the protein solution (PS) extracted under optimal conditions. For IP determination, 5 ml PS was diluted in distilled water (1:4 vol/vol) and the pH values adjusted with 0.5 or 3 N HCl and 0.5 or 3 N NaOH to obtain a pH range between 2.0 and 12.0. The turbidity of the solution at each concentration was then read with a spectrophotometer at 320 nm, and the pH value that gave the maximum turbidity was taken as the IP.

Protein solubility was determined to confirm the IP. Sample of 25 ml PS were adjusted to pH 2–12 with 0.5 or 1 N HCl and 0.5 or 1 N NaOH. The mixture was stirred at room temperature (approximately 25°C) for 60 min, using a magnetic stirrer, and then centrifuged at 5,046×g for 30 min at 4°C. The protein content in the supernatant was determined using the Bradford method (Bradford, 1976). Protein solubility was then calculated according to Equation (3):

$$\text{Solubility}\,(\%) = \frac{\text{Protein content of supernatant}}{\text{Total protein content}} \times 100 \quad (3)$$

2.5 | Extraction efficiency and protein isolate yield

Extraction efficiency was determined as the protein content extracted in the optimal conditions in relation to the initial protein content of the trub powder, expressed as percentage. Protein isolate yield was calculated as the final weight of the protein isolate lyophilized in relation to the initial weight of the trub powder, expressed as percentage.

2.6 | Trub protein isolate

The PS obtained under optimal conditions was adjusted to the IP (pH 3.9) with 1 N HCl (determined earlier). Protein precipitated was recovered after centrifugation for 20 min (10,180×*g*) at 4°C, mixed with distilled water, centrifuged again, and then lyophilized at −51°C (CHRIST, Alpha 1–4, LD Plus). Protein isolate (powder) was stored for phenolic compounds and antioxidant capacity analysis, and was solubilized in distilled water for protein content determination by Bradford method (Bradford, 1976).

2.7 | Protein solubility of the isolate

Protein solubility was determined in the protein isolate powder according to the method described by Klompong et al. (2007), with modifications. The pH of samples and distilled water (1:100; wt/vol) was adjusted to 2–12 with 0.5 or 1 N HCl and 0.5 or 1 N NaOH. The solutions were stirred at room temperature (approximately 25°C) for 60 min using a magnetic stirrer and then centrifuged at 5,046×*g* for 30 min at 4°C. The protein content in the supernatant was determined using the Bradford method (Bradford, 1976), and protein solubility was calculated according to Equation (3).

2.8 | Phenolic compounds and antioxidant capacity

2.8.1 | Preparation of extract

Trub protein isolate was homogenized (15 min) with distilled water (1:100 wt/vol), it was centrifuged at 5,046×*g* (15 min) at 4°C, and the supernatant was collected for analyses.

2.8.2 | Total polyphenol content (TPC)

The TPC was analyzed according to Singleton and Rossi (1965), with modifications. An aliquot (125 µl) of supernatant was mixed with Folin–Ciocalteu reagent (125 µl; 1:1 deionized water) and sodium carbonate (2,250 µl; 28 g/L), homogenized and incubated for 30 min in the dark at room temperature. The absorbance was measured at 725 nm, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of trub protein isolate.

2.8.3 | Flavonoid content

An aliquot (300 µl) of supernatant was mixed with $AlCl_3$ 5% (150 µl) and methanol 100% (2,550 µl) and incubated for 30 min in the dark. The absorbance was measured at 425 nm (Buriol et al., 2009). The results were expressed as quercetin equivalents (mg QE/g trub protein isolate).

2.8.4 | Antioxidant capacity by ABTS and DPPH assays

$ABTS^{+\cdot}$ solution (1960 µl) was mixed with an aliquot (40 µl) of supernatant according to Re et al. (1999), with modifications; and the absorbance was measured at 734 nm after reaction 6 min. DPPH assay was performed according to Li et al. (2009), with modifications. Supernatant (150 µl) was mixed with DPPH solution (2.85 ml; 60 µM) and incubated (30 min) in the dark; and the absorbance was measured at 515 nm.

DPPH and ABTS free-radical scavenging results were evaluated and expressed as an Trolox equivalent antioxidant capacity (TEAC) per 100 g of dry matter of sample (µmol/100 g).

2.9 | Statistical analysis

The experiments were repeated three times with three replicates and was analyzed in duplicate. Data were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine differences between mean experimental values at the 95% confidence level ($p < .05$). Statistica v12.0 software (Statsoft, Tulsa, USA) was employed for the statistical analysis of RSM models.

3 | RESULTS AND DISCUSSION

3.1 | Extraction results and statistical analysis

ANOVA for independent variables was performed for the response surface quadratic polynomial model as shown in Table 2. The coefficient value (R^2), employed to judge the adequacy of the model, was 0.9994 for the protein content, and the adjusted R^2 (R^2-adj) was 0.9976, suggesting that the total variation of 99.76% in the protein content was attributed to the extraction conditions. The data on protein content of the trub extract were analyzed by multiple regression and fitted to a second-order polynomial equation as follows (Equation [4]):

$$\begin{aligned} Y = & -6819.44 + 1210.56X_1 - 52.48X_1^2 - 455.39X_2 + 26.52X_2^2 \\ & + 16.47X_3 - 0.0058X_3^2 + 52.39X_1X_2 - 2.47X_1X_2^2 - 1.05X_1^2X_2 \\ & - 2.87X_1X_3 + 0.12X_1^2X_3 + 0.14X_2X_3 \end{aligned} \quad (4)$$

TABLE 2 Analysis of variance (ANOVA) of the second-order polynomial model for optimization of protein extraction

Factor	Sum of squares (SS)	Degrees of freedom (*df*)	Mean square (MS)	*p* value (95%)
x_1	3,103.39	1	3,103.39	<.0001*
x_2	134.51	1	134.51	.0023*
x_3	13.93	1	13.93	.0916
$x_1.x_2$	146.39	1	146.39	.0020*
$x_1.x_2^2$	476.93	1	476.93	.0002*
$x_1^2.x_2$	13.85	1	13.85	.0923
$x_1.x_3$	38.79	1	38.79	.0210*
$x_1^2.x_3$	27.66	1	27.66	.0357*
$x_2.x_3$	448.84	1	448.84	.0002*
x_1^2	10,656.43	1	10,656.43	<.0001*
x_2^2	1602.37	1	1602.37	<.0001*
x_3^2	115.35	1	115.35	.0031*
Pure error	11.41	4	2.85	
Total	18,753.76	16		
R^2	0.9994			
R^2-adj.	0.9976			

Note: x_1: pH, x_2: concentration (g/L), x_3: extraction time (min).
*Terms with significant effect ($p < .05$).

3.2 | Influence of extraction factors on the protein content

The coefficients magnitude (Equation 4) indicates how the independent variables correlates with the response variable. The high significance ($p < .001$; Table 2) and the high coefficient value for the linear term pH demonstrates its positive and relevant correlation in protein extraction. For the quadratic pH term, the coefficient was highly significant, but negative (Equation 4), resulting in a parabolic trend (Figure 1a). Protein extraction was effective at a higher pH value, however, in the certain level (>pH 12.6) exhibited an inverse behavior.

The protein extraction was more efficient in a pH range of 12.2–12.6. An increase in the negative surface charge of proteins can cause electrostatic repulsion between oppositely charged side chains and hydration of charged residues, resulting in greater aqueous solubility (Damodaran, 2008; Jarpa-Parra et al., 2014). Trub is formed after the wort-boiling phase; that is, the proteins have been exposed to heat and denatured. Denaturation increases the hydrophobicity of the protein surface and alters the balance between protein-protein and protein-solvent interactions (Damodaran, 2008). Highest protein-protein interaction may explain the need for more negative charge to increase the ionic interaction between water and protein; and increase its aqueous solubility.

However, above pH 12.6 (Figure 1b) there may have been a strong electrostatic repulsion caused by the high net charge, resulting in a reduction in protein solubility and therefore lower content of extracted protein (Jyothirmayi et al., 2006; Pedroche et al., 2004).

Bitterness compounds were extracted from trub in distilled water at pH 5.59 in a previous study. Due its hydrophobic character, the proteins were concentrated during process and a protein concentrate (70.3%) was obtained (Saraiva, Anjo, et al., 2019). This demonstrates the relationship between pH and solubility; the increase in pH in this study improved the solubility of proteins in water and made possible its extraction.

The concentration variable showed significance ($p < .05$) and negative correlation in protein extraction for the linear term, the higher the concentration, the lower was protein extraction. At higher concentrations, there is a reduction in available solvent, decreasing the concentration gradient between liquid and solid, which decreases the mass transfer from the solid to the medium (Bird et al., 2006). Quadratic term of the concentration was positive, indicating that after a certain level, the concentration did not negatively affect the extracted protein content.

Extraction time variable had significant effect only for quadratic term, which had a negative effect. Negative coefficient indicates that after a certain extraction time (60 min), there was a decrease in protein content extracted. Time is not significant as long as it is not too long. Hadidi et al. (2021) also observed this behavior; and in the study by Jarpa-Parra et al. (2014), time also did not have a significant effect for the linear term.

