A techno-economic perspective on a microwave extraction process for efficient protein recovery from agri-food wastes

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ABSTRACT

Different agri-food wastes (brewers spent grain (BSG), spent coffee ground (SCG) and kale stems) have been proposed as excellent sources of protein-enriched extracts with an antioxidant capacity. The optimization of the microwave-assisted hydrothermal and alkali extraction has been compared in this study. From a technical and economic point of view, the extraction of BSG under optimal conditions (110 °C, 10 min and 0.5 M NaOH) provided the best extract with a content of 14.6 kg protein/100 kg BSG (dry matter), 13.8 g/L of total sugars and an antioxidant activity (DPPH method) of 17.1 mg trolox equivalents (TE)/g BSG. This extract had the lowest production cost (29.9 €/kg) and a minimum selling price of 51.7 €/kg, estimated for an extraction pilot plant of 15 kg/h of BSG. The microwave-assisted hydrothermal extraction of kale stems, a novel waste in the biorefinery context, also provides bioactive and green extracts of commercial interest. There is a need for specific research studies related to biorefining of agri-food wastes to produce proteins for food, contributing to the development of a future sustainable and climate-neutral agriculture. The proposed techno-economic assessment represents an important advance in research and scaling-up of microwave-assisted extraction processes for protein recovery from agri-food wastes.

1. Introduction

Enormous amounts of agri-food waste (AFW) are generated in various stages of the entire agri-food supply chain (including processing) (Bhat, 2021). AFW is an excellent source of bioactive compounds to exploit, including proteins, sugars, lipids, and phenolics (Popovic et al., 2022). According to Marić et al. (2018), Europe generates about 100 million tonnes of waste each year in the food processing industry. The concept of considering by-products as a raw material for the recovery and production of several co-products using green methods within the integrated biorefinery model has great interest and potential (Fierascu et al., 2018) concerning the circular economy policies. In this context, brewers’ spent grain (BSG) is the most abundant by-product in the beer brewing process and is available throughout the year (Parchami et al., 2021). This material consists of the barley grain husks obtained as solid residue after the production of wort. It comprises approximately 85% of the total waste generated in this industry (Li et al., 2021); producing 0.2 kg wet BSG per liter of beer (Parchami et al., 2021). In 2019, 38.2 million metric tons of wet BSG were produced worldwide (Parchami et al., 2021). This residue contains a relatively large amount of protein (18–31% w/w) and fiber, sugars, and minerals. This waste is normally only used as animal feed or is directly discarded (Li et al., 2021). Another interesting AFW is spent coffee grounds (SCG). Coffee is one of the most consumed commercial foods and the second most exported product by emerging countries (Ribeiro et al., 2021). The coffee industry produces a large amount of waste which, according to Valdés et al. (2020), may represent somewhere over 50% of the mass of all the coffee beans in the producing countries. The world production of coffee in 2018 was around 9.5 million tons (de Otalora et al., 2020) and approximately 0.91 g of SCG is produced for 1 g of coffee ground (Tun et al., 2020). SCG contains significant protein content (up to 12% w/w) (Mussatto et al., 2011; Ribeiro et al., 2021). On the other hand, kale, a vegetable from the Brassica genus, has been attracting attention for the last few decades due to its high antioxidant and dietary fiber content (Casajús et al., 2021). Brassica genus crops are one of the ten most economically essential vegetables in global agriculture and markets. In

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2012, the global production of *Brassica* crops was almost 92 million metric tonnes, grown in 150 different countries and occupying 5.4 million hectares. Spain, Mexico, Italy, France, and the USA produce over 0.2 million metric tonnes per year. Around 7% of this vegetable is discarded as waste (Francisco et al., 2017). According to Megías-Pérez et al. (2020), the average composition of kale is water (89%), fiber (4%), proteins (3%), lipids (1.5%), and low molecular weight carbohydrates (1%). So fresh kale has moderate levels of protein (1.6–5.9 g/100 g).

In recent years, the food industry has focused on studying the transition from the use of animal proteins to plant-based proteins (Yang and Sagis, 2021). This development is due to the environmental aspects of meat production and the need for new protein sources for the higher global population (Parchami et al., 2021). Extracted proteins have properties that are both biofunctional (nutritional properties for application in feed/food and pharmaceutical sectors) and techno-functional (structural technical applications such as packaging with solubility or network formation and viscosity) (Yadav et al., 2020). So it is necessary to find an alternative, less resource-intensive source of protein for food, as well as for other applications.

