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Leaf Protein Concentrate of Rye (*Secale cereale* L.): A Green Innovation for Future Food and Feed

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Abstract. The concept of a green biorefinery offers promising pathways for the sustainable production of alternative protein sources for food and feed, utilizing green biomass derived from protein-rich plants and grasses. A central challenge in this approach is the reliable provision of high-quality protein. To address global protein shortages, various plant sources such as alfalfa, broccoli, green pea, and triticale have been investigated. In the present study, we evaluated the potential of rye (*Secale cereale* L.) as a source for leaf protein concentrate (LPC) production, employing two isolation methods, i.e., microwave coagulation (MW) and lactic acid fermentation (LA) of green juice (GJ) after wet-fractionation. The green juice powder (P-GJ) was used as a control. The results revealed that different amounts and compositions of LPC are generated by the methods applied. The P-GJ resulted in lower protein content (14.06%) and higher levels of most antioxidant capacity, i.e., total phenolic contents (TPC) 182.51 mg GAE/g and water-soluble antioxidant capacity (ACW) 8.038 µg/mg ascorbic acid. The LA method resulted in a lower yield and protein content in comparison to the MW method. On the other hand, the MW method increased the protein content by 35.55% compared to LA-LPC and 106.47% compared to P-GJ. LA and MW LPC showed no significant differences ($p<0.05$) in antioxidant capacity, which is 144.98-161.05 mg GAE/g for TPC and 7.83-9.28 mg GAE/g for TFC, while ACL and ACW were 3.50-6.69 µg/mg ascorbic acid and 2.08-2.09 µg/mg ascorbic acid, respectively. The yield of dried LPCs were between 3-4% of the GJ, with a protein content of 21-29%. It can be concluded that LPC of rye has potential as a raw material for food and feed. However, the harvest time and plant development stage as well as pressing techniques need to be optimized in order exploit the methods.

1. Introduction

The Food and Agriculture Organization (FAO) has provided projections indicating a consistent increase in global protein consumption. From 2022 to 2031, the anticipated total protein consumption worldwide is estimated to reach 409,437 tons, reflecting a modest annual growth rate of 1.15%. Regional variations are notable, with North America projected to grow at 0.81%, Latin America at 1.36%, Europe at 0.07%, Africa at 1.98%, Oceania at 1.54%, and Asia at 1.5%. Notably, Indonesia is expected to experience a significant increase in protein consumption, projected at 2.19% [1]. This suggests that regions or countries with the highest projected growth rates in protein consumption tend to have lower consumption levels currently [2,3].

Meeting the protein needs of a growing population is typically achieved through diverse sources, including livestock (both ruminants and poultry), fish, edible insects, vegetables, single-cell proteins [4], and various green [5–8]. While meat remains the primary protein source, livestock production systems' sustainability is increasingly called into question due to their dependence on extensive land use, high energy demands, and significant feed requirements. Consequently, exploring alternative and high-quality protein sources for human and animal food has become imperative. Green plant biomass, specifically young green plants harvested before flowering, presents a viable potential source of protein, offering benefits in both quantity and quality [6,9,10].

Utilizing proteins from green biomass, particularly those high in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), presents a promising avenue for sustainable nutrition. RuBisCo constitutes approximately 50% of the proteins found in green biomass and plays a critical role in the carbon fixation process during photosynthesis [11,12]. These proteins are nutritionally beneficial and possess favorable functional properties, thereby establishing their viability as food ingredients [8,13]. Importantly, no reported negative impacts on human or livestock health have been associated with consuming plant-derived proteins from green biomass. Using such biomass as a protein source offers significant environmental advantages, including a 17% reduction in feed quantity when included in diets [14], along with a rapid harvesting timeline and ease of handling due to its abundant availability.

Extensive research has been conducted on legumes and cereal crops in nations such as the Netherlands, Hungary, the United States, and Sweden, leading to notable findings [6]. The yield and quality of protein extracted from these biomass sources are influenced by the isolation technologies employed and the type of green biomass utilized [15–18].