Temperature was not used as a variable and was fixed at 80°C throughout the design in this study. Temperature was defined using preliminary tests, which demonstrated the trend greater protein extraction at higher temperatures. Protein isolates are usually obtained at temperatures lower than in this study (Coelho et al., 2019; Hadidi et al., 2021; Hadnadev et al., 2018; Jarpa-Parra et al., 2014), however, trub is a by-product obtained after the wort boiling step, its proteins already been exposed to higher temperature and time than those evaluated in this study.

3.3 | Response surface methodology

The fitted polynomial equation was plotted as three-dimensional response surfaces (Figure 1) to visualize the relationships between the independent variables and the response variable. Relative effects of two independent variables on protein content are shown, while the third is maintained at the central level.

All linear interaction terms were significant, showing a simultaneous effect on protein extraction. Effect of pH and concentration modulated the response in a positive way [Figure 1(a),(b)]. When pH and concentration were increased simultaneously, the individual effects of these parameters on protein extraction were added. This effect also occurs between the variables concentration and extraction time [Figure 1(e),(f)]. The pH and extraction time variables, when they are increased simultaneously, they have a negative effect on protein extraction, as their individual effects cancel each other out [Figure 1(c),(d)].

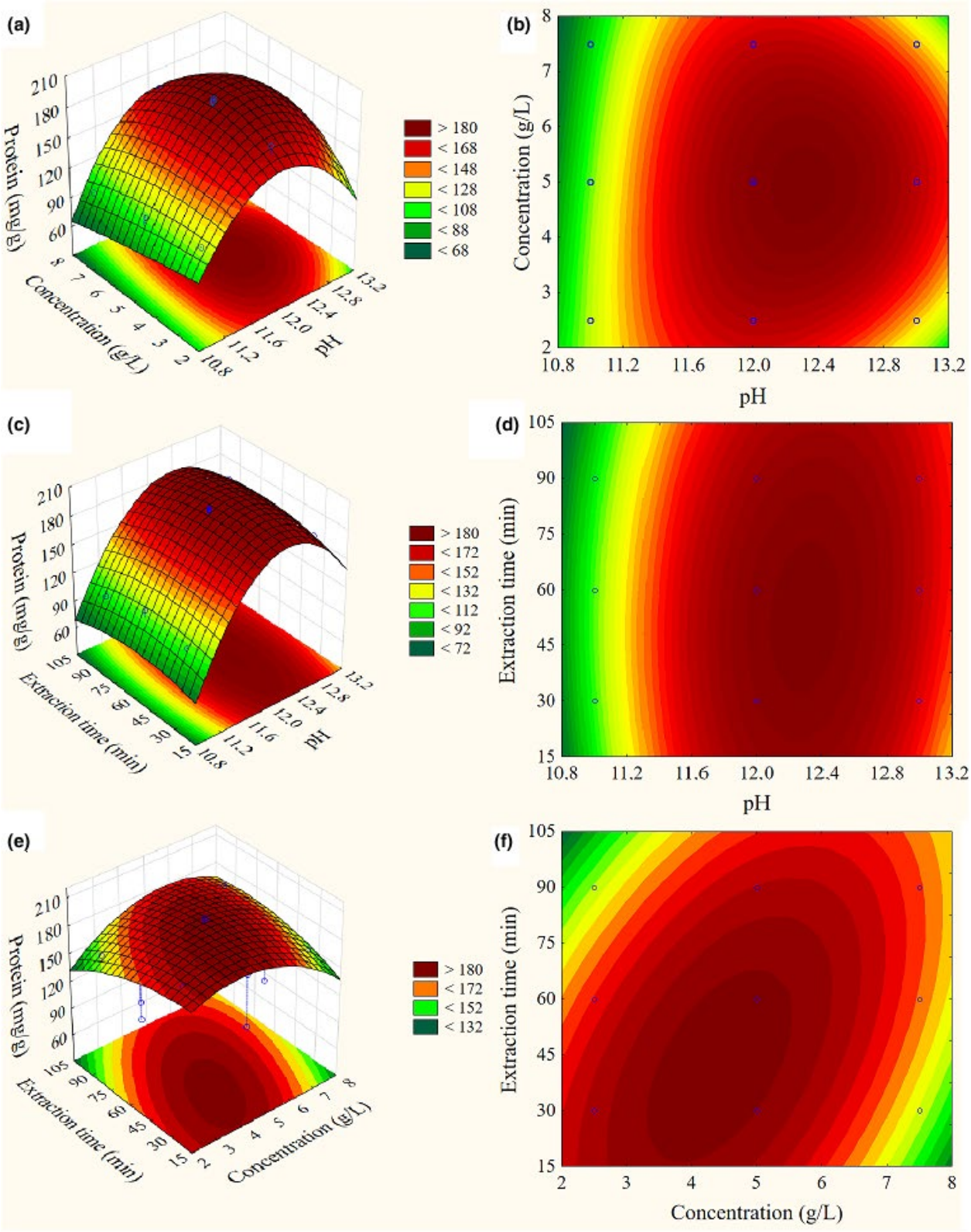

FIGURE 1 3D surface response and contour graphics of trub powder protein extraction; (a, b) Effect of concentration (g/L) and pH on protein extraction with an extraction time of 60 min; (c, d) Effect of time (min) and pH on protein extraction with a concentration of 5 g/L solvent; (e, f) Effect of extraction time and concentration on protein extraction at pH 12.0

In the interactions between linear and quadratic terms, the effects were also significant, except for the quadratic term of pH with the linear term of concentration. The negative effect of the linear term of pH with the quadratic term of concentration shows that increase in concentration results in a decrease in protein extraction, which is related to the concentration gradient and mass transfer. The interaction between the quadratic pH term and the linear time of extraction has a positive effect on protein extraction.

Results showed that the studied variables were significant in the extraction of trub proteins, mainly pH and concentration. Obtaining a protein isolate from this by-product using response surface methodology had not been studied before, and the alkaline extraction was chosen because it is a simple and widely used methodology in the vegetable proteins extraction.

3.4 | Optimal extraction conditions

According to the desirability function, the optimal extraction conditions were pH 12.31, concentration 5.31 g/L, and extraction time 51.78 min. The extraction was performed in triplicate under these conditions, and 196.38 ± 1.79 mg protein/g of trub powder was obtained, which is within the confidence interval of the model.

3.5 | Isoelectric point and protein solubility

Proteins extracted from trub presented IP in the pH 3.9 (Figure 2b). Proteins from food generally have lower solubility at pH 4 to 5, and greater solubility at alkaline pH, due to the characteristic of being acidic proteins (Damodaran et al., 2008). Amino acids analyze of trub (Saraiva, Anjo, et al., 2019), showed that Asp and Glu residues sum aregreater than Lys, Arg, and Hys residues sum, characterizing their proteins as acidic. This result is in accordance with Figure 2, which shows the lowest trub solubility at pH 4 (Figure 2c). The minimum solubility indicates no electrostatic repulsion, thus favoring the precipitation of particles (Damodaran et al., 2008; Jarpa-Parra et al., 2014), which for trub occurs at pH 3.9 (Figure 2b). As the trub is formed by a mixture of proteins that vary with the type of raw material used (malt), different IPs can be found.

Protein extraction generally occurs in alkaline medium, at pH 8 to 9, such as for soybean vegetable protein, but this range can be altered by processes such as thermal denaturation and use of inorganic solvents (Damodaran et al., 2008).

3.6 | Extraction process and protein isolate characterization

The protein extracted under the optimal conditions and precipitated at the IP (3.9) resulted in a protein isolate with 94.56% ± 0.63 protein and 5.23% ± 0.02 moisture (in dry matter basis; Table 3). This result was superior to that obtained in a previous study with

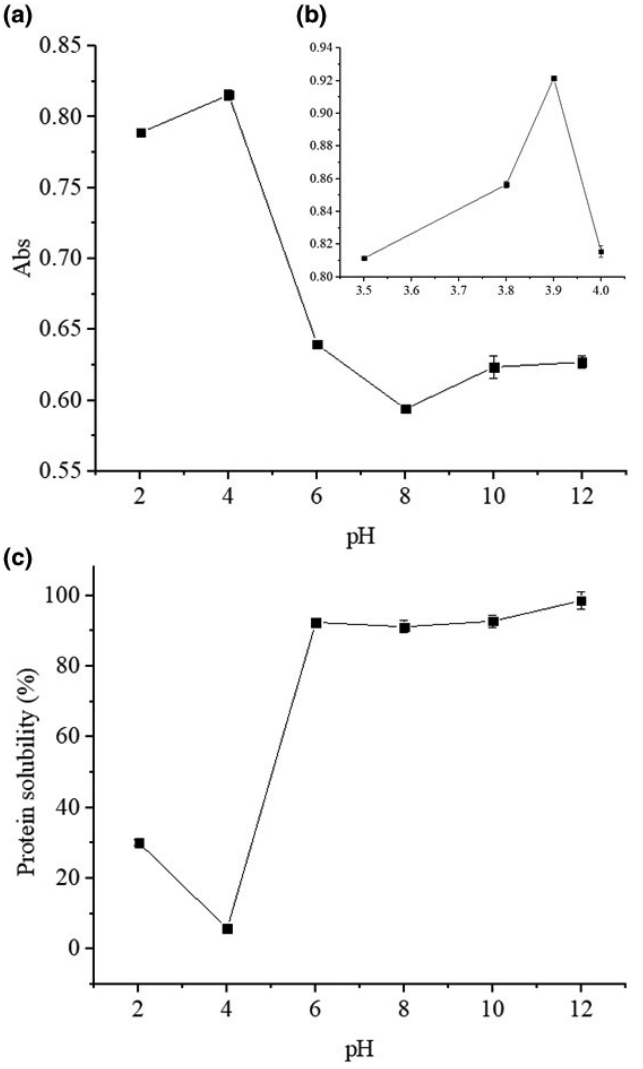

FIGURE 2 (a) Isoelectric point determination in the pH 2–12 range, (b) isoelectric point determination in the pH 3.5–4 range, and (c) solubility profile determination in the pH 2–12 range

70.34% proteins (Saraiva, Anjo, et al., 2019). The conditions studied were similar, except for the pH variation, an important condition for obtaining a water-soluble protein isolate. The protein isolate water solubility increased from 60 to more than 90% in the 6–12 pH range (Figure 3), which allows these proteins application in different products, mainly hydrophilic, different from the previous study (Saraiva, Anjo, et al., 2019).