The conventional protein extraction method has some drawbacks, such as the fact that it requires a large amount of water and energy, the protein extraction yield decreases when high purity protein extracts are obtained, or the process may alter the protein structure (Yang and Sagis, 2021). The problem for extracting protein in this type of waste with high yields is that several components, such as cellulose and lignin, form a complex network and trap the protein inside (Li et al., 2021). Therefore, chemical treatments have been applied. Conventional alkali extraction has been proved to be an appropriate protein extraction method, but it also has the disadvantage of a long extraction time (Li et al., 2021). For this reason, physical methods are generally used together with the chemical method to overcome the abovementioned disadvantages. Microwave-assisted extraction (MAE) could be a cost-effective, efficient and straightforward method to assist in protein extraction. Microwave technology has been considered a green and eco-friendly method to disrupt the cell wall with relatively low energy input, a rapid treatment time and the avoidance of the utilization of hazardous substances. Microwaves interact selectively with polar molecules and induce intracellular heating. This heat and pressure located in the cell walls lead to cell disruption allowing and improving the extraction of intracellular proteins. MAE was thus applied to enhance the efficacy of protein extraction and the co-extraction of phenolic compounds from several AFW, such as BSG, SCG, and kale stems.

The present work attempts to study the use of several wastes from the food and beverage industry (BSG, SCG, and kale stems) to achieve bioactive protein-enriched extracts with antioxidant activity. In this context, the MAE process was proposed, in which the influence of three parameters (temperature, time, and NaOH concentration), as well as the comparison of alkali and hydrothermal extraction, are evaluated using the response surface methodology. The evaluated maximizing response was the protein recovery. On the other hand, the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH method) were measured at the optimum value for each scenario evaluated. In addition, a preliminary economic study was carried out to compare the optimal scenarios of protein extraction from AFW. For this purpose, the total costs of the extraction and a versatile plant with a capacity for 15 kg/h of agro-waste of protein recovery from AFW, along with the market value of the protein that could be theoretically produced, were estimated.

This research study represents a significant advance in the development of new strategies for the production, extraction, processing and marketing of new alternative proteins from new sources that could be considered for animal feed and direct human consumption. It is worth mentioning that this study is the first to compare, from a technical and economic point of view, the microwave-assisted hydrothermal and alkali extraction of protein from various AFW. Moreover, a few references were found related to protein extraction from BSG and SCG, but none about kale waste for its valorization.

2. Materials and methods

2.1. Raw materials

In this case, AFW was used, namely BSG, SCG, and kale stems. The SCG was provided by PROSOL Productos Solubles (Venta de Baños, Spain), the BSG was donated by the Brewery Mahou San Miguel (Burgos, Spain), and the kale stems were supplied by NaturSnacks (Pedrajas de San Esteban, Spain). The three raw materials were dried at 60 °C in an oven and milled using a coffee grinder (Taurus Aromatic, 150 W). In this way, a particle size lower than 1 mm and moisture content lower than 3% for the three cases was achieved.

2.2. Microwave-assisted alkali extraction

A multiwave PRO SOLV reactor 50 Hz with Rotor type 16HF100 (Anton Paar GmbH, Austria, Europe) was used to extract the protein from the AFW, with a solid to liquid ratio of 10% (w/v). It was operated with continuous temperature control of the applied microwave energy (for more details, see López-Linares et al., 2019).

The raw materials and solvent were mixed (5 g dry weight raw material and 50 mL of solvent) in each of the pressure vessels of the multiwave reactor. The reactor warmed up and the extraction time was initiated when each run attained the required temperature. When the experimental runs were finished, the microwave equipment cooled the pressure vessels of the reactor down to a temperature of 50 °C. The slurry was vacuum filtered (when the solvent was water), or centrifuged at 10,000 rpm for 10 min (in the case of alkali solvent), to separate the solid and liquid phases. In addition, the solid phase was washed with distilled water and dried at 50 °C for 48 h. The solid was then weighed to determine the solid recovery (SR) (g solid fraction/100 g dry raw material). The TPC, TFC, DPPH, and total sugar content were determined in the liquid phase. Finally, the protein in the solid phase was analyzed.

2.3. Experimental design

In order to select the optimal conditions for protein extraction using microwave-assisted (alkali or hydrothermal) treatment from the three chosen raw materials, a central composite experimental design was used. The factors were temperature, time, and sodium hydroxide concentration. According to literature (Qin et al., 2018; Contreras et al., 2019; Du et al., 2020; Samalsale and Sothernvit, 2021; Ribeiro et al., 2021), higher protein extraction yields were achieved operating in a basic medium, being NaOH the most widely employed solvent. Solvent concentrations used are usually less than 1 M and extraction temperatures below 120°C in order to avoid possible protein denaturation, degradation or precipitation processes. Short extraction times are required using microwave technology (<15 min). Table 1 shows the coded and uncoded values of factors in the experimental designs whose data were processed and analyzed with the software Statgraphics Centurion XVIII.

