processes

MDPI

Article

From a Single-Stage to a Two-Stage Countercurrent Extraction of Lipids and Proteins from Full-Fat Chickpea Flour: Maximizing Process Extractability and Economic Feasibility

Kazunori Machida [1,†], Fernanda Furlan Goncalves Dias [2,†], Zhiliang Fan [1] and Juliana Maria Leite Nobrega De Moura Bell [1,2,*]

[1] Department of Biological and Agricultural Engineering, University of California, One Shields Avenue, Davis, CA 95616, USA
[2] Department of Food Science and Technology, University of California, One Shields Avenue, Davis, CA 95616, USA
* Correspondence: jdemourabell@ucdavis.edu
† These authors contributed equally to this work.

Abstract: The mainstream adoption of chickpea proteins and lipids requires a thorough understanding of the impact of critical extraction parameters (enzyme use, reaction time, and solids-to-liquid ratio—SLR) and modes of extraction (single-stage extraction—SSE and countercurrent extraction—CCE) on the simultaneous extraction of lipids and proteins from full-fat chickpea flour and economic process feasibility. A kinetics study revealed that 68.5% oil and 87% protein extraction yields can be achieved using 0.5% protease at pH 9.0, 50 °C, 60 min, and 1:10 SLR, highlighting the role of proteolysis and an adequate incubation time on overall extractability. An increased gradient concentration between the matrix and aqueous media solutes at a lower SLR (1:15), and reduced slurry viscosity increased oil and protein extractability to 80 and 91%, respectively. The high-water usage in the SSE was addressed by the development of a two-stage CCE that reduced water usage by 47% while increasing oil and protein extractability to ~96%. Higher extractability and reduced water usage in the two-stage CCE resulted in a higher net gross profit, thus outweighing its higher operating costs. The results presented herein further widen the scope of bioprocessing standards for full-fat chickpea flour and add to the elucidation of the impact of key processing conditions on the extractability and economic feasibility of the production of chickpea ingredients for subsequent food/nutraceutical applications.

Keywords: full-fat chickpea flour; oil and protein extraction; enzymatic extraction; economic analysis

Citation: Machida, K.; Furlan Goncalves Dias, F.; Fan, Z.; De Moura Bell, J.M.L.N. From a Single-Stage to a Two-Stage Countercurrent Extraction of Lipids and Proteins from Full-Fat Chickpea Flour: Maximizing Process Extractability and Economic Feasibility. *Processes* **2022**, *10*, 2349. https://doi.org/10.3390/pr10112349

Academic Editor: Agnieszka Zgoła-Grześkowiak

Received: 27 September 2022
Accepted: 3 November 2022
Published: 10 November 2022

1. Introduction

Consumers' desire for plant-based diets and the development of new plant-based products by the food industry are driving the surge in pulse crop production such as chickpeas, lentils, dry beans, and peas [1]. The shift toward a plant-based diet is evidenced by the plant protein market growth, which is expected to rise from USD 17,222.9 million in value in 2019 to USD 27,965.9 million by 2027 [2]. The increasing interest in plant-based products has been primarily attributed to the health benefits associated with the consumption of plant-based diets (i.e., reduction of cardiovascular diseases, gluten-free) and with the sustainability aspect associated with the production of plant-based ingredients [3,4]. An environmental impact review of the consumption of plant-based ingredients has shown a significant reduction in the production of greenhouse gases and water usage compared to that of traditional food diets and systems [5,6]. This rapid growth in the plant-based market has spurred additional interest in developing and improving processing techniques for the production of highly extractable and functional macronutrients that can supply current plant-based demand.

Chickpeas are an example of a pulse crop whose production in the U.S. increased from 60 million pounds in 2000 to 283 million kg in 2019 [7]. Like other pulse crops, the consumption of chickpeas is supported by its nutritional value. Chickpeas are a source of proteins (19%), carbohydrates (60%), lipids (6%), and dietary fiber (17%) [8]. In addition to its high protein content, chickpea proteins have a subtle taste and desired functional properties [9], making chickpeas a worldwide staple crop that can be used as a matrix to deliver highly sought-after products and/or ingredients for the manufacture of food products [10].

Chickpea protein extraction is traditionally accomplished by the use of an upstream lipid removal step involving defatting the chickpea flour with organic solvents such as hexane. To address the environmental, safety, and public concerns associated with the use of hexane, flammable solvent-free extraction processes such as the aqueous extraction process (AEP) and enzyme-assisted aqueous extraction process (EAEP) have been used to simultaneously extract proteins, lipids, and carbohydrates, among other minor compounds, from several food matrices [11,12]. The immediate advantage it carries is the complete elimination of the upstream lipid removal step by either solvent extraction or mechanical pressing. Instead, AEP and EAEP rely on the solubilization of proteins into the extraction medium (water), which then favors the washing out of oil droplets through the porous structure [13,14]. Enzyme addition to the AEP leads to the EAEP, where proteases and carbohydrases are commonly used to further catalyze the extraction of lipids and proteins [15]. Oil and protein extractability in the EAEP can significantly increase due to the enzymatic hydrolysis of the lipid body membrane, proteins, and cell walls [16]. Under controlled conditions (i.e., pH, temperature, reaction time, enzyme concentration), water is mixed with full-fat flour, resulting in a slurry with the dispersed food matrix. The slurry is subsequently centrifuged to fractionate the insoluble fraction (the fiber-rich fraction containing unextracted proteins and lipids) from the liquid phase containing the extracted compounds. The liquid phase is further fractionated by density difference into free oil, cream (an emulsion that contains most of the extracted oil), and skim (protein-rich phase) [13,17].

We have previously evaluated the effectiveness of different enzymes in assisting the extractability and distribution of lipids, proteins, and carbohydrates from full-fat chickpea flour [18]. The AEP (pH 9, 1:10, SLR 50 °C, 60 min) achieved oil and protein extraction yields of 48 and 63%, while the EAEP (AEP conditions with an additional 0.5% (*w/w*, weight of enzyme/weight of flour) protease) resulted in oil and protein extraction yields of 77 and 86%, respectively. Besides increasing the overall process extractability, the use of proteases and carbohydrases assists the extraction-generated proteins with higher solubility and digestibility, in addition to releasing new oligosaccharide molecules in the protein-rich extract [18]. However, the successful development of extraction methods for new protein sources depends on the development of fundamental knowledge of the impact of the key processing conditions employed (i.e., pH, time, type of enzyme and concentration, solids-to-liquid ratio (SLR), temperature [13]) on the extractability, composition, and functional properties of the extracted compounds. Enzyme specificity and concentration not only play a key role in the overall extractability of lipids, proteins, carbohydrates, and other minor soluble compounds from the matrix but also affect the separation of the fractions obtained (cream, skim, and insoluble fractions), the strength of the cream emulsion produced (thus affecting the final recovery of the extracted oil), the functional and biological properties of the extracted proteins, and the recovery of the extracted protein (i.e., by isoelectric precipitation or ultrafiltration) [12,13,15,19,20]. Common processing challenges in AEP and EAEP include maximizing the overall processing extractability of the desired compounds; achieving adequate distribution of the extracted compounds (i.e., more oil in the cream less oil in the skim, and more protein in the skim); recovering the extracted oil, which is mostly entrapped in the cream emulsion, through the development of chemical or enzymatic strategies to breakdown the emulsion to free the cream oil; and minimizing the water usage in the process without a reduction in extractability [15,21,22].

Higher extraction yields in AEP and EAEP have often been accomplished by the use of single-stage extractions, which require higher water usage. In general, a low solids-to-

liquid ratio (SLR) (1:10) has been used to maximize the process extractability of many food matrices [23,24]. The use of low SLR (i.e., a higher amount of water) has been shown to favor protein solubilization and, to a lesser extent, the washing or extractability of lipids. As an example, reducing the amount of water used in the single-stage EAEP of soybeans from 1:10 to 1:5 resulted in the reduced extractability of lipids and proteins from soybean flakes, as demonstrated by the increase in the lipid (from 5 to 10%) and protein (from 12 to 20%) contents of the insoluble fraction (extraction byproduct) [21].

Countercurrent extraction approaches with the use of flammable solvents such as hexane have been widely used by the oil industry to reduce solvent use without a loss in the process extractability [23]. Because of increasingly restrictive environmental, safety, and consumer concerns regarding the use of flammable solvents such as hexane, this strategy has also been applied in the AEP and EAEP of soybeans, defatted sunflower seed meal, and Antarctic Krill [21,25,26]. Countercurrent extraction systems rely on contacting the freshest feed (starting material) with a richer extraction medium originating from a previous extraction, where the feed continues to be extracted until near-depleted feed contacts the fresh extraction medium. The development of a two-stage countercurrent extraction EAEP for extruded soybean flakes proved to be successful not only in reducing the amount of water used in the process by approximately one-half but also in increasing protein (from 87 to 96%) and oil (from 96 to 98%) extraction yields compared with the single-stage extraction process [21].

While previous studies have unveiled the impact of AEP and EAEP for many other legumes and oilseeds, there is limited information about the effects of key extraction parameters such as SLR, the amount and type of enzyme, and the extraction pH on the overall extractability of lipids and proteins from chickpea full-fat flour. As a consequence, there is also a lack of economic analysis to compare the processing feasibility of current processing practices (upstream lipid removal by solvent extraction followed by the aqueous extraction of chickpea proteins) with the use of AEP and EAEP, which simultaneously extract chickpea lipids, proteins, and carbohydrates without the use of flammable solvents.