Proteins isolated from by-products of primrose oil processing obtained by alkaline extraction and deamidation show solubility greater than 80% at pH values above 8 (Hadidi et al., 2021), for hemp protein isolates prepared by the micellization and isoelectric precipitation procedures, the solubility was higher than 80% only in the pH 10 (Hadnadev et al., 2018), behavior similar to that obtained in this study.

The extraction process presented a yield of 19.89% (Table 3). Similar value was presented by protein isolate from hemp seed meal obtained by micellization (19.24%), and a higher value was obtained by isoelectric precipitation (24.24%) (Hadnadev et al., 2018). At the

TABLE 3 Protein content, moisture, extraction characterization, bioactive compounds, and antioxidant activity of trub protein isolate

Protein content (% dry matter basis)	94.56 ± 0.63
Moisture (% dry matter basis)	6.01 ± 0.02
Extraction yield (%)	19.89 ± 0.19
Extraction efficiency (%)	80.45 ± 0.06
Bioactive compounds	
Phenolic compounds (mg GAE/g)	35.36 ± 0.10
Flavonoids (mg QE/g)	4.06 ± 0.12
Antioxidant capacity	
ABTS assay (TEAC μmol/100 g)	186.07 ± 0.17
DPPH assay (TEAC μmol/100 g)	63.03 ± 0.51

Abbreviations: ABTS, free radical scavenging ABTS; DPPH, free radical scavenging DPPH; GAE, gallic acid equivalent; QE, quercetin equivalent; TEAC, trolox equivalent antioxidant capacity.

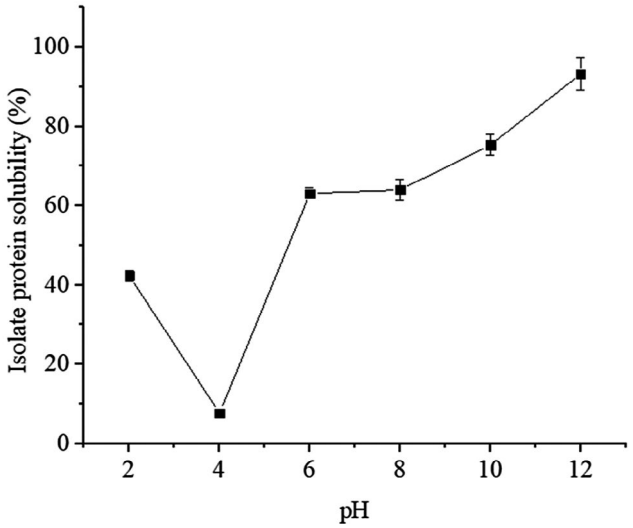

FIGURE 3 Solubility profile of trub isolate protein at different pH values

first, the protein content was higher, demonstrating that higher protein content are associated with the smaller yield extraction. Protein isolates from alfalfa seeds presented lower yield values (16.76 and 15.29%) (Sahni et al., 2020), but protein content of 94.13% and 96.77%, respectively. Maximum yield obtained in the protein extraction optimization from primrose oil processing by-products was 26.4% with 86.1% of protein content (Hadidi et al., 2021). The yield value can be influenced by the coproducts extracted with the protein, the initial protein content of the raw material, and the type of process used.

At pH 12.31, the protein extraction efficiency was 80.45% (Table 3). Other studies have also shown a high protein extraction efficiency under alkaline conditions (pH 12), reaching greater than 85% protein extraction (Gerzhova et al., 2016; Gillberg & TÖRnell, 1976; Quinn & Jones, 1976; Tzeng et al., 1988).

3.7 | Bioactive compounds and antioxidant capacity

Trub protein isolate showed high antioxidant capacity by ABTS and DPPH assays, 186.07 and 63.03 TEAC μmol/100 g, respectively (Table 3). The Alfafa protein isolate, another plant-based protein, also showed high values from processed and unprocessed seeds, 202.07 and 164.34 TEAC μmol/100 g (respectively) for the ABTS assay; and 141.78 and 132.93 TEAC μmol/100 g (respectively) for the DPPH assay (Sahni et al., 2020). The greater antioxidant capacity obtained by the ABTS method in relation to the DPPH method can be explained by the differing reaction mechanisms of these methods. $ABTS^{+}$ is capable of reacting with a greater range of antioxidants, and its reactions occur by electron transfer, whereas reactions with the DPPH radical occur by transfer of hydrogen atoms, showing greater selectivity (Marecek et al., 2017). The trub protein isolate presented high content of bioactive compounds, 35.46 mg GAE/g total phenolic compounds and 4.06 mg QE/g flavonoids. Alfafa protein isolate presented a smaller value of phenolic compounds (19.08 and 16.25 mg GAE/g, for unprocessed and processed seeds, respectively), however, the flavonoids content obtained was higher (7.70 and 6.48 mg QE/g for unprocessed and processed seeds, respectively; Sahni et al., 2020).

Phenolic content from hops and malt in beer contribute to beer quality and are associated with beer style produced (Harborne, 1989). These compounds are also found in brewery by-products (Saraiva, Anjo, et al., 2019; Saraiva, Agustinho, et al., 2019), and of the 49 phenolic compounds identified in 22 beers, 11 individual compounds were determined, such as gallic acid, (−) catechin, epicatechin, ferulic acid, chlorogenic acid, morin, rutin, quercetin, caempherol, naringenin, and luteolin (Marova et al., 2011). Among phenolics in beer, the flavonoids found in hops (such as xanthohumol and related prenylflavonoids) and malt (such as phenolic acids, flavonols, flavonols) are important for beer quality control. Some specific flavonoids amount can be correlated with the beer production technology and the quality of the material used (Marova et al., 2011).

Bioactive compounds and antioxidant capacity of trub were observed in the previous study (Saraiva, Anjo, et al., 2019). Results obtained show that these compounds can be coextracted with the proteins, by the capacity of polyphenol-protein interaction (Bandyopadhyay et al., 2012). Studies show that this interaction can increase the antioxidant capacity of proteins (Matumoto-Pintro et al., 2017; You et al., 2014), in addition, protein antioxidants can be used in functional foods, providing additional nutritional value (Hernández-Ledesma et al., 2007).

4 | CONCLUSION

The optimum region of extraction process was obtained successfully through the response surface methodology, and the optimal extraction condition was determined at a pH of 12.31, concentration of 5.37 g/L, with 51.78 min of extraction at 80°C (fixed temperature). Under these conditions, it was possible to obtain a protein isolate of

vegetable source from a by-product, which presented water solubility over a wide pH range. In addition, the trub protein isolate has antioxidant capacity and bioactive compounds, increasing the opportunities for its application in foods.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing: Bianaka Saraiva. *Formal analysis; Investigation; Methodology; Writing-review & editing*: Fernando Antônio Anjo. *Conceptualization; Data curation; Methodology; Supervision; Writing-review & editing*: Ana Carolina Pelaes Vital. *Conceptualization; Data curation; Investigation; Supervision; Visualization; Writing-review & editing*: Paula Matumoto-Pintro.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ARTICLE 2

Brewing by-product valorization: trub debittered for nutritional and quality improvement of pasta

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Abstract

Brewery by-product trub has been little valued due to bitter compounds presence. Processes have been studied for these compounds extraction, but their limited solubility reduces the extraction efficiency. This study presents optimize a bitter compounds extraction from trub by response surface methodology, and use of debittered trub (DT) as new ingredient to enrich pasta. Bitterness extraction process was evaluated at different pH values (7, 10, 13), time (20, 40, 60 min) and extraction steps (1, 2, 3) using the Box-Behnken design. Optimal condition to obtain DT was pH 10 and extraction in two steps of 40 min, obtaining a yield greater than 40%. Extraction process effects on global bitterness content, protein conformation, technological properties, antioxidant capacity, chemical composition and trub color were evaluated. DT-enriched and control pasta were evaluated for nutritional composition, quality properties, texture and scanning electron microscopy. After bitter compounds extraction, DT protein content increased by 85.70%, which generated an increase of 33.51% of pasta protein with 10% DT addition. The trub protein structures were altered with process, also modifying its technological properties. Interaction of trub and wheat proteins resulted in a more compact structure, and DT water absorption capacity provided texture changes, as lower hardness and chewiness of enriched pasta in relation to control, and in an increase in cohesiveness. After bitter compounds extraction, trub use for pasta enrichment improved its nutritional and quality properties, enabling trub valorization and its use as vegetable proteins alternative source.