2.4. Analytical methods

2.4.1. Raw material composition

The composition of the proposed raw materials, i.e.,, extractives, structural carbohydrates (cellulose and hemicellulose), lignin, and ash content were measured using the analytical methodology of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2005, 2008, 2011). The experiments were carried out in triplicate and the averages of the results are shown.

The total protein content of the raw material and extracted solids was analyzed by the Kjeldahl acid digestion method of Prabhuzantye et al. (2019). The SR from the extractions was determined according to
2.4.2. Chemical characterization of the liquid extracts

The total sugar concentration was determined by the Phenol-Sulfuric Acid Method, a colorimetric method that uses D-glucose as standard (Nielsen, 2017). The results are expressed as g of total sugar L⁻¹ of the liquid extract.

The Folin-Ciocalteu method (Singleton and Rossi, 1965) was used to analyze the TPC. This method uses gallic acid as standard, and the results are expressed as mg gallic acid equivalents (GAE) g⁻¹ of the dry raw material.

On the other hand, to analyze the TFC, the colorimetric method described by Zhishen et al., (1999) was employed, using catechin as standard. The TFC is expressed as mg of catechin equivalents (CE) g⁻¹ of the dry raw material.

In order to measure the antioxidant capacity of the liquid extracts obtained from the treatment with microwaves, the DPPH radical scavenging method described by Brand-Williams et al. (1995) was used. The standard used was Trolox (6-hydroxy-2, 5,7,8-tetramethylchromene-2-carboxylic acid), and the results are shown as mg of Trolox equivalents (TE) g⁻¹ of the dry raw material.

The analytical determinations were carried out in triplicate, and the average results were indicated. Relative standard deviations were below 2%.

2.4.3. Calculation of protein extraction yield

The protein extraction yield was calculated as the ratio of the extracted protein to the initial protein in the raw material (RM), using Eq. (1).

\[
\text{Protein extraction yield (\%)} = \frac{\text{Initial protein in RM}}{\text{solid protein}} \times 100
\] (1)

2.5. Definition of scenarios

The protein extraction process from AFW has two stages: the first is a solid-liquid MAE, obtaining a slurry stream. The next stage separates the liquid and solid phases to obtain two streams; the liquid stream being rich in proteins and phenols, while solid stream is waste.

Four scenarios have been considered to evaluate the best operating conditions for protein extraction. In addition, an economic evaluation was carried out. Scenario 1 (BSG-NaOH), Scenario 2 (SCG-NaOH), and Scenario 3 (kale-NaOH) consist in a MAE using an alkali solution as the solvent. Scenario 4 (kale-H₂O) comprises a MAE using water as the solvent. After extraction, all four scenarios consider a liquid and solid phase separation stage.

The operating conditions of the MAE were selected based on previously published results and previous experimental results (data not shown). Two response variables have been compared: the protein...
recovery in other wastes (agro-industrial and fruit/vegetable wastes) and the solvent used (alkali extraction or water). The process flow considered is presented in Fig. 1.

2.6. Economic evaluation

A preliminary and comparative economic study of an industrial plant for protein extraction-purification from different AFW was carried out. A plant with a production capacity of 15 kg/h of raw material (BSG, SCG, and kale stems) and a humidity of 20% w/w was selected as the basis for the economic analysis. This flow rate was chosen based on the lowest production of the three raw materials in Castile & León (Spain) to ensure continuous year-round production. In this case, only 280 t of kale were produced in this region in 2020 (MAPA, 2021). In order to estimate the minimum selling price of protein, two stages have been considered. First, the upstream (extraction + centrifugation) has been rigorously designed based on the laboratory data using the optimal conditions for each scenario; the related equipment costs were also calculated. Second, the cost of the purification section (precipitation and spray-drying) was estimated. The downstream processes are the most expensive part of the protein extraction-purification process, and their associated cost can be around 70% of the total plant costs (Kruschitz and Nidetzky, 2020; Łojewska et al., 2016). The theoretical protein production was calculated on this basis, considering that the total precipitated protein was 70% of the theoretical. The market value of the bioactive protein extract was estimated in order to verify whether the process could become economically viable and if the selling price could be competitive.