The overall goal of this work was to evaluate the impact of the amount of enzyme used to assist the extraction, reaction time, and amount of water used in the process on oil and protein extractability and processing economics. The specific objectives of this work were to: (a) evaluate different concentrations of protease (0 to 0.75%) and extraction times (15 to 75 min) in the single-stage EAEP of chickpea full-fat flour; (b) determine the impact of increasing the solids-to-liquid ratio (1:15 to 1:6) on the overall extractability of the single-stage EAEP of chickpea full-fat flour; (c) evaluate the effectiveness of a two-stage countercurrent extraction in reducing water usage while maintaining or increasing oil and protein extractability, and (d) evaluate the economic impact of implementing the two-stage countercurrent EAEP vs. the single-stage EAEP.

2. Materials and Methods

2.1. Chickpea Flour and Enzyme Use in the EAEP

Steamed chickpea flour of Kabuli chickpeas was kindly provided by Natural Products, Inc. (Grinnell, IA, USA). The starting material was analyzed as described in Section 2.5 and contained 7.4 ± 0.1% oil, 25.9 ± 0.07% protein, and 4.7 ± 0.1% moisture. FoodPro Alkaline Protease (also known as Protex 6), a commercial bacterial alkaline endoprotease from *Bacillus licheniformis* with pH activity from 8.0 to 10.5, temperature from 45 °C to 75 °C, and enzyme activity of 580,000–650,000 DU/g (Genencor Division of Danisco, Rochester, NY, USA), was used for enzymatic extractions.

2.2. Effect of Enzyme Concentration and Reaction Time on the Extractability of the Single-Stage Enzyme-Assisted Extraction Process of Chickpea Flour

Enzyme kinetic studies were conducted to determine the effects of the amount of protease used to assist the extraction and reaction time on the total oil (TOE) and protein (TPE) extraction yields. Extractions were performed by dispersing 50 g of chickpea flour into 500 mL of water to achieve a 1:10 solids-to-liquid ratio (SLR). The addition of FoodPro

Alkaline Protease at concentrations of 0% (AEP, control), 0.3%, 0.5%, and 0.7% and extraction times of 15, 30, 45, 60, and 75 min was evaluated. The slurry pH was adjusted to pH 9.0 to favor protein solubility and extractability [27] and was kept at 50 °C under constant stirring of 120 rpm. The insoluble fraction (spent solids) was separated from the liquid (extracted compounds) by centrifugation of the slurry at 4000× *g* for 30 min at 4 °C. Subsequent fractionation of the liquid fraction was accomplished by allowing the liquid fraction to settle overnight at 4 °C in a separatory funnel. After overnight settling, the liquid fraction was separated into skim (protein- and carbohydrate-rich fraction), cream (oil-rich fraction), and free oil, which were stored and analyzed for the distribution of the extracted oil and protein (Figure 1A). Total oil (TOE) and protein extraction yields (TPE) were determined according to Equations (1) and (2), respectively:

Total Oil Extraction Yield

$$TOE\ (\%) = \left[100 - \left(\frac{Oil\ (g)\ in\ Insoluble}{Oil\ (g)\ in\ chickpea\ flour}\right)\right] \times 100 \quad (1)$$

Total Protein Extraction Yield

$$TPE\ (\%) = \left[100 - \left(\frac{Protein\ (g)\ in\ Insoluble}{Protein\ (g)\ in\ chickpea\ flour}\right)\right] \times 100 \quad (2)$$

Each extraction condition was carried out in triplicate.

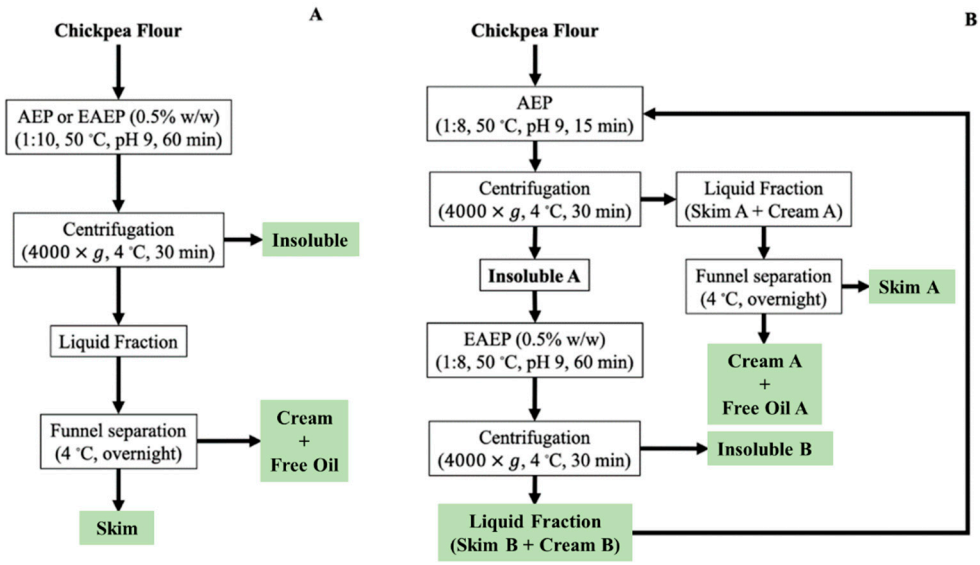

Figure 1. Simplified processing schematic of the single-stage (**A**) and two-stage (**B**) countercurrent EAEP of full-fat chickpea flour.

2.3. Effects of Solids-to-Liquid Ratio (SLR) on the Extractability of the Single-Stage Enzyme-Assisted Aqueous Extraction Process of Chickpea Flour

After the identification of the amount of enzyme and the reaction time leading to higher oil and protein extractability (Section 2.2), the effects of different SLRs on the extraction kinetics of the single-stage EAEP were evaluated. Extractions were performed using the selected amount of enzyme and the reaction time from Section 2.2, except for the SLRs, which were 1:6, 1:8, 1:10, and 1:15 (weight of flour/weight of water). Each extraction condition was performed in triplicate, and the overall processing efficiency (TOE and TPE) was calculated according to Equations (1) and (2), as described in Section 2.2.

2.4. Two-Stage Countercurrent Enzyme-Assisted Aqueous Extraction Process of Full-Fat Chickpea Flour

A two-stage countercurrent EAEP was developed to overcome the reduction in the overall process extractability when a higher SLR is used in the single-stage EAEP (Figure 1B). Each two-stage countercurrent EAEP run was performed in triplicate, with each EAEP run being composed of three samples of chickpea flour that were sequentially extracted. Each sample was subjected to two extractions in batch mode. Extractions were also conducted without an enzyme (AEP, control) to compare the enzyme use impact throughout the process. The first extraction of the first run was performed by dispersing 50 g of chickpea flour into water to achieve a 1:8 SLR. The slurry pH was adjusted to 9.0, and the reaction was performed at 50 °C for 15 min under constant stirring. After the first extraction, the slurry was centrifuged to separate the liquid fraction A (skim A + cream A, which left the process) from the first insoluble fraction (insoluble A), which was used as the substrate for the second extraction. The second extraction was performed by dispersing the first insoluble fraction into water to achieve a 1:8 SLR, adjusting the slurry pH to 9.0 before the addition of 0.5% protease (*w/w*, weight of enzyme/weight of flour), and keeping the slurry at 50 °C for 60 min under constant stirring. The resulting slurry was centrifuged to separate the second insoluble fraction (insoluble B) from the liquid fraction B (skim B + cream B), which was subsequently used to slurry the first extraction of the second sample, where fresh incoming chickpea flour was used. This was the first time that the enzyme used in the second extraction was recycled into the first extraction of the following sample. The second extraction of the insoluble fraction of the first extraction of the second sample was performed as described for the first sample, and this procedure was repeated for the third sample. The sequential extraction of the three samples, constituting one complete run, was repeated three times. Only samples (insoluble, cream, and skim) collected from the third sample of each extraction run were analyzed to ensure that the enzyme had been adequately recirculated throughout the process.

Oil and protein yields in the insoluble, skim, and cream fractions of the third sample of each run were calculated according to Equations (3) and (4).

$$Oil\ distribution\ in\ the\ fractions\ (\%) = \left(\frac{Oil\ (g)\ in\ fraction}{Oil\ (g)\ in\ chickpea\ flour}\right) \times 100 \quad (3)$$

$$Protein\ distribution\ in\ the\ fractions\ (\%) = \left(\frac{Protein\ (g)\ in\ fraction}{Protein\ (g)\ in\ chickpea\ flour}\right) \times 100 \quad (4)$$

2.5. Proximate Analysis

Cream, skim, insoluble, and starting materials (chickpea flour) were analyzed regarding dry matter, oil, and protein contents. Dry matter content was measured by weighing after drying the samples in a vacuum oven (AOCS method 925.09) [28]. Oil content was determined by using the Mojonnier acid hydrolysis (AOCS method 989.05) [28], and protein content was determined by using the Dumas method and a conversion factor of 6.25 (Vario MAX cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). The extraction yields of oil, protein, and solids were expressed as percentages relative to their initial amounts in the chickpea flour. All analyses were conducted in duplicate, and a mass balance was provided for all extracted compounds.