Keywords: process optimization; Box-Behnken design; bitter compounds; nutritional enrichment; vegetable protein.

1. Introduction

The search for alternative sources of nutrients has increased considerably in recent years, driven by increase in food demand until 2050 (FAO, 2017). Food production resources are limited, both threaten food sustainability systems at large (Alexandratos and Bruinsma, 2012). An alternative for obtaining nutrients is recycling of agricultural and agro-industrial by-products. Among them, brewer's spent grain, residual yeast and hot trub are generated in beer production, which from 1998 to 2020, increased about 40% global production (TSP, 2021).

Hot trub is a precipitate formed mainly by proteins coagulated during wort boiling. During this process, proteins can to adsorb bitter compounds (from hops added before) and decant them with trub, imparting by-product bitter taste (Askew, 1964; dos Santos Mathias et al., 2015; Spetsig, 1968). Trub has soluble and insoluble bitter compounds, as lupulones, iso-α-acids generate after α-acids thermal isomerization during boiling wort, and humulones (α-acids) (Haseleu et al., 2009; Palamand and Aldenhoff, 1973). Among these bitter compounds are iso-α-acids, humulinones, hulupones and polyphenols (Kowaka and Kokubo, 1977; Wolfe et al., 2012). Soluble and insoluble bitter compounds content in trub varies according to way in which hops are used and brewing process, but insoluble bitter compounds content is always high (Spetsig, 1968). Trub composition is rich in nutrients, such as proteins and carbohydrates (dos Santos Mathias et al., 2015; Saraiva et al., 2019[b]), and can also be reused in food.

Technologies should be used to allow agro-industrial by-products to be transformation into ingredients or additives for food industry. These can be used in nutritional enrichment of products such as pasta, ice cream, extruded snacks and hamburgers (Balli et al., 2021; Jozinović et al., 2019; Saraiva et al., 2020, 2019[a]; Vital et al., 2020), among than, pasta

has been used as a vehicle for nutritional enrichment with new food ingredients. It is a low-cost, long shelf-life product, and its consumption has increased in recent years (Mercier et al., 2016). Pasta can its nutritional potential improved with trub addition. Therefore, objective of this research was to optimize bitter compounds extraction from trub obtaining the debittered trub (DT), and using it in nutritional enrichment in pasta to evaluate the effect on nutritional and technological properties.

2. Materials and methods

2.1.Raw material and chemicals

Trub (moist) was obtained from a traditional Pilsner-type brewery from Industrial Norte Paranaense de Bebidas (INBEB; Londrina, PR, Brazil), shortly after wort boiling stage. This by-product was dried at 55 °C (24 h) with air circulation, ground, standardized at 60 mesh, and stored at 8 °C for further analysis.

Bradford reagent, Folin–Ciocalteu reagent, gallic acid, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylcromane-2-carboxylic acid (Trolox), potassium persulphate, sodium hydroxide (NaOH), hydrochloric acid (HCl), and sodium carbonate (Na_2CO_3) were from Sigma–Aldrich (USA). Methyl alcohol, and ethyl alcohol were of analytical grade.

2.2.Optimization of bitter compounds extraction

Box-Behnken Design (BBD) and Response Surface Methodology (RSM) were used to optimize bitter compounds extraction parameters, access the interactions between independent variables, and predict the optimal experimental conditions. The independent

variables [pH (X_1), extraction steps (X_2), and extraction time (X_3)] were used for high, intermediate, and low levels (+1, 0, −1, successively), and extraction temperature (90 °C) and concentration (25 g/L) were fixed. The process variables and temperature were defined based on previous study (Saraiva et al., 2019[b]), and preliminary tests of variables levels. Extraction yield and global bitterness were used as dependent variables.

BBD matrix of three-factor, with 15 runs/experiments and two replicates in the central points were used to allow for estimation squares pure error sum. The levels of variables were 7, 10, and 13 (X_1), 1, 2, and 3 (X_2), and 20, 40, and 60 min (X_3) (Table 1). After extraction step each, the sample was centrifuged (MPW 351R, Warszawa, Poland) at 9,650*xg* for 10 min (22 °C). Supernatant was recovered for global bitterness analysis, and precipitate recovery was lyophilized for yield analysis.

2.3.Global bitterness and yield value analysis

Global bitterness was measured according to Philpott et al. (1997). An aliquot of iso-octane (2,2,4-trimethylpentane; 4 mL) was mixed with HCl (3M; 200 µL) and sample (2 mL). After homogenization (30 min) and centrifugation at 960*xg* for 10 min, the supernatant was read at 275 nm in the spectrophotometer (EvolutionTM 300; Thermo Fisher Scientific, Cambridge, UK). The result was expressed in International Bitterness Units (IBU). The extraction yield was evaluated by weight of DT (g) concerning initial trub weight (g) and was expressed in percentage.

2.4.Optimal extraction conditions

Optimal condition of bitter compounds extraction from trub was determined by desirability function aiming to maximize the global bitterness extraction and the yield. Responses of predicted and experimental obtained in the optimal conditions were

compared for evaluate to model efficiency. Trub obtained after extraction process in the optimal conditions was called debittered trub (DT), it was lyophilized and used for further analyses.

2.5.UV–Visible spectroscopy

Absorption spectra of trub and DT were recorded using a UV-Vis spectrophotometer model EvolutionTM 300. Scan speed and bandwidth were 240 nm min^{-1} and 1 nm, respectively. The extraction was performed in isooctane (1:50 w/v) and each sample (2 mL) was diluted in acidified isooctane (4 mL) by 3M HCl (200 µL) (Philpott et al., 1997). The baseline was performed with isooctane and spectral scan was acquired in the range of 400-210 nm.

2.6.Phenolic compounds and antioxidant capacity

Bioactive compounds extraction was performed by trub and DT samples homogenization (10 min) with distilled water (1:10 w/v), centrifuged at 960*xg* (10 min), and supernatant was collected. Total phenolic compounds (TPC), DPPH and ABTS radical scavenging capacity were determined (Saraiva et al., 2019[b]). TPC results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample. ABTS and DPPH results were expressed as Trolox equivalent antioxidant capacity (TEAC - µmol) per 100g of sample.

2.7.Fourier Transform Infrared Spectroscopy (FTIR)

Trub and DT samples were evaluated by FTIR on a Vertex 70v FTIR spectrometer (Bruker Optik GmbH, Ettilingen, Germany) equipped with attenuated total reflectance (ATR) accessory with a diamond crystal. Spectra were obtained from 400 to 4000 cm^{-1}

with a spectral resolution of 4 cm^{-1}, being each spectrum an average of 128 scans, collected in triplicate at room temperature. Spectra underwent baseline correction and vector normalization. Amide I band deconvolution was performed using Gaussian Fit to determine protein conformation of trub and debittered trub.

2.8.Technological properties

Water absorption index (WAI), water solubility index (WSI) and oil absorption capacity (OAC) were determined in the trub and DT (Anderson et al., 1969; Lin et al., 1974).

2.9.Chemical composition and color

Chemical composition of trub and DT was determined by moisture, crude protein (Kjeldahl method), total fat (Soxhlet method), ash, and crude fiber analysis (AOAC, 2005; AOCS, 1996). Total carbohydrate was estimated by difference, and caloric value (kcal/100 g) was determined by Li et al. (2014). Results were expressed on a dry basis. Color was measured by CIELab system. Parameters L* (100 = white; 0 = black), a* (+, red; -, green) and b* (+, yellow; -, blue) were determined using a colorimeter with illuminant C (Chroma Meter CR-400; Minolta, Mahwah, New Jersey, USA).

2.10.Pasta production

Control pasta (30 cm long, 0.6 cm wide and 0.25 cm thickness) was prepared by wheat flour and distilled water (2:1 w/w) homogenization. The enriched pasta were prepared by replacing wheat flour for DT at different concentrations (5%, 7.5%, and 10% w/w). The mass remained at rest for 30 min, was molded and cut in pasta machine, pre-dried for 12 min at 30 °C and then dried 60 °C for 180 min according to Vital et al. (2020). Pasta was stored in polyethylene bags for 24 h until analyses. In the formulations with DT addition

more water was added to obtain a homogeneous mass, while for control 50g of water were used, 53.75, 54.50 and 55.25g were used in formulations with 5, 7.5 and 10% of DT.

2.11.Scanning electron microscopy

Fresh pasta structure was analyzed by scanning electron microscope (SEM) (QUANTA 250) at 15 kV. The samples were frozen by liquid nitrogen and lyophilized (Christ Alpha 1-4 LD plus, Marin Christ, Germany). Small fraction of samples were mounted on an aluminium stub using carbon tape and coated with a gold layer (Sputter coater, Baltec SCD 050, Balzers, Liechtenstein).

2.12.Cooking quality and chemical composition of pasta

Optimal cooking time (1:20 pasta:water), water absorption capacity and cooking loss were determined using AACC Method 66-50 (AACC, 2000). Centesimal composition and color were performed as described above in dried pasta.