The Lang factors method, extensively used in industrial engineering to calculate the different plant costs, was applied for this preliminary economic study. A complete method is described in the literature (Sinnott, 2005). First, the upstream equipment was designed and the associated equipment cost (PCE) was estimated using the CAPCOST software. To calculate the total plant PCE, the upstream PCE was divided by 0.3 in order to be able to apply the Lang Factor method to calculate the Total Investment Cost (TIC) of the whole process. After that, the TIC was calculated using the solid-liquid criteria of the Lang factor method. Eqs. (2)–(4) were used to estimate the plant costs.

Physical Plant Cost (PPC) = PCE * 3.15
Fixed Capital Cost (FCC) = PPC * 1.40
TIC = FCC * 1.05

The costs of the proposed raw materials were estimated from the literature: process water: 3.16 €/m³ (Aquavall, 2017), and NaOH: 4 €/kg (Sinnott, 2005). The average cost considered for the AFW was 20 €/t because the range of the BSG cost was 20 – 35 €/t (Fernández-Delgado et al., 2019), while the SCG cost was around 20–60 $/t (Atabani et al., 2019, Kamil et al., 2019), though no data were found for kale.

The following assumptions were necessary to estimate the plant profits and the minimum selling price of the protein extract. All scenarios and equipment amortization had a plant lifetime of 10 years. The annual production costs per kg of protein were estimated considering that the plant works 8000 h/y. Finally, the minimum sale price could be calculated, considering a net present value (NPV) of the plant of 0 € and an internal rate of return (IRR) of 10% (Fernández-Delgado et al., 2022).

Fig. 1. Flow diagram of the proposed scenarios. Flow diagrams elaborated according to UNE ISO 10628:2015.
3. Results and discussion

3.1. Characterization of agri-food wastes

Firstly, the composition achieved for SCG was the following (% w/w dry matter): cellulose, 16.3 ± 0.1; hemicellulose, 27.7 ± 0.7; acid-insoluble lignin (AIL), 38.5 ± 0.7; acid-soluble lignin (ASL), 0.7 ± 0.1; extractives, 12.4 ± 0.4 (glucose in extractives, 0.0 ± 0.0); ash, 0.1 ± 0.0; acetyl groups, 0.4 ± 0.0 and protein, 12.1 ± 0.4 (Lopez-Linares et al., 2021). On the other hand, the composition was (% w/w dry matter) for BSG: cellulose, 32.6 ± 0.6; hemicellulose, 23.2 ± 0.1, AIL, 13.0 ± 0.5 ASL, 1.3 ± 0.0; extractives, 14.2 ± 0.3 (glucose in extractives, 0.8 ± 0.0); ash, 13.0 ± 0.1; acetyl groups, 0.8 ± 0.0 and protein, 22.04 ± 0.2. Finally, the composition for kale stems was (% w/w dry matter): cellulose, 15.0 ± 0.0; hemicellulose, 13.0 ± 0.1, AIL, 2.1 ± 0.3; ASL, 1.8 ± 0.0; extractives, 46.9 ± 0.7 (glucose in extractives, 7.5 ± 0.6); ash, 19.3 ± 0.1; acetyl groups, 0.3 ± 0.0; and protein, 15.7 ± 0.2.

3.2. Effect of operation conditions on protein extraction yield

In order to evaluate the effect of the operating conditions on the protein extraction yield, the results of scenarios 1, 2, and 3 were analyzed. The central composite experimental designs analyzed the effects of three factors: namely temperature, time, and the NaOH concentration. Table 2 shows the experimental results obtained for the content of solid protein after the MAE and the protein extraction yield responses for each experimental run and each scenario.

As can be appreciated in Table 2, the protein extraction yield ranged between 14.1% (run 19) and 93.7% (run 11) for alkali extraction and between 9.6% (run 0.2) and 93.7% (run 11) for BSG, between 9.6% (run 0.2) and 93.7% (run 11) for alkali extraction and between 68.6% (run 19) and 95.4% (run 15) for conventional alkali extraction (86.16 vs. 45.71%), at a concentration of 110 mM NaOH and 1:15 (w/v) solid to liquid ratio for 20 min under ultrasound treatment. According to Parchami et al. (2021), 48% of the initial protein in BSG was solubilized using a hydrothermal pretreatment (180 °C and 30 min), which is a lower value than that mentioned before using alkalis. In this context, Contreras et al. (2019) also observed a significant positive effect when NaOH was added as a solvent, with concentrations up to 0.4 M; pointing out that the solid-to-liquid ratio, extraction time, pH, temperature, and alkali concentration are crucial conditions, with the absolute amount of applied alkali being the critical factor.