2.6. Viscosity Analysis

The viscosity of slurries with different SLRs (1:6, 1:8, 1:10, 1:15) was determined using a viscometer (Ametek Brookfield DV2T Rotational Viscometer, Middleborough, MA, USA). The slurries evaluated in this experiment were produced with 600 g of water basis. A total of 600 g of DI water was placed in a 600 mL beaker, and the corresponding flour amounts were added for each SLR by weight: 40 g of flour for 1:15, 60 g of flour for 1:10, 75 g of flour for 1:8, and 100 g of flour for 1:6. Each slurry was raised to 50 °C and pH 9.0. The viscosity analysis of each slurry was performed with the use of the LV-1 spindle, and an appropriate

rpm was selected such that the % torque would fall within the acceptable range of 10–100%. The viscosity of each SLR sample, at the appropriate rpm, was measured in duplicate.

2.7. Techno-Economic Analysis

The economic impact of replacing the single-stage EAEP (Scenario1) with a two-stage countercurrent EAEP (Scenario 2) was evaluated by estimating the extra capital cost associated with implementing the two-stage countercurrent EAEP vs. the single-stage EAEP and then determining the payback time of the extra capital cost by the increased net gross profit as a result of implementing the two-stage countercurrent EAEP. SuperPro Designer v12.0 (Intelligen, Inc., Scotch Plains, NJ, USA) was used to model the industrial-scale extraction process for the single-stage EAEP and the two-stage countercurrent EAEP of chickpea flour according to the processes described in Figure 1, with the assumption that 100% of the skim protein and cream oil could be recovered. The revenue, operating costs, and capital costs were evaluated and compared.

The payback time was calculated using Equations (5)–(7) [29].

$$\Delta Capital\ Investment = Investment\ Scenario\ 2 - Investment\ Scenario\ 1 \quad (5)$$

$$\Delta annual\ gross\ Profit = Profit\ of\ Scenario\ 2 - Profit\ of\ \ Scenario\ 1 \quad (6)$$

$$Payback\ Time = \frac{\Delta Capital\ Investment}{\Delta Annual\ Gross\ Profit}\ years \quad (7)$$

2.8. Statistical Analysis

Each extraction process was performed in triplicate, and each sample was analyzed in duplicate. Replicates of each measurement were analyzed by ANOVA with generalized linear models from the Statistica software (version 13.5.0.17 1984-2018, TIBCO Software Inc., Palo Alto, CA, USA). Multiple comparisons of least-square means were made by Tukey's adjustment, with the level of significance set at $p < 0.05$. Statistical significance differences were denoted by different letters, with the letter "a" being assigned to the highest value.

3. Results and Discussion

3.1. Effect of Enzyme Concentration and Reaction Time on the Extractability of the Single-Stage Enzyme-Assisted Extraction Process of Chickpea Flour

Previous work demonstrated that the use of enzymes to assist the extraction of full-fat chickpea flour was effective not only in significantly increasing oil and protein extractability but also in producing extracts containing more digestible proteins and new oligosaccharides [18]. The use of 0.5% of FoodPro Alkaline Protease, regardless of the use of a carbohydrase pretreatment or not, increased oil extractability from 49.8 to 77.2% and protein extractability from 62.8 to 83.5% compared with the AEP, respectively.

To improve upon the extraction processing conditions that were previously identified, we determined the effects of different FoodPro Alkaline Protease concentrations and reaction times with respect to oil and protein extraction kinetics. The oil and protein extraction yields from the kinetics study are described in Figure 2.

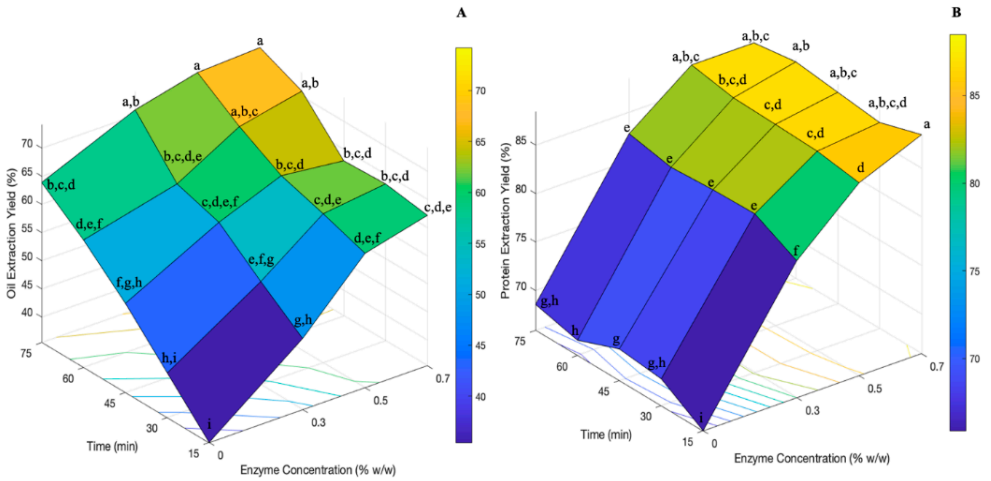

Figure 2. Total oil (**A**) and protein (**B**) extraction yields at each time point and enzyme concentration. Different letters indicate statistically significant differences by a two-way ANOVA followed by Tukey's test at $p < 0.05$.

For each enzyme concentration evaluated, oil extraction yields increased with higher reaction times, while, for each reaction time, the use of higher enzyme concentrations favored oil extraction yields (Figure 2A). However, the statistical significance of the increments observed varied within the experiments. For example, the use of 0.5% enzyme significantly increased the TOE from 59.3 to 73.7% when the reaction time increased from 15 to 75 min, although the extraction yields were not statistically different ($p < 0.05$) between 60 min (68.5%) and 75 min (73.7%). At a 60 min reaction time, the oil extractability significantly ($p < 0.05$) increased from 58.1 to 68.5% when the enzyme concentration increased from 0 to 0.5%, with no statistically significant improvement in extractability being observed when the enzyme concentration increased from 0.5% (68.5) to 0.7% (70.9%). Overall, the use of a higher extraction time (60–75 min) and a higher amount of enzyme (0.5–0.7%) led to increased oil extractability (up to 74.2% for 0.7% of enzyme and a 75 min reaction time). The higher oil extractability observed at longer reaction times and higher enzyme amounts can be due to the increased exposure of the food matrix to the protease, which favors lipid extraction. As previously described, proteases can actively hydrolyze the lipid body membrane and release lipids into the extraction medium [23]. In addition, protein removal from the matrix by solubilization or proteolysis leaves behind a more porous structure that facilitates the release of the oil from the matrix [19]. Increased enzyme concentrations allow for higher proteolysis, while increased reaction times allow the protease to fully interact with available substrates. Based on the extraction kinetics results, similarly high oil extraction yields (not statistically different at $p < 0.05$) were obtained at the following extraction conditions: 74.2% TOE (0.7% of enzyme and 75 min), 68.5% TOE (0.5% of enzyme and 60 min), 71.0% TOE (0.3% enzyme and 75 min), and 70.9% TOE (0.7% enzyme and 60 min). Based on the lack of a statistical difference among the extraction yields above, the extraction conditions with reduced enzyme use and reaction times (0.5% of enzyme and 60 min or 0.3% of enzyme and 75 min) could be used as optimum conditions to maximize the oil extractability from full-fat chickpea flour.

When looking at the impact of enzyme concentration and reaction time on protein extractability (Figure 2B), enzyme concentration was the major processing parameter significantly impacting protein extractability, with minimum to no changes in protein extractability being observed within the different reaction times evaluated. The effect of reaction time was only statistically significant at enzyme concentrations varying from 0 to 0.5%, although the magnitude of the increase in protein extractability observed was small, as demonstrated by the general slope, which is not nearly as pronounced as the one describing the impact of reaction time on oil extractability.

For example, at 75 min of reaction time, total protein extractability (TPE) increased from 68.6 to 87.6% when the enzyme concentration increased from 0 to 0.5%, with no significant increase in extractability being observed when the enzyme concentration was further increased from 0.5% (87.6%) to 0.7% (87.9%). At 0.5% enzyme, the TPE increased minimally (although statistically significant at $p < 0.05$) from 85.8 to 87.6% when the reaction time increased from 15 to 75 min, although the extraction yields between 30 min (85.8%) and 75 min (87.6%) were not statistically different. According to Figure 2B, the highest TPE (88.5%) was achieved by the use of 0.7% enzyme and a 15 min reaction time. However, a similar TPE (87.6%) was also achieved by the use of a smaller amount of enzyme (0.5%) and a longer reaction time (75 min).

Because the feasibility of EAEP depends on maximizing the extractability of both lipids and proteins from the chickpea flour, the use of 0.5% of protease and a 60 min reaction time, which favored the simultaneous extractability of lipids and proteins, was selected for subsequent experiments. The results presented herein are in agreement with our previous work, where oil and protein extraction yields from full-fat chickpea flour of 77.2 and 83.5%, respectively, were achieved when 0.5% of the enzyme and a reaction time of 60 min were used.