2.13.Pasta texture analysis

Texture was performed on cooked pasta at its optimal cooking time using a Brookfield-CT III texture analyzer (Middleborough, USA) with TA/1000 cylindrical probe, compressing one pasta strand to 50% of initial pasta thickness at 1 mm/s constant speed and 10 g Trigger (Vital et al., 2020). Hardness, chewiness, cohesiveness, elasticity, and adhesiveness parameters were evaluated. Firmness was evaluated by cut five pasta strands (5 cm) with the Warner–Bratzler blade (AACC, 2000), 10g Trigger at 2 mm/s.

2.14. Statistical analysis

Statistica v12.0 software (Statsoft, Tulsa, USA) was employed for statistical analysis of RSM models, as to constructing the experimental design, data analysis, and model building. All experiments were carried out in a randomized three times, and were analyzed in triplicate. Responses functions investigated were fitted to a second-order polynomial model. Experimental model statistical significance was performed by analysis of variance (ANOVA), and chosen polynomial model fitting quality was evaluated from R squared coefficients (R^2).

Pasta attributes were evaluated with SPSS program (v.23.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows by ANOVA using general linear model (GLM), and means were compared by Tukey's test. Data were expressed as mean ± standard deviation of three replicates and experiment was repeated twice.

3. Results and discussion

3.1. Extraction results

The global bitterness and yield values obtained in each extraction condition are shown in Table 1. R^2 value was employed to judge model adequacy (Table 2), for global bitterness was 0.9974, and the adjusted R^2 (R^2-adj) was 0.9896, and for extraction yield R^2 was 0.9994 and R^2-adj 0.9977, suggesting 98.96% and 99.77% total variation, respectively, were attributed to extraction factors. Global bitterness and extraction yield data were carried out by multiple regressions to fit the second-order polynomial equation as following Eqs. (1) and (2):

Extraction yield (%)

$$= -20.0340 + 20.5467X_1 - 1.18078{X_1}^2 \quad (1)$$

Global bitterness (IBU)

$$= -240.348 + 50.70X_1 + 67.709X_2 - 2.262{X_1}^2 \quad (2)$$

3.2.Factors influence on the bitter compounds extraction

Regression coefficients for response surface polynomial model of independent variables and *p*-values of linear and quadratic terms were shown in Table 2. Three-dimensional response surface (Fig. 1) show the relative effects of two independent variables on bitter compounds extraction and yield value, while the variable third is maintained at the central level.

The pH was a significant ($p<0.05$) factor for dependent variables in linear and quadratic terms, and extraction steps were significant only global bitterness in linear term. Extraction time and interaction variables were not significant for dependent variables evaluated.

Positive coefficient for pH linear term demonstrates that when value increase of 7-10, the global bitterness extracted and extraction yield increased (Fig. 1A and B), however, the pH increase of 10-13, resulted in decrease values, due to pH quadratic effect. From a certain pH value, quadratic term is more significant than linear term; as this coefficient is negative, global bitterness extraction and extraction yield are reduced. The extraction steps also presented a positive linear correlation with global bitterness extracted, however, a greater number of extractions resulted in reduced yield value (Table 1).

Optimal conditions for bitter compounds extraction from trub given by desirability function were pH 10, 2 extraction steps, and 40 min extraction time. The predicted

maximal global bitterness extraction was 98.08 IBU and the predicted maximal extraction yield was 43.16%, similar values to experimentally obtain under these conditions (Table 1).

3.3.Bitter compounds reduction analyses

Reduction in bands intensities at 230 and 275 nm from trub to DT demonstrate bitter compounds reduction by extraction process employed (Fig. 2). Global bitterness analysis quantifies chemical compounds that contribute to beer bitterness, including iso-α-acids, polyphenols, hulupones and humulinons (products from β-acids and α-acids oxidation respectively) (Kowaka and Kokubo, 1977). Absorption band observed at 275 nm be associated with these bitter compounds, mainly iso-α-acids (Philpott et al., 1997); while band at 230 nm is associated with α-acids (Kornyšova et al., 2009). Phenolic compounds, which also contribute to bitterness, were reduced after the extraction process (Table 3) due to their solubility in water (Karakaya, 2004; Kowaka and Kokubo, 1977). The reduction in antioxidant capacity of DT by ABTS and DPPH methods is associated with phenolic compounds reduction, expressed in Gallic acid (Table 3), phenolic acid that already has reported antioxidant potential (Yen et al., 2002).

Trub has soluble and insoluble bitter substances (Spetsig, 1968). Lupulones and other bitter compounds of low solubility can be solubilized by suspension in air-treated alkaline water at 80 °C (Spetsig, 1968). Oxidation reduces iso-α-acids concentration by degradation products formation, and makes soluble the insoluble substances. The alkaline pH (10) used in this extraction process allowed greater removal bitter substances from trub than in previous study performed in natural pH (Saraiva et al., 2019[b]).

3.4. Secondary structure and technological properties

Spectrum collected for trub and DT was plotted only from 1600 to 1700 cm^{-1} as showed in Figure 3B), since this region is amide I characteristic. Amide I is the most sensitive probe of proteins secondary structure when compared to other bands associated with proteins, such as amide II and III, as this spectral range is sensitive to such structures, showing greater absorption (Alhazmi, 2019). This characteristic absorption can be deconvoluted through Gaussian Fit, as follows: range from 1670 to 1678 cm^{-1} associated with the β-turn (Alhazmi, 2019), 1650 to 1660 cm^{-1} attributed to the α-helix (Alhazmi, 2019; Bier et al., 2013), 1640 to 1648 cm^{-1} linked to random coil structures (Bier et al., 2013) and finally, between 1614 to 1637 cm^{-1} related to β-sheet (Alhazmi, 2019).

Each protein conformation was calculated using fit area compared to amide I total area. Results show that predominant structures of DT were β-turn and random coils, with lower contribution of α-helix after bitter compounds extraction (Table 3). Increase in β-turn and random coil structures while there was a reduction in the α-helix implies a more compact conformation state, as was observed for bioconjugated albumin by Shang et al. (2007).

Use of alkaline pH in compounds extraction may have favored protein unfolding, leading to exposure to hydrophobic groups, an increase in protein-protein interactions, and thus a solubility reduction (Damodaran, 2007). High temperature use also favors hydrophobic interactions (protein-protein), with maximum strength between 70-80 °C (Damodaran, 2007). Hydrophobic interactions are endothermic, therefore, stabilize with heating, demonstrating that the studied bittering extraction conditions resulted in DT technological properties changes, such as reduction of WSI and increase of WAI and OAC (Table 3). These properties are associated with DT application foods, can be used to enrich ice cream, breads, hamburgers, among other foods.

3.5.Chemical composition and color of trub and debittered trub

Chemical composition of trub was altered after bitter compounds extraction (Table 3). Crude protein, fiber, and ash increased in DT; and total fat and carbohydrates decreased, while caloric value did not presented difference ($p<0.05$). Protein increased in 85.70%, and carbohydrates reduced in 29.82% in DT; behavior also observed by FTIR-ATR spectra collected in spectral range of 400 to 4000 cm^{-1} (Fig. 3A).

Absorption broadband (Fig. 3A) present between 3000 and 3600 cm^{-1} indicates overlapped stretching modes of hydroxyl and NH groups (Lin et al., 2021; Socrates, 2001). Bands observed in spectral range of 2800 to 3000 cm^{-1} correspond to aliphatic vibrations attributed to stretching CH_2 and CH_3 chains (Socrates, 2001). The band at 1737 cm^{-1} is associated to C=O stretching, while 1600 and 1700 cm^{-1} corresponds to primary amide, which is directly associated with proteins (Lin et al., 2021; Socrates, 2001). The secondary amide presents absorption close to 1538 cm^{-1} (Lin et al., 2021; Socrates, 2001), while total carbohydrates absorb from 946 to 1182 cm^{-1} (Feng et al., 2020).

The range associated with carbohydrates and primary amide, indicated by gray line in Fig. 3A), had their areas calculated by integration, limited by a baseline plotted between each interval. Figure 3C) shows calculated area by integration for each spectral region in compare to protein quantification by Kjeldahl method and total carbohydrate calculated by difference (Table 3). This comparison reveals an analogous behavior between the data acquired by different techniques, indicating a protein content increase ($p<0.05$) and total carbohydrates content reduction ($p<0.05$) in DT when compared to trub.

Color of trub was modified by bitter compounds extraction. DT presented a darker color, with L* and a* smaller values (Table 3). The decrease in luminosity may be related to Maillard reaction (non-enzymatic darkening) that can occur in amino acids and

reducing sugar presence under heating and alkaline media (Damodaran, 2007; Ellis, 1959).

3.6.Chemical composition and color of pasta

Use an ingredient with high crude protein content (45.72%; Table 4) resulted in pasta protein enrichment, increase of 33.51% in the formulation with 10% DT compared to control; and fiber that was not present in control pasta was added by DT use, contributing to its increase nutritional content. Other plant proteins have been used for pasta nutritional enrichment, as non-commercial asparagus flour (Vital et al., 2020), mushroom powder, Bengal gram flour, defatted soy flour (Kaur et al., 2013), chickpea flour, red lentil flour, white lentil flour, green gram flour, soy flour (Bhatt et al., 2015), and leaves of *Pereskia aculeata* Miller (*ora-pro-nobis*) (Sato et al., 2019).