Table 2

<table>
<thead>
<tr>
<th>Run</th>
<th>Scenario 1 (BSG-NaOH)</th>
<th>Scenario 2 (SCG-NaOH)</th>
<th>Scenario 3 (kale-NaOH)</th>
<th>Scenario 4 (kale-H₂O)</th>
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<tr>
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<td>Yield (%)</td>
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<td>89.9</td>
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where "T" is the temperature (°C), "t" is the time (min), and "C" is the NaOH concentration (M).

In all the modeling, the values of R² and adjusted R² (Eqs. (1)–(3)), as well as the confidence levels (90%, p < 0.05), show a reasonable adjustment between the experimental and predicted data.

As observed in Eqs. (5)–(7) and Table 2, the most significant effect is the NaOH concentration in the three scenarios, this effect being positive for scenarios 1 and 3, and negative for scenario 2. In the case of BSG and kale (scenarios 1 and 3), in order of importance, the temperature and time were also positive effects, but there was a big difference between these and the NaOH concentration effect (and even more for BSG). Thus, high values of NaOH concentration could lead to an increase in the protein extraction yield for BSG and kale. The time effect was insignificant in SCG (scenario 2).

On the other hand, concerning the interactions between the different factors (Eqs. (5)–(7) and Fig. 2), a slight positive interaction between the temperature and the NaOH concentration factors can be observed in scenarios 1 and 2 (BSG and SCG).

This trend can also be observed in the Deleu et al. (2019) study, indicating that the alkali extraction conditions generally increase the protein extraction yield by breaking down the matrix in which proteins are present and making the protein of cereals and pseudo-cereals more soluble. In this way, proteins from barley were also extracted using the alkaline extraction (23°C, 0.5 M NaOH and 2 h), achieving 57.1% of protein recovery yield, with a protein content of 33 g/100 g raw material (Houde et al., 2018). Li et al. (2021) demonstrated that ultrasound alkali extraction improves the protein yield from BSG versus conventional alkali extraction (86.16 vs. 45.71%), at a concentration of 110 mM NaOH and 1:15 (w/v) solid to liquid ratio for 20 min under ultrasound treatment. According to Parchami et al. (2021), 48% of the initial protein in BSG was solubilized using a hydrothermal pretreatment (180 °C and 30 min), which is a lower value than that mentioned before using alkalis.

3.3. Effect of solvent type on extraction yield

In this case, to evaluate the effect of the solvent type on the protein extraction yield, the experimental results of scenarios 3 and 4 were analyzed. The protein concentration and protein extraction yield responses for each experiment and scenario are shown in Table 2.

As can be seen in Table 2, and comparing the experimental values for scenarios 3 and 4, the protein extraction yield ranged between 69.4% (run 16) and 95.4% (run 15) for alkali extraction and between 68.6%
(run 6) and 73.0% (run 5) for aqueous extraction. The average protein extraction yield around the central point (scenario 3: 90°C, 10 min and 1 M NaOH (runs 1 and 2); and scenario 4: 90°C and 10 min (runs 2 and 3)), was 90.5% for alkali extraction (scenario 3) and 71.9% for water extraction (scenario 4).

The protein extraction yield responses were also adjusted by the second-order polynomial equation (Eq. (8) for scenario 4):

\[
\text{Protein extraction yield} = 46.039 + 0.437 T + 0.594 t - 0.002 T^2 \\
(R^2 = 0.962; \ R^2 \text{ adjust} = 0.917)
\]

where "T" is the temperature (°C), and "t" is the time (min). The values of \( R^2 \) and adjusted \( R^2 \) (Eq. (4)), as well as the confidence levels (90%, \( p < 0.05 \)), also show a reasonable adjustment between the experimental and predicted data in this case.

As mentioned above and looking at the equations (Eqs. (7) and (8)) and Table 2, the most significant positive effect in the case of alkaline extraction is the NaOH concentration, and very slightly the temperature and time. On the other hand, in aqueous extraction, the most significant positive effect is the time, followed by the temperature (both very similar), while very low effects were found by comparing with the NaOH concentration effect observed for alkaline extraction. This behavior can also be seen in Fig. 2(C-D), respectively. This is due to the NaOH acting as a facilitating agent in the extraction process when alkalis are used. In contrast, the effect is compensated over time in aqueous extraction, according to Contreras et al. (2019), which indicates that alkaline extraction generally shows higher yields than acid or hydrothermal extraction. Moreover, it is worth highlighting that, by comparing with conventional extraction methods, microwave-assisted extraction is able to increase the protein extraction yield by up to 1.54 times (Contreras et al., 2019).