The results presented herein are in agreement with the ones presented for other food matrices such as soybeans, where the use of an intermediate enzyme concentration (0.5%) favored the oil and protein extraction of the EAEP of the almond cake [19]. Although the extraction kinetics experiments presented herein were useful in determining the necessary amount of enzyme and the reaction time needed to maximize the extractability of lipids and proteins from chickpea flour using the single-stage EAEP, the impact of different SLRs, which impact the overall process extractability and economics, had yet to be evaluated for the single-stage EAEP of chickpea flour.

3.2. Effects of Solids-to-Liquid Ratio (SLR) on the Extractability of the Single-Stage EAEP of Chickpea Flour

Building on the results of the impact of the amount of enzyme and reaction time on extraction kinetics, where 0.5% of the enzyme and a reaction time of 60 min were selected for subsequent extractions, we evaluated how different SLRs impact the oil and protein extractability from chickpea flour. It is well known that, to a certain extent, higher water usage (low SLR) leads to enhanced protein solubilization into the extraction medium and, to a lesser extent, also enhances oil extractability [21]. The effects of increasing SLRs from 1:15 to 1:6 on the oil and protein extractability of the single-stage EAEP of chickpea flour are shown in Figure 3A,B.

As expected, oil and protein extraction yields significantly increased as the SLR decreased from 1:6 to 1:15. Although the oil and protein extraction mechanisms are different, often, conditions that favor protein extractability also favor lipid extractability, although to different extents [19]. Oil extraction yields increased from 55.1 to 80.1% when the SLR decreased from 1:6 to 1:15, in agreement with the trend observed for protein extractability. Because of the insolubility of lipids in water, lipids are washed from the porous structure within the chickpea flour matrix into the aqueous solvent [15], which is enhanced by the reduced viscosity of the extraction medium when the SLR decreases. In addition, oil extraction yields can benefit from a reduced SLR because of the reduced interfacial coverage of lipid bodies by adsorbed proteins [11], which are released into the extraction medium, thus reflecting higher protein extractability.

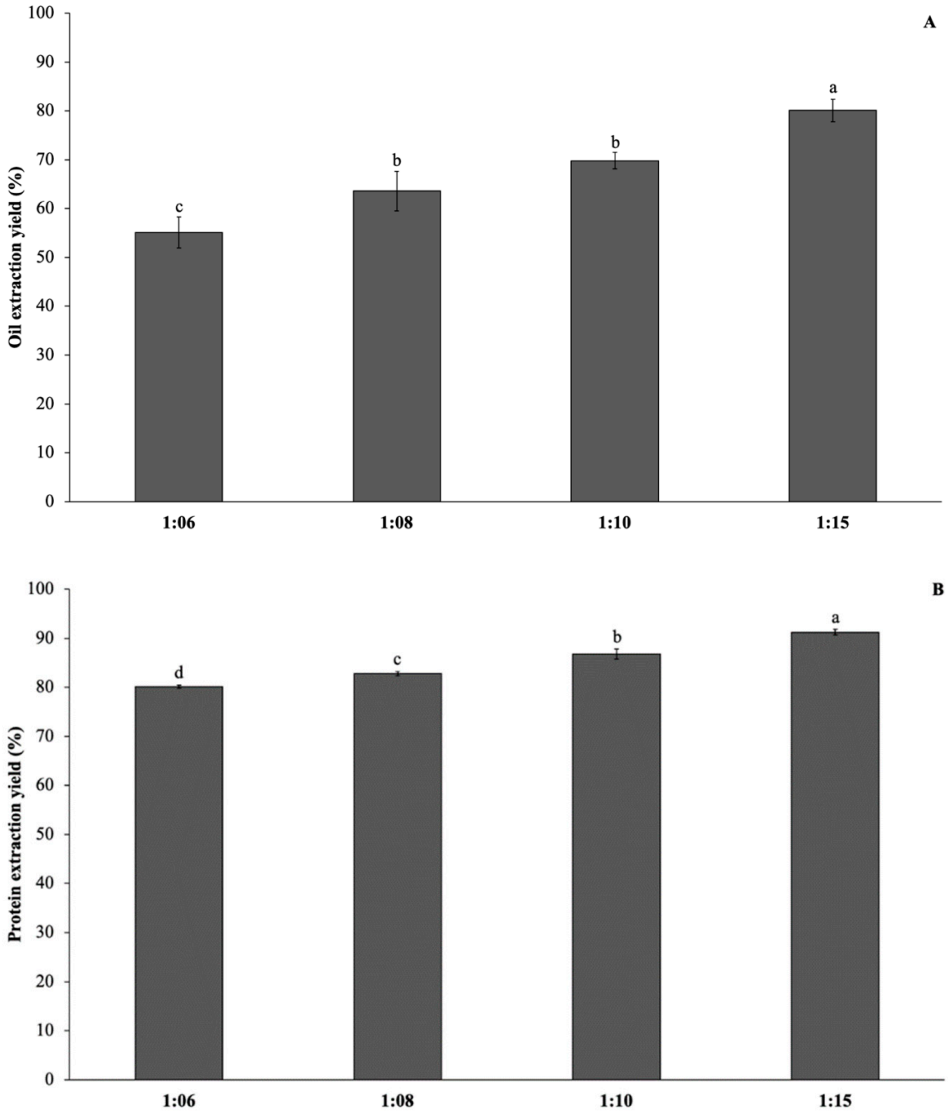

Figure 3. Total oil (**A**) and protein (**B**) extraction yields at different solids-to-liquid ratios (SLR). Different letters indicate statistically significant differences by one-way ANOVA followed by Tukey's test at $p < 0.05$. Extractions were performed at pH 9.0, 0.5% of protease (w/w), 50 °C, 60 min.

As depicted in Figure 3B, protein extraction yields significantly increased from 80.14 to 91.20% when the SLR decreased from 1:6 to 1:15. At a lower SLR (1:15), the higher amount of water increases the gradient between the matrix solute and the aqueous media solute [11], thus favoring the extractability of the solute. In addition, higher water usage also reduced the slurry viscosity, as demonstrated by viscometry analysis (Table 1). The slurry viscosity decreased by ~50% when the SLR decreased from 1:6 (10.18 cP) to 1:15 (5.15 Cp), in agreement with the higher oil and protein extraction yields achieved when decreasing SLR from 1:6 to 1:15 (Figure 3A,B). For soluble materials such as proteins, dissolution and diffusion are highly favored under a low SLR and reduced viscosity, leading to an increase in extraction yields [30].

Table 1. Effects of SLR on slurry viscosity at different RPMs. Different letters within the same RPM row indicate statistically significant differences by one-way ANOVA followed by Tukey's test at $p < 0.05$.

	Viscosity (cP)			
RPM/SLR	**1:6**	**1:8**	**1:10**	**1:15**
150	10.18 ± 0.08 [a]	7.69 ± 0.07 [b]	6.90 ± 0.17 [c]	5.16 ± 0.11 [d]
200	12.20 ± 0.03 [a]	9.05 ± 0.04 [b]	7.97 ± 0.02 [c]	6.12 ± 0.04 [d]
250	13.96 ± 0.04 [a]	10.20 ± 0.07 [b]	8.88 ± 0.01 [c]	6.80 ± 0.07 [d]

Our results are in agreement with previous results [19,21,22] which demonstrated that higher extraction yields were achieved at lower SLRs for soybeans, almonds, and almond cake, respectively.

Considering the enzyme kinetics and SLR study results, higher oil (80%) and protein (91%) extraction yields can be achieved by using 1:15 SLR, 0.5% protease, pH 9.0, 50 °C, and a reaction time of 60 min, being higher than the 68.5% oil extraction and 86.7% protein extraction achieved under optimum extraction conditions (1:10 SLR, 0.5% protease, pH 9.0, 50 °C, and a reaction time of 60 min) identified in the enzymatic/reaction time kinetics study. However, the higher extractability observed was achieved at the expense of a lower SLR (1:15 vs. 1:10), which unquestionably has an impact on processing economics. High water usage in the AEP/EAEP leads to higher volumes of effluent that need to be handled, centrifuged, and subsequently spray-dried to produce protein concentrates and isolates, increasing the use of unit operations that are energy-intensive [31,32]. To address the challenge of maintaining extractability while reducing water usage, a two-stage countercurrent EAEP was developed with the use of 1:8 SLR, which had an intermediate loss in extractability but could bring a significant reduction in water usage compared with a 1:15 SLR. Therefore, the extraction conditions for the development of the two-stage countercurrent EAEP were: 0.5% enzyme, 1:8 SLR, pH 9, and 50 °C for 15 (first extraction) and 60 min (second extraction).

3.3. Two-Stage Countercurrent AEP and EAEP of Chickpea Flour

To reduce the amount of water used in the single-stage EAEP without a loss in oil and protein extractability, a two-stage countercurrent extraction process was developed using 0.5% of enzyme, 1:8 SLR, pH 9.0, 50 °C and 15 (first extraction) and 60 min (second extraction). A control experiment was performed under the same conditions, except for the lack of enzyme use (AEP).