DT addition presented an effect on color of pasta as can be observed in Table 4. The L* was reduced, and a* increased with DT concentration increasing. For b* parameter, the formulations with DT addition did not present differences ($p<0.05$) in each other, only in relation to control. DT has a darker color (Table 3) than wheat flour (L* value 52.63). Enriched pasta are usually color-modified with new ingredients addition (Mercier et al., 2016; Sato et al., 2019; Vital et al., 2020).

3.7.Scanning electron microscopy of pasta

Scanning electron microscopy (SEM) was used to study control and enriched fresh pasta (10% of DT) microstructure (Fig. 4). Images showed that starch was more incorporated into control pasta protein matrix (Fig. 4A), formed only by gluten proteins, when compared to enriched pasta matrix (Fig. 4C) formed also by gluten-free proteins. Competition between different protein sources causes starch granules not to be properly

incorporated into protein matrix (Khatkar and Kaur, 2018). From SEM images, it was possible to observe the variation in starch granules shape, from smooth surface lenticular (Fig. 4A) to ovoid and circular (Fig. 4C) shapes with few protrusions or cavities, which are typical of wheat starch (Jane et al., 1994; Yonemoto et al., 2007), which may occur due to starch-protein interaction strength.

Pasta with trub addition presented a more compact structure of protein matrix with smaller empty spaces (Fig. 4D) in relation to control (Fig. 4B). Interaction of protein matrix with starch granules was improved with mustard protein (Alireza Sadeghi and Bhagya, 2008) and seaweed (Prabhasankar et al., 2009) addition in pasta; however, in this study, a greater interaction between trub and wheat proteins was observed.

3.8.Quality properties of pasta

The optimal cooking time, cooking loss, and water absorption did not present difference ($p<0.05$) between control and formulation with 5% of DT (Table 4), however, in above concentration, cooking time increased. Paste with enrichment usually reduces the cooking time, due to starch dilution, which reduces the amount of water needed for gelatinization; but some ingredients addition capable of competing with starch for water can delay this process (Mercier et al., 2016), as observed in this study.

Adding a pasta high-protein content ingredient makes it harder for starch granules to swell, as non-gluten proteins compete with starch during cooking (Simonato et al., 2019).

Pasta water absorption increased with DT enrichment in 7.5 and 10%. DT has a WAI three times greater than wheat flour (2.11% ± 0.02), and this competition for water contributed in slower gelatinization and longer cooking time of pasta with DT.

Enrichment can weaken the dough gluten network and result in greater cooking loss due amylose leaching in cooking water (Islas-Rubio et al., 2014; Petitot et al., 2010); but

DT addition did not increase cooking loss (Table 4), which may be related to the strengthening of the protein matrix (Fig. 4).

3.9.Texture of pasta

DT enrichment modified pasta texture properties (Table 5). Hardness and chewiness correspond to the force needed to compress and chew a pasta strand (Epstein et al., 2002) are associate to water absorption high during pasta cooking (Table 4).

When gluten-free ingredients are used in pasta fortification, gluten strength dilutes and likely weakens pasta overall structure (Petitot et al., 2010). Trub addition, which has proteins coming mainly from barley, is able to maintain and even increase gluten strength ($p<0.05$), as shown by the cohesiveness.

Elasticity was affected by enrichment in relation to control, but not by DT different concentrations, while adhesiveness reduced with concentrations increase. Although hardness decreased, the firmness evaluated in cut was lower only for 7.5%, which presented lower cohesiveness. The DT addition resulted in soft pasta, without becoming crumbly and sticky, important properties during pasta preparation and consumption.

4. Conclusions

The bitter compounds extraction process was optimized and the optimal extraction condition was pH 10 performed in two steps of 40 min at 90 °C (fixed temperature). Alkaline solution resulted in higher bitterness extraction with high process yield. Debittered trub used to enrich pasta increased the protein, fat, and fiber content, improving its nutritional content. Pasta presented soft and cohesive texture, greater water

absorption without changing cooking loss. Debittered trub can be used to enrich foods; adding value to by-product and increasing its ways of use.

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.

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Figure caption

Fig. 1. Response surface plots for global bitterness extraction (A) and yield value (B); (a) Effect of pH and extraction steps; (b) Effect of time (min) and pH; (c) Effect of extraction time and extraction steps.

Fig. 2. UV–Visible scan spectroscopy of trub and debittered trub (DT).

Fig. 3. A) Average FTIR-ATR spectra of trub and debittered trub (DT); B) FTIR-ATR spectrum of amide I region of trub and DT, in which the contribution of each protein conformation was obtained through Gaussian fit: (I) Gaussian fit sum; (II) β-turn; (III) α-helix; (IV) Random coil; (V) β-sheet. C) Behavior of crude protein and carbohydrates from quantitative method and integrated band area. Capital letters refer to the FTIR method and lowercase letters refer to the quantitative method. Different letters indicate a significant difference ($p<0.05$) between the values of proteins or carbohydrates evaluated in each method.

Fig. 4. Scanning electron microscopy (SEM) of fresh pasta control with magnification x5000 (A) and x500 (B) and enriched pasta with 10% of trub debittered with magnification x5000 (C) and x500 (D). P: protein; S: starch.

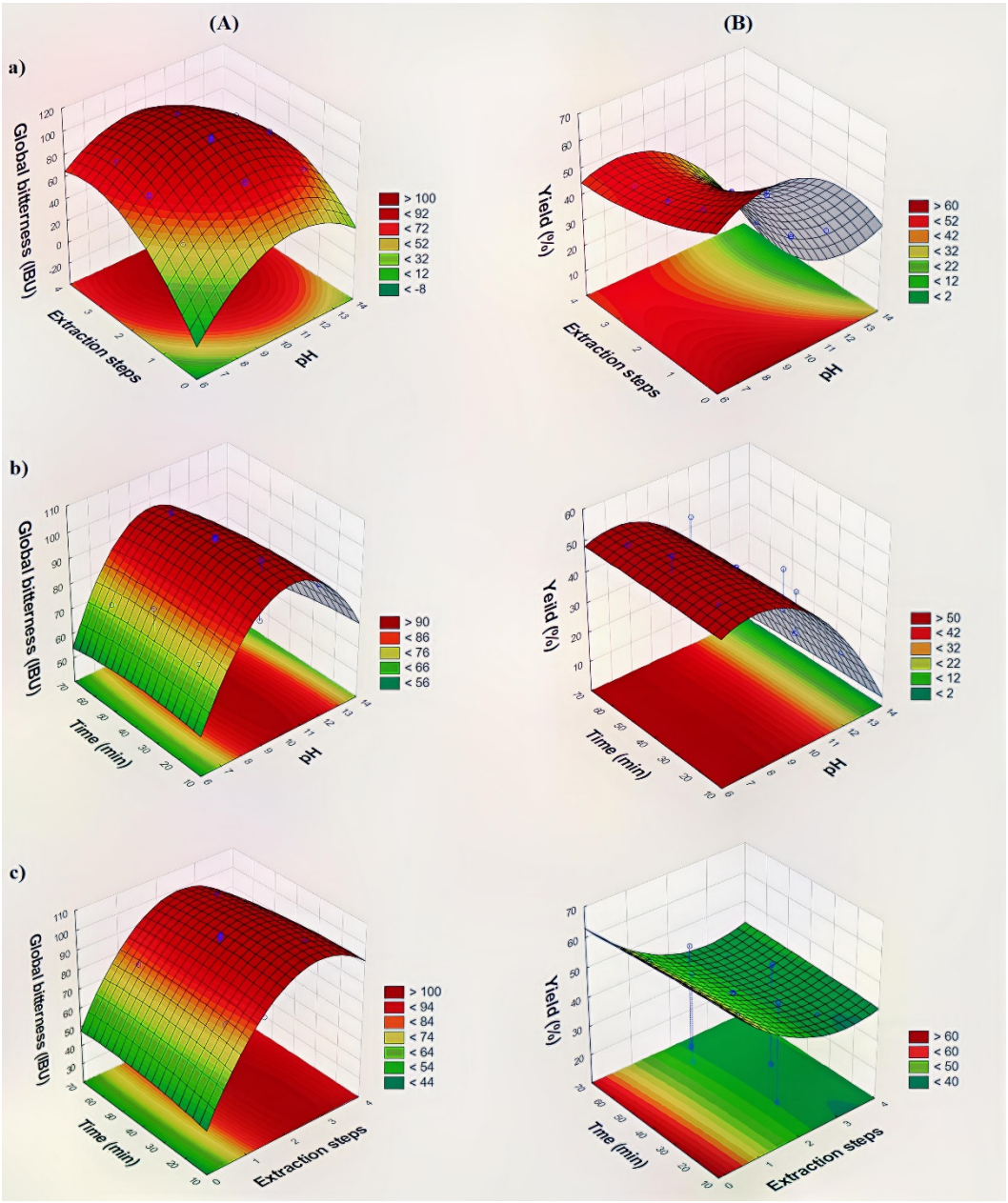
(A)
(B)
a)
Global bitterness (IBU)
Extraction steps
pH
> 100
< 92
< 72
< 52
< 32
< 12
< -8
Yield (%)
> 60
< 52
< 42
< 32
< 22
< 12
< 2
b)
Global bitterness (IBU)
Time (min)
pH
> 90
< 86
< 76
< 66
< 56
Yield (%)
> 50
< 42
< 32
< 22
< 12
< 2
c)
Global bitterness (IBU)
Time (min)
Extraction steps
> 100
< 94
< 84
< 74
< 64
< 54
< 44
Yield (%)
> 60
< 60
< 50
< 40

Fig. 1.