3.4. Optimization of extraction conditions

The MAE optimization from three AFW (BSG, SCG, and kale) was carried out, maximizing the protein extraction yield as the studied response. Thus, the optimal experimental conditions found by the model for the four scenarios (temperature, time, and NaOH concentration in the case of alkali extraction) are included in Table 3.

The model was validated by performing a confirmatory experimental run in optimal conditions. As can be observed in Table 3, a reasonable adjustment of the model was found for the four scenarios, since the deviations between the predicted and experimental values were less than 3% in all four cases. By comparing the three AFW (BSG, SCG and kale), it can be observed that the best protein extraction yields (92–95%) were achieved for BSG and kale when the microwave assisted alkaline extraction was carried out. However, a protein extraction yield lower than 59% was obtained for SCG using the same alkaline extraction method. On the other hand, by comparing both the hydrothermal and alkaline extraction methods for kale, both assisted by the microwave technique; an increase in the protein extraction yield of up to 22.64% was attained through the alkaline extraction technique. As described previously, alkaline extraction generally shows higher protein extraction yields than acid or hydrothermal extraction (Contreras et al., 2019).

In addition, under these optimal extraction conditions, the TPC, TFC,
antioxidant capacity, and total sugar content were determined (Table 3). As can be appreciated, except for SCG, about 14–15 g/L total sugars can be obtained for both BSG and kale raw materials, independently of the catalyst (water or alkali) used. Regarding TPC, similar concentrations (about 48–52 mg GAE/g RM) were attained for BSG and SCG, while lower values were obtained for kale (~34 mg GAE/g RM), using both hydrothermal and alkaline extraction techniques. Nevertheless, SCG was the AF with the highest TPC (15.95 mg CE/g RM), followed by BSG and kale (8.68 and 0.98 mg CE/g RM, respectively). Concerning the antioxidant activity (DPPH), up to 7.6 and 17.1 mg TE/g RM could be got from BSG and kale by microwave-assisted alkaline extraction. Therefore, in conclusion, by comparing the three AF used, BSG could be an interesting raw material for protein production, as well as total sugars and phenolic and antioxidant compounds.

On the other hand, concerning both the hydrothermal and alkaline extraction methods performed with kale, the results of total sugars, TPC, TFC and antioxidant compounds obtained (Table 3) show that hydrothermal extraction was able to get better results for TPC (34.32 vs. 20.87 mg GAE/g RM) and TFC (2.46 vs. 0.98 mg CE/g RM), as happened for the protein extraction yield described before, but with much lower DPPH values (1.71 vs. 7.57 mg TE/g RM). The total sugar content was similar (about 15 g/L) for both extraction techniques.

Finally, by comparing the results obtained in this work (Table 3) with the literature, considering BSG for instance, similar results (90–95%) were attained by Qin et al. (2018) using dilute acid (11,400 mg H2SO4/g BSG, 121 °C for 1 h), or sequential alkaline (110 mM NaOH, 1:20 w/v, 50 °C and 200 rpm) and dilute acid (1 M H2SO4, 25 °C, 250 rpm for 1 h, followed by autoclaving at 121 °C for 1 h) extraction, but lower values (64–66%) by hydrothermal extraction (2.5% w/v, 60°C, 24 h). A much lower protein extraction yield (48%) and concentration (27 g/L) was also achieved by Parchami et al. (2021) using hydrothermal extraction (180 °C and 30 min). Du et al. (2020) also obtained a low protein extraction yield (21.4%, 6.8% and 7.2%) using three different extraction methods: alkaline (40°C, 120 min and 0.1 M NaOH), aqueous (40°C and 120 min), and subcritical water extraction (200°C and 20 min).

With regard to SCG, similar results for protein extraction yields (about 59%) to those obtained in this work (61%, Table 3) were achieved by an acid extraction process (using 0.1 M HCl and 0.1 M NaCl, at 1:12, w/v) at 4 °C for 12 h (Ribeiro et al., 2021). Moreover, the presence of phenolic (1755.76 μmol GAE/g) and antioxidant compounds (ABTS: 441 μmol TE/g SCG; FRAP: 611 μmol TE/g SCG) was also detected. This therefore means that the protein extraction can be suitably carried out using alkali and acid, which may be due to the structure of the SCG. Samsalee and Sothornvit (2021) got a lower protein content (34%) by ultrasonic-assisted extraction (40% amplitude, 20 min and pH 11 using 0.7 M NaPO4). However, much higher TPC and DPPH values (304.81 mg GAE/g RM and 933.92 mM TE/g RM) were obtained.