Figure 4A,B describe the effects of the two-stage countercurrent EAEP and AEP on oil and protein extraction yields, as well as on their distribution among the fractions generated by the extraction processes of chickpea flour. By contacting fresh incoming chickpea flour with the skim fraction arising from the second extraction from a previous extraction (where an enzyme addition happens), the two-stage countercurrent EAEP (0.5% enzyme) extracted 95.3% of the chickpea flour oil at a 1:8 SLR, compared to 61.7% for the two-stage countercurrent AEP (control, no enzyme) (Figure 4A), highlighting the effectiveness of using a protease to increase TOE yields. As previously discussed, the use of protease during the extraction can benefit oil extractability by either hydrolyzing the lipid body membrane (i.e., oleosin) or hydrolyzing the protein matrix, which, in turn, favors the release of the oil from the matrix [23,33]. The extracted oil in the two-stage countercurrent was distributed as 46.4% in the cream and 47.9% in the skim in the EAEP, in contrast to 20.6% in the cream and 46.7% in the skim for the AEP. Such change in the distribution of the extracted oil demonstrates that the additional oil extracted by the two-stage countercurrent EAEP, compared with the two-stage countercurrent AEP (control), was present in the cream fraction, which could therefore be recovered as free oil for subsequent use if adequate demulsification strategies are developed to breakdown the cream emulsion and release the oil. Since there are no viable methods available to recover the diluted oil present in the skim fraction, shifting more oil from the skim to the cream becomes necessary to maximize

the recovery of the extracted oil [20]. The TOE yields presented herein were measured for the third sample of each run when the enzyme had been adequately recycled throughout the process. The TOE was also measured for each of the three samples composing each extraction run. Average TOE of 88.1, 91.9, and 95.8% and 51.2, 60.2, and 73.1% were achieved for samples 1, 2, and 3 from the three EAEP and AEP runs, respectively (Figure 5A). The increased extractability from the extraction of sample 1 to sample 3 indicates the impact of the adequate recycling of the second skim, containing the enzyme and extracted solutes, into the subsequent extraction (first extraction of incoming flour), favoring overall extractability. The TOE in the two-stage countercurrent EAEP (1:8 SLR) of 95.3% was much higher than the optimum 80.1% TOE yield achieved by the single-stage EAEP at a 1:15 SLR, indicating the effectiveness of the two-stage countercurrent EAEP in reducing the amount of water used in the process by 47% while increasing the TOE by 18.9%.

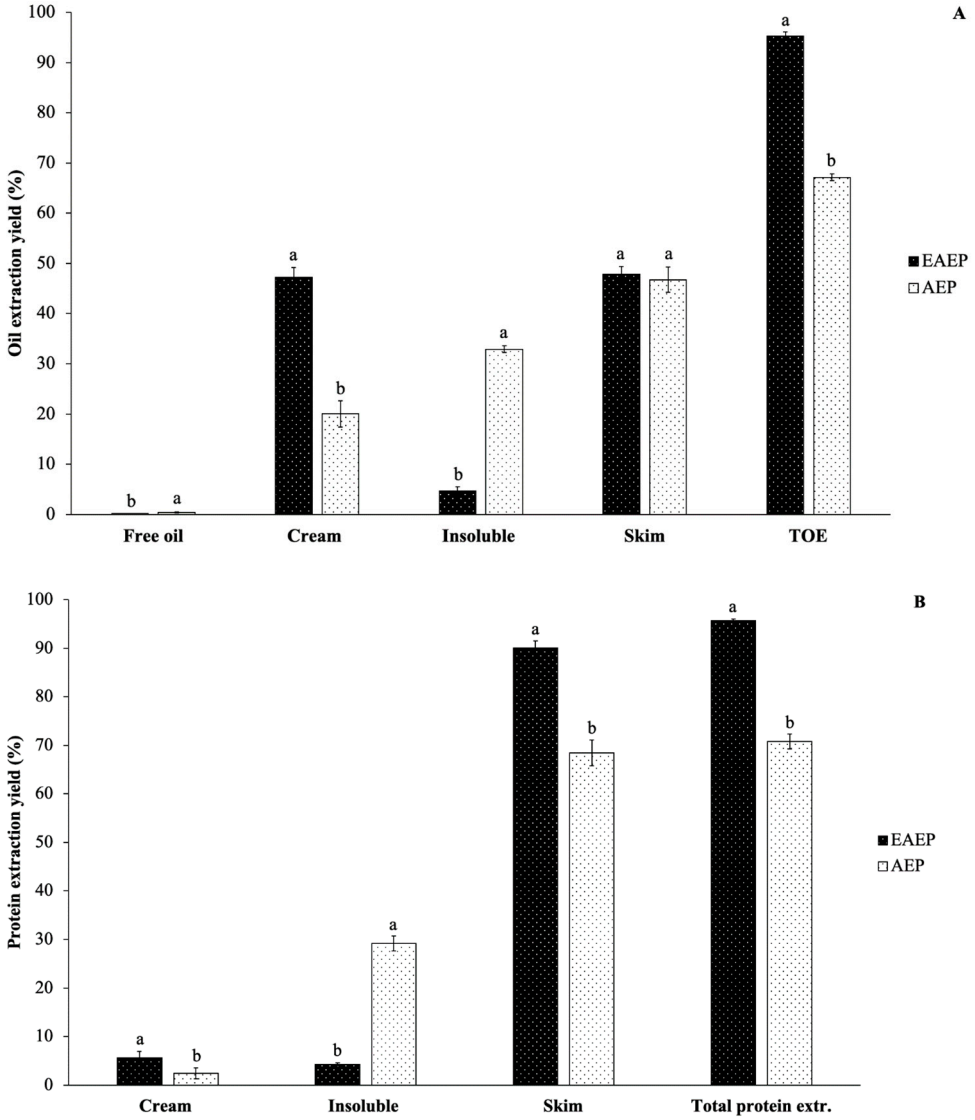

Figure 4. Oil (**A**) and protein (**B**) extraction yields and their distribution in the fractions. Different letters indicate statistically significant differences by one-way ANOVA followed by Tukey's test at $p < 0.05$.

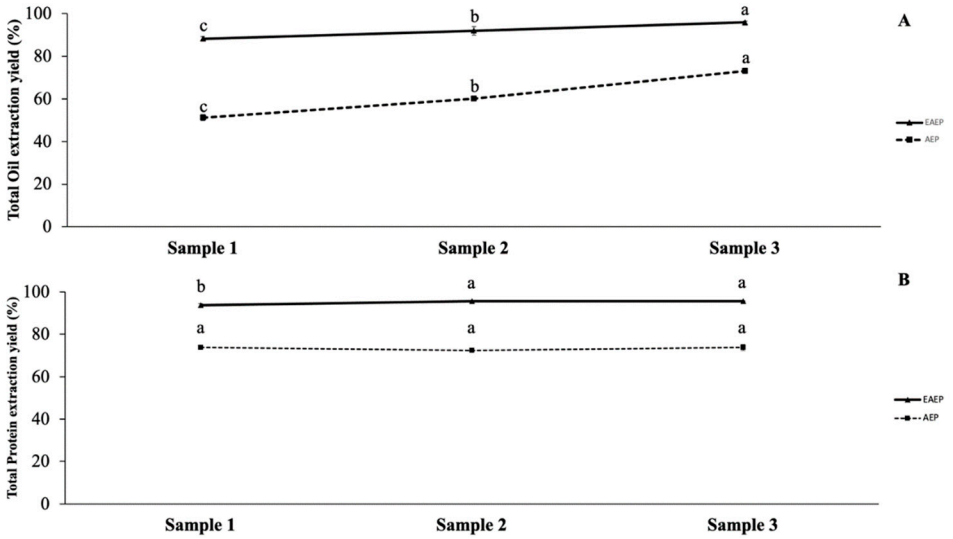

Figure 5. Total oil (**A**) and protein (**B**) extraction yields for the three samples composing each extraction run. Different letters indicate statistically significant differences by one-way ANOVA followed by Tukey's test at $p < 0.05$.

As observed for the TOE, the two-stage countercurrent EAEP achieved a significantly higher total protein extractability (TPE) than the two-stage countercurrent AEP (control) (Figure 4B). The two-stage countercurrent EAEP (0.5% enzyme) extracted 95.7% of the chickpea protein, compared to 68.4% for the two-stage countercurrent AEP (control, no enzyme) (Figure 4B). The increased TPE yields for the EAEP can be attributed to the ability of proteases to hydrolyze large protein bodies into smaller peptides, which become more soluble in the aqueous media, thus improving protein extractability [34,35]. The extracted protein in the two-stage countercurrent was distributed as 90.1% in the skim and 5.6% in the cream for the EAEP, in contrast to 68.4% in the skim and 2.4% in the cream for the AEP. The increased protein content in the skim can lead to higher protein purity when producing concentrates or isolates, which would be the main goal of the downstream processing of the skim. The two-stage countercurrent EAEP achieved TPE yields of 95.7% at a 1:8 SLR, being higher than the optimum 91.2% TPE yield achieved by the single-stage EAEP at 1:15 SLR. The two-stage countercurrent EAEP was effective in reducing the amount of water used in the process by 47% while increasing TPE yields by ~5% compared with the single-stage EAEP. The higher protein extractability and the lower usage of water of the two-stage countercurrent EAEP result in a skim fraction that has a significantly higher protein concentration (3.7%) than that of the single-stage EAEP (1.3% for EAEP) at optimum extraction conditions (0.5% enzyme, 60 min, 1:15 SLR). As previously mentioned, this can be beneficial for reducing the downstream processing costs of the skim for the industrial production of protein concentrates and isolates. The TPE yields presented herein were measured for the third sample of each run, when all fractions had been adequately recycled throughout the process. TPE yields were also measured for each sample composing each of the three runs. The TPE increased from 93.7%, 95.6%, and 95.7% from sample 1 to sample 3, reflecting the adequate recycling of the skim containing the enzyme used in the previous second extraction by the extraction of the third sample. The third sample was the first sample to be extracted by recycling the skim fraction that was generated from the second sample extraction, which had enzyme used in both the first and second extractions. That was evidenced by the higher protein extractability of sample 3. Although the TPE was not statistically different among samples 2 and 3, only samples from the third sample were collected and analyzed to calculate protein extraction yields. The TPE yield in the two-stage countercurrent EAEP (1:8 SLR) of 95.70% was much higher than the optimum 91.2% TPE yield achieved by the single-stage EAEP (1:15). Our results demonstrate the effectiveness

of countercurrent extraction conditions for improved yields of both oil and protein and reduced water usage.