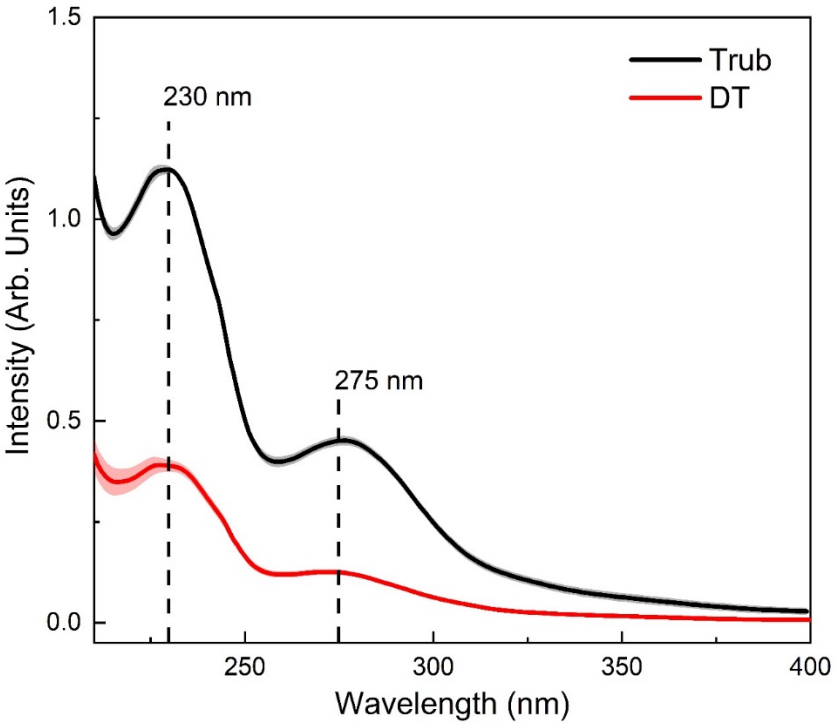
1.5
1.0
0.5
0.0
Intensity (Arb. Units)
230 nm
275 nm
Trub
DT
250
300
350
400
Wavelength (nm)

Fig. 2.

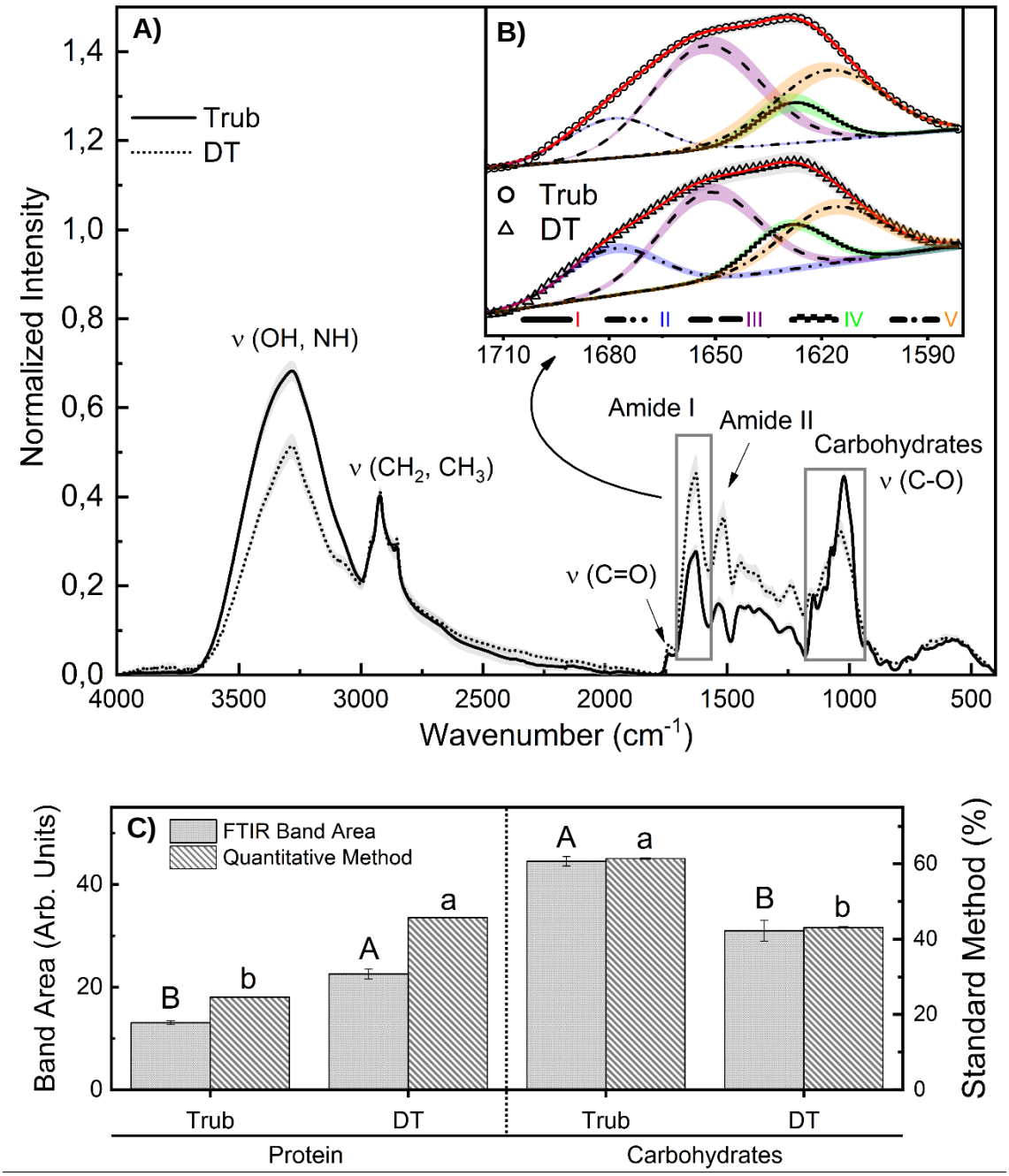
A)
B)
Trub
DT
Normalized Intensity
Wavenumber (cm-1)
ν (OH, NH)
ν (CH2, CH3)
ν (C=O)
Amide I
Amide II
Carbohydrates
ν (C-O)
I
II
III
IV
V
C)
FTIR Band Area
Quantitative Method
Band Area (Arb. Units)
Standard Method (%)
Protein
Carbohydrates

Fig. 3.

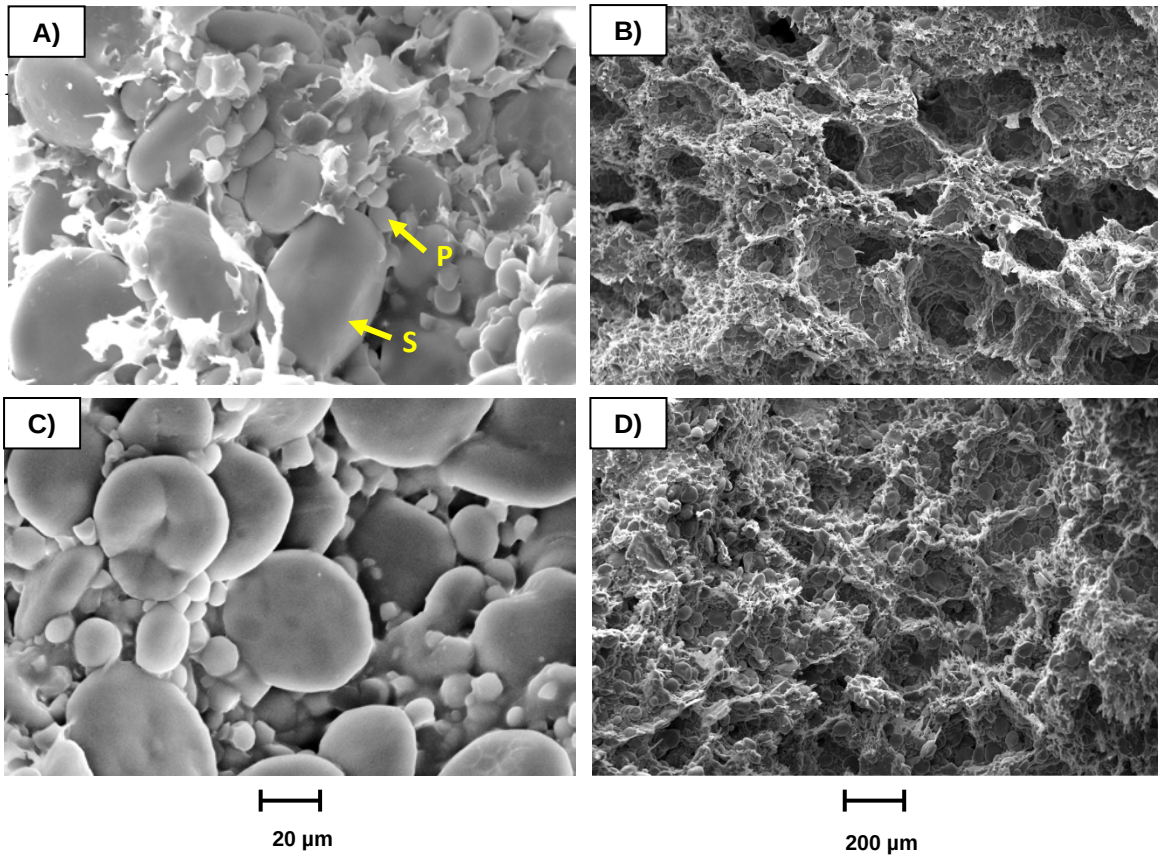

Fig. 4.