As pointed out above, similar or relatively higher protein extraction yields are achieved in this study. However, the bioactive liquid extracts obtained in this work are characterized by containing an appreciable amount of antioxidant compounds and carbohydrates, giving them greater added value for their possible commercial application in the food, pharmaceutical and cosmetic industries.

3.5. Economic evaluation

3.5.1. Investment and production costs

A preliminary economic study compared the optimal protein recovery strategies from BSG, SCG, and kale wastes. Table 4 summarizes the results for the Lang Factor method for the four proposed scenarios.

The total cost of the equipment of scenarios 1, 2, and 3, based on NaOH-extraction, is identical (153,000 €), regardless of the raw material used because the necessary equipment is the same in these scenarios. However, the cost of scenario 4, which uses water as a solvent, is lower (150,000€), because the solvent storage tank does not require corrosion-resistant construction materials, and this scenario does not have the NaOH solvent preparation stage before MAE.

This cost directly affects the TIC, 690,000 € (scenario 4) to 710,000 € (scenarios 1, 2, and 3). On the other hand, the determining factor to estimate production costs is the raw material, mainly the amount of NaOH required in each scenario. Achieving the lowest cost of the raw material and the lowest annual production cost required, the production cost of the protein extract may thus be reduced. So, as shown in Table 4, scenario 4 had the lowest production cost (438,000 €/yr) as the extraction is only performed with water. If the NaOH-extraction scenarios are compared, scenario 1 had a lower production cost (524,000 €/yr) in comparison to scenarios 2 and 3 (647,000 €/yr), since scenario 1 needs a lower concentration of NaOH (0.5 M) in contrast to scenarios 2 and 3, which use almost 3-times more of NaOH during the extraction (1.3 M). Otherwise, the production cost per kg of protein was associated with the initial protein concentration of the AFW and with the extraction yield achieved under optimum operating conditions. So, scenario 1, whose yield was higher than scenarios 2 and 3, had the lowest production costs per kg of protein (29.9 €/kg versus 51.1 –104.6 €/kg). On the other hand, comparing the kale-extraction scenarios (3 and 4), the production cost depends principally on the solvent used. In this case, scenario 4, using water as a solvent, had a lower production cost (45.5 €/kg) versus scenario 3 with NaOH solvent (51.1 €/kg).

Finally, the minimum selling price for the protein, shown in Table 4, is the selling price from which the plant would begin to be profitable. As can be seen, the lowest selling price for the protein obtained is associated with scenario 1, with 51.7 €/kg corresponding to the BSG-NaOH extraction process and a higher yield (14.6 kg protein/100 kg DM). In comparison, the highest price was reached in scenario 2 (SCG-NaOH), with a selling price of 168.6 €/kg, whose overall extraction yield is the lowest (5.2 kg protein/100 kg DM). In any case, these prices are above the estimated prices for the sale of protein found in the literature, indicating that the proposed processes are not profitable. The market price depends on the raw material and the protein properties (Baker and Charlton, 2020). For example, Muneer et al. (2021) estimated that the protein market price varied from 2 to 15 €/kg. However, this process could prove profitable if not only the precipitated protein is taken into account. The extracts obtained after extraction contain significant

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Characterization of optimal extracts.</th>
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<tbody>
<tr>
<td></td>
<td>Scenario 1 (BSG-NaOH)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>110</td>
</tr>
<tr>
<td>Time (min)</td>
<td>9.98</td>
</tr>
<tr>
<td>NaOH concentration (M)</td>
<td>0.50</td>
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<tr>
<td>Protein extraction yield (%)</td>
<td>93.99</td>
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<tr>
<td>Confirmatory experimental protein extraction yield (%)</td>
<td>92.05</td>
</tr>
<tr>
<td>Total sugars (g/L)</td>
<td>13.84</td>
</tr>
<tr>
<td>TPC (mg GAE/g RM)</td>
<td>48.42</td>
</tr>
<tr>
<td>TFC (mg CE/ g RM)</td>
<td>8.68</td>
</tr>
<tr>
<td>DPPH (mg TE/g RM)</td>
<td>17.10</td>
</tr>
</tbody>
</table>
amounts of sugars and antioxidant compounds, demonstrating an adequate antioxidant capacity. For example, the extract from scenario 1 has a sugar content of 13.84 g/L, a TPC of 48.42 mg GAE/g RM, a TFC of 8.68 mg CE/g RM, and a DPPH antioxidant capacity of 17.10 mg TE/g RM. These concentrations would positively affect the selling price of the bioactive extracts obtained. In this way, the profitability of the process would be significantly improved.