The total oil and protein extraction yields presented herein are consistent with previous studies which found higher extraction yields with a concurrent reduction in the amount of water used in the process in the two-stage countercurrent EAEP of soybeans, mustard flour, and green coffee flour [21,36,37]

The two-stage EAEP achieved extraction yields of 95.3% for oil and 95.7% for protein, which are greater than the ones achieved by the single-stage EAEP using a 1:10 SLR, where oil and protein extraction yields of 77.2 and 83.5% were achieved (23.5 and 14.6% improvement for oil and protein extractability, respectively). The use of a two-stage countercurrent EAEP led to oil and protein extractability increments of 25.7 and 10.1% for green coffee [37] and 2.1 and 5.8% for soybeans [21], respectively, when compared with their respective single-stage EAEP. The aforementioned extractability increments reported in the literature were lower than the ones achieved in our study, which could be related to the use of different matrices in the studies described above and the different extraction conditions employed in the single-stage and two-stage countercurrent EAEPs among the studies (i.e., SLR, reaction time, enzyme use). With the application of the two-stage extraction process, water usage decreases, and the amount of water per extraction is significantly reduced. Additionally, because the two-stage countercurrent EAEP of chickpeas was able to improve extraction yields by such a wide margin over the single-stage EAEP, it makes the two-stage EAEP a potential alternative strategy to maximize protein and oil extractability.

3.4. Techno-Economic Analysis

A SuperPro process model was developed to compare the economics of the single-stage EAEP to those of the two-stage countercurrent EAEP for lipid and protein production from chickpea flour. It was assumed that the single-stage EAEP and the two-stage countercurrent EAEP were operated at the same processing capacity of 500 kg chickpea flour per hour continuously. Only the extraction and centrifugation steps were considered and modeled for both processes. The process flow diagram of the two processes were presented in Figure 6A,B. All materials added to the process were at room temperature when added to the blending tanks. The slurry was heated to 50 °C, and the materials were mixed for a specific residence time. Once the extraction was completed, the slurry was separated by centrifugation, and the liquid phase was cooled down to 25 °C. Optimum extraction yields (80.1% for oil and 91.2% for protein at a 1:15 SLR and 95.3% for oil and 95.7% for protein at a 1:8 SLR) were used to model the single-stage EAEP and two-stage countercurrent EAEP, respectively. It is worth mentioning that potential differences in extraction yields and the ease of separation of fractions through centrifugation, which might alter the distribution of the extracted compounds within the fractions generated by the process, should be acknowledged when considering process scale-up. While the experimental data used for the technical-economic analysis were based on lab-scale experiments using 500 g of flour per extraction, the economic analysis was conducted at an industrial scale, using 500 kg of flour per hour. However, based on the existing literature [21,31] for soybean processing, the oil and protein extraction yields and cream demulsification yields at the pilot scale (75 kg of soybeans) were similar to the ones obtained at the lab scale (2 kg of soybeans) for the integrated two-stage countercurrent extraction, where the enzyme added during the demulsification was further recycled into the two-extraction stages. While AEP and EAEP generate a protein-rich fraction (skim) that can be subjected to isoelectric precipitation and/or spray-dried or freeze-dried to produce protein concentrates or isolates, the extracted oil is entrapped in an oil-rich fraction (cream) that requires the development of a demulsification process (i.e., chemical or enzymatic) to break down the emulsion and free the oil for subsequent use as food or fuel [17]. The use of enzymatic demulsification was shown to be effective in breaking down the emulsion produced by the two-stage EAEP of soybeans and in reducing the overall enzyme use in the process by 35% through the recycling of the enzyme used in the cream demulsification into the extraction [31]. Further

research is needed to optimize key demulsification parameters (i.e., enzyme use, incubation time, reaction pH) to maximize the release of the cream oil for chickpeas. In the current model, we assumed that both single-stage and two-state countercurrent processes had the same operational and capital costs and excluded them from the current model.

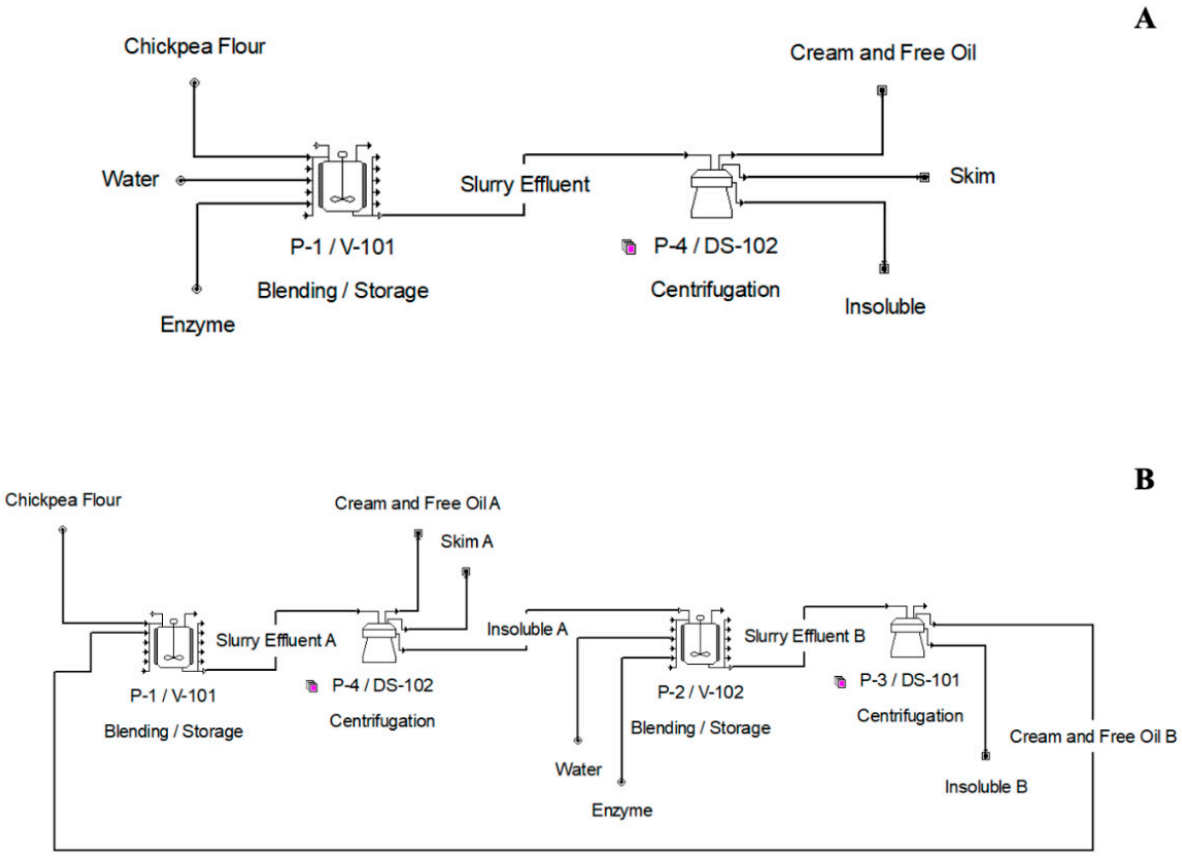

Figure 6. Modeling the techno-economic analysis of single-stage EAEP (**A**) and two-stage countercurrent EAEP (**B**) of chickpea full-fat flour.

Revenue and operational costs were evaluated based on the material and energy balance of the process model. Revenues come from the sale of proteins and lipids, assuming their full recovery (Table 2). Chickpea proteins have a wide range of applications in the food industry due to their renowned mild taste and high solubility and digestibility, which can justify the higher price point for chickpea proteins compared with other plant-based proteins [18]. A high-quality chickpea protein concentrate can be sold at USD 20 per kilogram [38]. Chickpea oil is a desirable cosmetic; however, because of its scarcity and reduced production scale, its current price is approximately USD 63 per kilogram [39]. The unit price for chickpea oil was set to be a quarter of the retail price (USD 15 per kg). The price points for chickpeas were USD 2.43 per kilogram of flour (NPI Soy FOB, Grinnell, IA, USA) and USD 23 per kilogram of the enzyme [40], which were the costs of the materials used in the lab-scale research. Additionally, the price for water was calculated, using local water rates (Davis, CA, USA), to be USD 0.0011795099 per liter of water [41]. Labor and utilities (power, steam, chilled water) costs were provided by the SuperPro Designer database.