Tables

Table 1. The experiment data for bitter extraction process conditions using Box-Behnken design and experimental values.

	Factors			Responses	
Run	pH (X_1)	Steps (X_2)	Time (min) (X_3)	Global bitterness (IBU)	Extraction yield (%)
1	7	1	40	50.95	56.33
2	13	1	40	68.00	26.91
3	7	3	40	83.90	47.61
4	13	3	40	79.95	12.77
5	7	2	20	74.18	50.51
6	13	2	20	83.40	16.54
7	7	2	60	73.75	50.45
8	13	2	60	77.68	15.37
9	10	1	20	79.73	52.71
10	10	3	20	102.93	40.19
11	10	1	60	80.93	51.00
12	10	3	60	100.00	40.36
13	10	2	40	97.63	42.58
14	10	2	40	100.00	43.90
15	10	2	40	100.95	44.14
16	10	2	40	100.88	44.08
17	10	2	40	98.40	43.86

Table 2. Regression coefficients for response surface polynomial model and statistical information of bitter compounds extraction.

Source	Global bitterness (IBU)		Yield extraction (%)	
	Coefficient	*p* value (95%)	Coefficient	*p* value (95%)
Intercept	-240.3480	0.0026	-20.0340	0.2659
x_1	50.7000	0.0019	20.5467	0.0024
x_2	67.7090	0.0215	4.5002	0.6027
x_3	-0.1000	0.8759	0.0554	0.8404
$x_1.x_2$	-3.1920	0.3106	-2.2130	0.1357
$x_1.x_2^2$	-0.0040	0.9911	0.3995	0.0583
$x_1^2.x_2$	0.0730	0.5684	0.0081	0.8802
$x_1.x_3$	0.1010	0.4415	-0.0130	0.8109
$x_1^2.x_3$	-0.0060	0.3545	0.0004	0.8759
$x_2.x_3$	-0.0520	0.2399	0.0235	0.2181
x_1^2	-2.2620	0.0027	-1.1808	0.0013
x_2^2	-7.5710	0.1032	-1.4738	0.3962
x_3^2	-0.0030	0.2184	-0.0004	0.6203
Pure Error	2.2361		0.4160	
R^2	0.9974		0.9994	
R^2-adj.	0.9896		0.9977	

Table 3. Properties of trub and debittered trub (DT).

	Trub	DT
Phenolic compounds and antioxidant capacity		
Total phenolic compounds (mg GAE/g)	2.40 ± 0,11[a]	0.64 ± 0.01[b]
ABTS (TEAC µmol /100 g)	82.03 ± 1.59[a]	24.53 ± 0.88[b]
DPPH (TEAC µmol /100 g)	36.08 ± 0.35[a]	4.43 ± 0.14[b]
Secondary structure content (%)		
β-turn	14.25 ± 0.69[b]	18.72 ± 0.36[a]
α-helix	46.21 ± 1.15[a]	39.92 ± 0.36[b]
Random coil	11.87 ± 0.44[b]	15.23 ± 0.93[a]
β-sheet	27.67 ± 1.36[a]	26.13 ± 0.86[a]
Technological properties		
WAI (g/g)	4.61 ± 0.34[b]	6.14 ± 0.01[a]
WSI (%)	34.16 ± 0.66[a]	1.24 ± 0.04[b]
OAC (g/g)	2.09 ± 0.01[b]	3.66 ± 0.06[a]
Chemical composition		
Moisture (%)	9.85 ± 0.02[a]	7.00 ± 0.03[b]
Crude protein (%)	24.62 ± 0.01[b]	45.72 ± 0.04[a]
Total fat (%)	2.00 ± 0.16[a]	0.93 ± 0.08[b]
Carbohydrates (%)	61.39 ± 0.17[a]	43.08 ± 0.13[b]
Crude fiber (%)*	3.84 ± 0.11[b]	9.27 ± 0.04[a]
Ash (%)	2.14 ± 0.01[b]	3.27 ± 0.02[a]
Caloric value (kcal/100g)	362.00 ± 0.86[a]	363.57 ± 0.39[a]
Color		
L*	69.44 ± 0.06[a]	52.63 ± 0.08[b]
a*	1.97 ± 0.02[b]	4.49 ± 0.03[a]
b*	14.26 ± 0.05[a]	9.32 ± 0.08[b]

Results are expressed in dry matter as mean ± standard deviation. Different letters in the same line are significantly different ($p<0.05$). GAE: gallic acid equivalent, WAI: water absorption index, WSI: water solubility index. OAC: oil absorption capacity, ABTS: free radical scavenging ABTS, DPPH: free radical scavenging DPPH, TEAC: trolox equivalent antioxidant capacity. *Crude fiber is included in the total carbohydrates.

Table 4. Chemical composition, color and quality properties of control and enriched pasta with debittered trub (DT).

	Control	5%	7.5%	10%
Chemical composition				
Moisture (%)	12.78 ± 0.03^{a}	12.12 ± 0.04^{b}	11.85 ± 0.02^{c}	11.73 ± 0.05^{c}
Crude protein (%)	11.19 ± 0.17^{d}	12.94 ± 0.07^{c}	13.81 ± 0.06^{b}	14.94 ± 0.03^{a}
Total fat (%)	0.11 ± 0.00^{d}	0.15 ± 0.01^{c}	0.18 ± 0.01^{b}	0.20 ± 0.02^{a}
Crude fiber (%)	0.00 ± 0.00^{d}	0.47 ± 0.02^{c}	0.71 ± 0.01^{b}	1.01 ± 0.03^{a}
Ash (%)	0.49 ± 0.03^{c}	0.63 ± 0.01^{b}	0.67 ± 0.01^{ab}	0.72 ± 0.01^{a}
Carbohydrate (%)	75.43 ± 0.15^{a}	74.16 ± 0.03^{b}	73.49 ± 0.08^{c}	72.41 ± 0.01^{d}
Caloric value (kcal/100g)	347.46 ± 0.02^{c}	349.77 ± 0.13^{b}	350.82 ± 0.10^{a}	351.20 ± 0.16^{a}
Color				
L*	90.80 ± 0.33^{a}	75.89 ± 0.42^{b}	73.69 ± 0.42^{c}	71.35 ± 0.55^{d}
a*	-1.82 ± 0.10^{d}	1.58 ± 0.05^{c}	1.86 ± 0.08^{b}	2.09 ± 0.07^{a}
b*	13.07 ± 0.80^{a}	7.99 ± 0.24^{b}	8.13 ± 0.20^{b}	8.01 ± 0.17^{b}
Quality properties				
Optimal cooking time (min)	19.25 ± 0.35^{c}	20.00 ± 0.00^{c}	24.25 ± 0.35^{b}	28.00 ± 0.00^{a}
Cooking loss (%)	5.70 ± 0.48^{a}	5.88 ± 0.46^{a}	5.80 ± 0.45^{a}	5.83 ± 0.31^{a}
Water absorption (%)	132.06 ± 1.20^{c}	136.66 ± 2.99^{c}	154.37 ± 1.85^{b}	160.73 ± 3.37^{a}

Results are expressed as mean ± standard deviation. Different letters in the same line are significantly different ($p<0.05$).

Table 5. Texture analyses of control and enriched pasta with debittered trub (DT) after cooking.

	Control	5%	7.5%	10%
Hardness (kg)	6.57 ± 0.16^{a}	5.39 ± 0.16^{b}	4.37 ± 0.22^{c}	3.53 ± 0.05^{d}
Chewiness (kJ)	35.05 ± 1.50^{a}	26.03 ± 1.07^{b}	22.40 ± 1.41^{c}	18.82 ± 0.86^{d}
Cohesiveness	0.68 ± 0.02^{ab}	0.66 ± 0.02^{ab}	0.65 ± 0.02^{c}	0.69 ± 0.01^{a}
Elasticity (mm)	0.77 ± 0.00^{b}	0.85 ± 0.01^{a}	0.84 ± 0.03^{a}	0.82 ± 0.01^{a}
Adhesiveness (kJ)	3.17 ± 0.22^{a}	1.64 ± 0.05^{b}	1.27 ± 0.09^{c}	0.95 ± 0.06^{d}
Firmness (kg)	1.06 ± 0.01^{a}	1.08 ± 0.01^{a}	1.00 ± 0.00^{b}	1.06 ± 0.00^{a}