3.5.2. Sensitivity analysis

Based on the economic evaluation, a sensitivity analysis was performed to analyze the influence of the most critical parameters that could affect the NPV (Fig. 3). For the evaluated scenarios, the key parameters that significantly affect the NPV are the protein selling price and the total direct costs.

Among the raw materials costs, those of the AFW and water are insignificant in the NPV variation in the scenarios evaluated (Fig. 3). On the contrary, the variation in the cost of NaOH affects scenarios 1, 2, and 3 (NaOH-extraction). The NPV can vary by up to 360,000 € when the cost of the NaOH changes by 50% (incremental and decremental) (Fig. 3. C). On the other hand, scenario 1 (BSG-NaOH) is the least affected by the variation in the cost of the NaOH, since the NPV only decreases to 140,000 € when the cost of the NaOH increases by 50%.

Concerning the plant profits, the only income is generated from selling the protein, while the sensitivity analysis demonstrates that the NPV value is susceptible to changes in this price. For example, a 50% increase in the protein sale price can increase the NPV by 1280,000 € to 1675,000 € (Fig. 3. B-D). However, the increment in the protein price is unfeasible from an economic point of view, and it is necessary to reduce the selling price in order to be competitive. For example, in the case of scenario 1 (BSG-NaOH), which has the lowest selling price (51.7 €/kg) (Table 4), the sensitivity analysis shows that if the protein were to be sold with a competitive price (15 €/kg), the plant losses would be above 2100 k€.

Some researchers have demonstrated the economic viability of the MAE technology for other applications (Zhang et al., 2014; Fernández-Delgado et al., 2022). Therefore, an alternative to obtaining a viable and competitive extraction process could be to optimize the MAE process to obtain extracts enriched not only in proteins, but also in other bioactive compounds of interest for food and biomedical applications. As seen before, these extracts contain a significant sugar concentration and antioxidant compounds, proving a suitable antioxidant capacity. In this way, the production costs could decrease significantly and the selling price of the whole process could be competitive in the food and pharmaceutical market.

### Table 4

| Economic evaluation of the proposed scenarios. |
| Units | Precipitated Protein | PCE | TIC | Production Cost | Minimum Selling Price |
| Yield kg/100 kg DM | Flow kg/h | € | € | €/year | €/kg |
| Scenario 1 (BSG-NaOH) | 14.6 | 2.2 | 153,000 | 710,000 | 524,000 | 29.9 | 51.7 |
| Scenario 2 (SGC-NaOH) | 5.2 | 0.8 | 153,000 | 710,000 | 647,000 | 104.6 | 168.6 |
| Scenario 3 (kale-NaOH) | 10.6 | 1.6 | 153,000 | 710,000 | 647,000 | 51.1 | 82.3 |
| Scenario 4 (kale-H$_2$O) | 8.0 | 1.2 | 150,000 | 690,000 | 438,000 | 45.5 | 82.9 |

**Fig. 3.** Sensitivity analysis for the proposed scenarios. (A) Scenario 1: BSG + NaOH; (B) Scenario 2: SCG + NaOH; (C) Scenario 3: kale + NaOH; (D) Scenario 4: kale + H$_2$O.
4. Conclusions

This study confirms that microwave-assisted hydrothermal and alkali extraction is a suitable technology for the efficient recovery of bioactive compounds from the agri-food wastes tested (BSG, SCG and kale stems). Technically and economically, microwave-NaOH extraction from BSG provides the best alternative for obtaining extracts of commercial interest enriched in protein (protein recovery yield of 94%), total sugars and antioxidant compounds. However, hydrothermal microwave extraction from kale stems could become a promising process that combines a novel waste in a biorefinery context, which contains an appreciable protein concentration (15.7% w/w) with a water-based cleaner solvent operating under mild process conditions.

CRediT authorship contribution statement

Cristina Barrios: Investigation, Methodology, Writing – original draft. Marina Fernanda Delgado: Investigation, Methodology, Writing – original draft. Juan C. López-Linares: Investigation, Methodology, Writing – original draft, Supervision. María Teresa García-Cubero: Conceptualization, Supervision, Writing – original draft. Mónica Coca: Conceptualization, Formal analysis, Supervision. Susana Lucas: Conceptualization, Writing – review & editing, Project administration.

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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