Table 2. Price points, early production and consumption, yearly revenue and operating costs, and total net gross profit of single-stage EAEP and two-stage countercurrent EAEP of chickpea full-fat flour.

			Single-Stage EAEP		Two-Stage EAEP	
		Cost per Unit	**Units per Year**	**Cost per Year**	**Units per Year**	**Cost per Year**
Revenue	**Protein**	USD 20.00	902,021 kg	USD 18,040,420.00	966,849 kg	USD 19,336,980.00
	Oil	USD 15.00	70,091 kg	USD 1,051,365.00	144,433 kg	USD 2,166,495.00
Operating Costs	**Flour**	USD 2.43	3,960,000 kg	USD 9,622,800.00	3,960,000 kg	USD 9,622,800.00
	Enzyme	USD 23.00	19,800 kg	USD 455,400.00	19,800 kg	USD 455,400.00
	Water	USD 0.0011795	59,400,000 L	USD 70,062.89	31,680,000 L	USD 37,366.87
	Labor	USD 69.00	22,629 h	USD 1,561,401.00	45,257 h	USD 3,122,733.00
	Power	USD 0.10	1,877,163 kWh	USD 187,716.30	2,012,097 kWh	USD 201,209.70
	Steam	USD 12.00	2996 MT	USD 35,952.00	3934 MT	USD 47,208.00
	Chilled Water	USD 0.40	429,382 MT	USD 171,752.80	532,663 MT	USD 213,065.20
Net Gross Profit				**USD 6,986,700.01**		**USD 7,803,692.23**

When analyzing the revenue stream, the increased protein extractability achieved by the two-stage EAEP led to a slight 7.19% increase in revenue (Table 2). From analyzing the chickpea oil revenue, a 106.05% revenue increase was observed when moving from the single-stage EAEP to the two-stage countercurrent EAEP due to the significantly higher amount of oil extracted by the two-stage countercurrent EAEP. As discussed previously, the two-stage process improved oil extraction yields to a greater extent (from 80.1% to 95.3%) than it did for protein extraction yields (from 91.2% to 95.7%). When comparing the operating costs of the two processes, the cost of the flour and enzyme were the same, while the water cost was higher for the single-stage process. The use of the two-stage countercurrent EAEP enabled a 47% water cost reduction compared with the single-stage EAEP, reducing yearly water usage from 59,400 MT to 31,680 MT. Finally, labor and utility costs were different between the single-stage EAEP and two-stage countercurrent EAEP. We observed a large difference in labor costs, where more operational costs are present in the two-stage process due to the necessity of managing cyclical extractions and the additional reactor and centrifuge usage. The utilities for the two-stage process are also higher, but not to the same extent as the labor costs. Because the working volume of the two-stage extraction slurry is reduced compared with the single-stage process, the utility demand for the two-stage EAEP is only slightly higher because of the reduction in water usage. The increased revenue and decreased water usage of the two-stage countercurrent EAEP greatly outweighed its higher operating costs. The conservation of water is important in reducing usage demand and lessening the environmental impact of the extraction process and can also decrease the operational and energetic costs associated with the further processing separation of the fractions by centrifugation and water removal by spray-drying to produce protein powders. Reducing water usage is a key step in reducing the centrifugation costs for the aqueous materials and the protein recovery costs for processing the skim, which can satisfy commercial demands [21]. Overall, the annual net gross profit was greater for the two-stage process than that for the single-stage process.

Equipment costs were evaluated using the SuperPro database, as shown in Table 3. The two-stage process requires more equipment; hence, it has a higher capital cost investment compared to the single-stage process.

Table 3. Equipment costs of single-stage and two-stage countercurrent EAEP.

Single-Stage EAEP			Two-Stage Countercurrent EAEP		
Item	**Units**	**Total Cost**	**Item**	**Units**	**Total Cost**
Blending Tank	1	USD 271,000.00	Blending Tank	1	USD 206,000.00
Disk-stack centrifuge	4	USD 2,024,000.00	Blending Tank	1	USD 261,000.00
			Disk-stack centrifuge	3	USD 1,533,000.00
			Disk-stack centrifuge	2	USD 1,080,000.00
Net Equipment Costs		**USD 2,295,000.00**			**USD 3,080,000.00**

Overall, the two-stage countercurrent EAEP has a higher net gross profit than that of the single-stage EAEP, which can be attributed primarily to its higher extractability and the reduced amount of water used in the process. Using Equation (7), the payback time for the additional capital cost for implementing Scenario 2 vs. Scenario 1 was calculated to be 0.96 years. Its higher net gross profit outweighed the additional capital costs. The two-stage countercurrent EAEP is more advantageous compared with the single-stage EAEP from economic and environmental perspectives.

4. Conclusions

This study revealed the impact of critical extraction parameters (amount of enzyme, reaction time, and solids-to-liquid ratio) and modes of extraction (single-stage and countercurrent extraction) on the simultaneous extraction of lipids and proteins from full-fat chickpea flour and economic process feasibility. While extraction kinetics revealed that protein extractability was primarily favored by greater enzyme use, oil extractability, which benefits from the removal of the protein from the matrix, required greater enzyme use and longer reaction times. About 68.5% oil and 86.7% protein were extracted by the use of 0.5% protease, pH 9.0, 50 °C, a reaction time of 60 min, and a 1:10 SLR. A further evaluation of the impact of SLR demonstrated that a lower SLR significantly reduced the slurry viscosity, thus increasing the oil and protein extractability to 80 and 91%, respectively (1:15 SLR, 0.5% protease, pH 9.0, 50 °C, and a reaction time of 60 min). The development of a two-stage countercurrent extraction process was successful in reducing the amount of water used in the single-stage extraction while further improving the oil and protein extraction yields to 95.8 and 95.7%, respectively. The results of the techno-economic analysis of the single-stage and two-stage countercurrent EAEP showed that the two-stage countercurrent EAEP had a higher yearly net revenue but also a higher equipment investment cost. The results presented herein further widen the scope of processing standards for full-fat chickpea flour and add to the elucidation of the impact of key processing conditions on the extractability and economic feasibility of the production of chickpea ingredients for subsequent food/nutraceutical applications.

Author Contributions: K.M. and F.F.G.D.: Methodology, Data curation, Formal analysis, Writing—review & editing. Z.F.: Supervision, Writing—review & editing. J.M.L.N.D.M.B.: Conceptualization, Data curation, Supervision, Funding, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Pulse Crop Health Initiative (PCHI 2019), and the USDA National Institute of Food and Agriculture Hatch/Multi-State project (1023517).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. USDA ERS—Pulses Production Expanding as Consumers Cultivate a Taste for U.S. Lentils and Chickpeas. Available online: https://www.ers.usda.gov/amber-waves/2017/januaryfebruary/pulses-production-expanding-as-consumers-cultivate-a-taste-for-us-lentils-and-chickpeas/ (accessed on 16 June 2021).
2. Joshi, S. Plant Protein Market Growth Sturdy at 6.2% CAGR to Outstrip. Available online: https://www.globenewswire.com/news-release/2021/05/06/2224574/0/en/Plant-Protein-Market-Growth-Sturdy-at-6-2-CAGR-to-Outstrip-27-965-9-Million-by-2027-COVID-19-Impact-and-Global-Analysis-by-TheInsightPartners-com.html (accessed on 18 June 2021).
3. Wild, F.; Czerny, M.; Janssen, A.M.; Kole, A.P.W.; Zunabovic, M.; Domig, K.J. The Evolution of a Plant-Based Alternative to Meat. *Agro Food Ind. Hi Tech* **2014**, *25*, 45–49.
4. Naghshi, S.; Sadeghi, O.; Willett, W.C.; Esmaillzadeh, A. Dietary Intake of Total, Animal, and Plant Proteins and Risk of All Cause, Cardiovascular, and Cancer Mortality: Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies. *BMJ* **2020**, *370*, m2412. [CrossRef] [PubMed]
5. Mekonnen, M.M.; Hoekstra, A.Y. *Value of Water Research Report Series No. 48 Value of Water*; UNESCO-IHE: Delft, The Netherlands, 2010.
6. Sheldon, S.; Giles, D.-E.; Lazimy, U.; Capuano, J.D.; Freedgood, A. *Sustainability KPI Calculation Methodology*; Eat Just, Inc.: San Francisco, CA, USA, 2017.
7. USDA Commodity Highlights. Available online: https://www.ers.usda.gov/topics/crops/vegetables-pulses/commodity-highlights/ (accessed on 9 September 2021).
8. U.S. Department of Agriculture, A.R.Service. FoodData Central. Available online: https://fdc.nal.usda.gov/fdc-app.html#/food-details/173757/nutrients (accessed on 29 May 2020).
9. Ghribi, A.M.; Maklouf Gafsi, I.; Sila, A.; Blecker, C.; Danthine, S.; Attia, H.; Bougatef, A.; Besbes, S. Effects of Enzymatic Hydrolysis on Conformational and Functional Properties of Chickpea Protein Isolate. *Food Chem.* **2015**, *187*, 322–330. [CrossRef] [PubMed]
10. Duranti, M.; Gius, C. Legume Seeds: Protein Content and Nutritional Value. *Field Crops Res.* **1997**, *53*, 31–45. [CrossRef]
11. Campbell, K.A.; Glatz, C.E. Mechanisms of Aqueous Extraction of Soybean Oil. *J. Agric. Food Chem.* **2009**, *57*, 10904–10912. [CrossRef]
12. De Queirós, L.D.; Dias, F.F.G.; de Ávila, A.R.A.; Macedo, J.A.; Macedo, G.A.; Leite Nobrega de Moura Bell, J.M. Effects of Enzyme-Assisted Extraction on the Profile and Bioaccessibility of Isoflavones from Soybean Flour. *Food Res. Int.* **2021**, *147*, 110474. [CrossRef]
13. De Moura, J.M.L.N.; Campbell, K.; De Almeida, N.M.; Glatz, C.E.; Johnson, L.A. Protein Recovery in Aqueous Extraction Processing of Soybeans Using Isoelectric Precipitation and Nanofiltration. *JAOCS J. Am. Oil Chem. Soc.* **2011**, *88*, 1447–1454. [CrossRef]
14. Cheng, M.H.; Sekhon, J.J.K.; Rosentrater, K.A.; Wang, T.; Jung, S.; Johnson, L.A. Environmental Impact Assessment of Soybean Oil Production: Extruding-Expelling Process, Hexane Extraction and Aqueous Extraction. *Food Bioprod. Process.* **2018**, *108*, 58–68. [CrossRef]
15. Dias, F.F.G.; de Almeida, N.M.; de Souza, T.S.P.; Taha, A.Y.; de Moura Bell, J.M.L.N. Characterization and Demulsification of the Oil-Rich Emulsion from the Aqueous Extraction Process of Almond Flour. *Processes* **2020**, *8*, 1288. [CrossRef]
16. Nadar, S.S.; Pawar, R.G.; Rathod, V.K. Recent Advances in Enzyme Extraction Strategies: A Comprehensive Review. *Int. J. Biol. Macromol.* **2017**, *101*, 931–957. [CrossRef]
17. Wu, J.; Johnson, L.A.; Jung, S. Demulsification of Oil-Rich Emulsion from Enzyme-Assisted Aqueous Extraction of Extruded Soybean Flakes. *Bioresour. Technol.* **2009**, *100*, 527–533. [CrossRef] [PubMed]
18. Machida, K.; Huang, Y.P.; Furlan Gonçalves Dias, F.; Barile, D.; Leite Nobrega de Moura Bell, J.M. Leveraging Bioprocessing Strategies to Achieve the Simultaneous Extraction of Full-Fat Chickpea Flour Macronutrients and Enhance Protein and Carbohydrate Functionality. *Food Bioproc. Tech.* **2022**, *15*, 1760–1777. [CrossRef]
19. Souza, T.S.P.; Dias, F.F.G.; Koblitz, M.G.B.; de M. Bell, J. Aqueous and Enzymatic Extraction of Oil and Protein from Almond Cake: A Comparative Study. *Processes* **2019**, *7*, 472. [CrossRef]
20. de Almeida, N.M.; Dias, F.F.G.G.; Rodrigues, M.I.; Bell, J.M.L.N.D.M. Effects of Processing Conditions on the Simultaneous Extraction and Distribution of Oil and Protein from Almond Flour. *Processes* **2019**, *7*, 844. [CrossRef]
21. de Moura, J.; Johnson, L.A. Two-Stage Countercurrent Enzyme-Assisted Aqueous Extraction Processing of Oil and Protein from Soybeans. *JAOCS J. Am. Oil Chem. Soc.* **2009**, *86*, 283–289. [CrossRef]
22. Dias, F.F.G.; Taha, A.T.; de Moura Bell, J.M.L. Effects of enzymatic extraction on the simultaneous extraction of oil and protein from full-fat almond flour, insoluble microstructure, emulsion stability, and functionality. *Future Foods* **2022**, *5*, 100151. [CrossRef]
23. Campbell, K.A.; Glatz, C.E.; Johnson, L.A.; Jung, S.; De Moura, J.M.N.; Kapchie, V.; Murphy, P. Advances in Aqueous Extraction Processing of Soybeans. *JAOCS J. Am. Oil Chem. Soc.* **2011**, *88*, 449–465. [CrossRef]
24. De Moura, J.; Campbell, K.; Mahfuz, A.; Jung, S.; Glatz, C.E.; Johnson, L. Enzyme-Assisted Aqueous Extraction of Oil and Protein from Soybeans and Cream de-Emulsification. *JAOCS J. Am. Oil Chem. Soc.* **2008**, *85*, 985–995. [CrossRef]
25. Campbell, K.A.; Vaca-Medina, G.; Glatz, C.E.; Pontalier, P.Y. Parameters Affecting Enzyme-Assisted Aqueous Extraction of Extruded Sunflower Meal. *Food Chem.* **2016**, *208*, 245–251. [CrossRef]
26. Liao, X.Q.E.; Zhao, K.; Mac, J.; Xiangzhao, R. Multi-Stage Countercurrent Process for Extracting Protein from Antarctic Krill (Euphausia Superba). *J. Food Sci. Technol.* **2018**, *55*, 4450–4457. [CrossRef]

27. Dias, F.F.G.; de Moura Bell, J.M.L. Understanding the impact of enzyme-assisted aqueous extraction on the structural, physico chemical, and functional properties of protein extracts from full-fat almond flour. *Food Hydrocoll.* **2022**, *127*, 107534. [CrossRef]
28. *AOAC Official Methods Analysis*, 15th ed.; AOAC: Rockville, MD, USA, 1990. [CrossRef]
29. Cheng, M.; Rosentrater, K.A.; Wang, T. *Techno-Economic Analysis of Integrated Enzyme Assisted Aqueous Extraction of Soybean Oil Techno-Economic Analysis of Integrated Enzyme Assisted Aqueous*; ASABE: Orlando, FL, USA, 2016. [CrossRef]
30. Rosenthal, A.; Pyle, D.L.; Niranjan, K. Simultaneous Aqueous Extraction of Oil and Protein from Soybean: Mechanisms for Process Design. *Food Bioprod. Process. Trans. Inst. Chem. Eng. Part. C* **1998**, *76*, 224–230. [CrossRef]
31. De Moura, J.; Maurer, D.; Jung, S.; Johnson, L.A. Pilot-Plant Proof-of-Concept for Integrated, Countercurrent, Two-Stage, Enzyme-Assisted Aqueous Extraction of Soybeans. *JAOCS J. Am. Oil Chem. Soc.* **2011**, *88*, 1649–1658. [CrossRef]
32. Cater, C.M.; Rhee, K.C.; Hagenmaier, R.D.; Mattil, K.F. Aqueous Extraction-An Alternative Oilseed Milling Process. *J. Am. Oil Chem. Soc.* **1974**, *51*, 137–141. [CrossRef]
33. Rosenthal, A.; Pyle, D.L.; Niranjan, K. Aqueous and Enzymatic Processes for Edible Oil Extraction. *Enzyme Microb. Technol.* **1996**, *19*, 402–420. [CrossRef]
34. De Almeida, N.M.; De Moura Bell, J.M.L.N.; Johnson, L.A. Properties of Soy Protein Produced by Countercurrent, Two-Stage, Enzyme-Assisted Aqueous Extraction. *JAOCS J. Am. Oil Chem. Soc.* **2014**, *91*, 1077–1085. [CrossRef]
35. de Souza, T.S.P.; Dias, F.F.G.; Oliveira, J.P.S.; de Moura Bell, J.M.L.N.; Koblitz, M.G.B. Biological Properties of Almond Proteins Produced by Aqueous and Enzyme-Assisted Aqueous Extraction Processes from Almond Cake. *Sci. Rep.* **2020**, *10*, 10873. [CrossRef]
36. Tabtabaei, S.; Diosady, L.L. Aqueous and Enzymatic Extraction Processes for the Production of Food-Grade Proteins and Industrial Oil from Dehulled Yellow Mustard Fl Our. *FRIN* **2013**, *52*, 547–556. [CrossRef]
37. Souza Almeida, F.; Furlan Goncalves Dias, F.; Kawazoe Sato, A.C.; Leite Nobrega de Moura Bell, J.M. From Solvent Extraction to the Concurrent Extraction of Lipids and Proteins from Green Coffee: An Eco-Friendly Approach to Improve Process Feasibility. *Food Bioprod. Process.* **2021**, *129*, 144–156. [CrossRef]
38. Chickpea Protein Isolate Cost. Available online: https://www.alibaba.com/product-detail/High-Quality-Chickpea-Protein-Isolate_62290145129.html?spm=a2700.galleryofferlist.normal_offer.d_title.995b47369dwOfu (accessed on 9 September 2021).
39. Chickpea Oil 30 mL. Available online: https://www.hemanitrading.us/chickpea-oil-30ml/?gclid=Cj0KCQjwse-DBhC7ARIsAI8YcWLRrWcVOEFelb3dMX4rWU77suUIX-sboEZlW_N_Pxr4SLGuwo9OJ-QaAscdEALw_wcB (accessed on 9 September 2021).
40. PROTEX 6L Protease 25 kg. Available online: https://www.fishersci.com/shop/products/NC0603342/NC0603342 (accessed on 9 September 2021).
41. Water Rates. Available online: https://www.cityofdavis.org/city-hall/public-works-utilities-and-operations/water/water-rates (accessed on 9 September 2019).