The present investigation focuses on autumn-sown rye (*Secale cereale* L.) green biomass, which has been recognized for its utility as a winter cover crop in Debrecen, Hungary, attributed to its adaptability to lower ambient temperatures. Rye exhibits excellent early growth characteristics, indicating its potential as a nutritious green biomass option that minimizes planting time. Rye is often grown as a substitute for wheat in areas where the soil is not ideal for wheat, or where rye's higher nutritional value and higher resistance are beneficial. Rye is more tolerant of acidic, less fertile soils and colder climates than wheat. Furthermore, rye contributes positively to digestive health due to its elevated fiber content and is a rich source of essential nutrients, including B vitamins, calcium, niacin, and various minerals [19,20]. This investigation aims to evaluate the biomass yield and nutritional profile of LPC rye utilizing various protein isolation techniques. The data derived from these results are anticipated to provide valuable insights into the nutritional viability of LPC rye for food and feed applications, thereby contributing to the broader understanding of its utility in agricultural practices.

2. Materials and Methods

2.1 Experimental Design

Rye was cultivated in an open field at the Demonstration Garden of the Debrecen University, Hungary (47^{0}32'0" N; 21^{0}38'0" E) in the fall of 2022. Plants were planted in three replicate plots in 5 m^2 (2 x 2.5 cm^2) per plot. Seed of rye was sown at a rate of 20 g/m^2. The experimental plots received no fertilizers or pesticides before or during the growing period. Weeding was carried out manually. Plants were harvested before flowering (BBCH, GS39-41) to ensure optimal leaf protein content, and plant biomass was cut 5 cm from the ground.

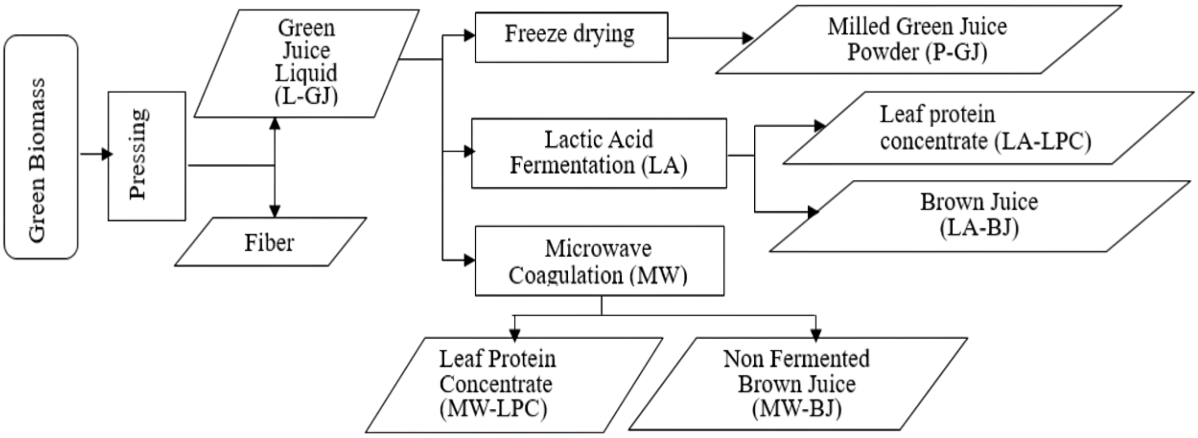


Figure 1. Chart flow of protein isolation technique of rye green biomass.

2.2 Green Biomass Conversion Process to Leave Protein Concentrate (LPC)

The transformation of green plant biomass into a protein concentrate typically occurs in several phases, which include selecting plant species, harvesting, and pressing [21]. Figure 1 illustrates the green biomass processing steps. The collected green biomass is categorized into roots, stems, and yellow-brown leaves. Before pressing (fractionation), the green biomass is rinsed.

The fractionation procedure begins with the extraction of green biomass using a juicer (Angle Twin Screw 5500, Angle Ltd., Anyang, South Korea) to produce liquid green juice (L-GJ) and separate it from the pulp (fiber solid fraction). The L-GJ is immediately placed in a freezer at -20 °C to prevent nutritional degradation before further processing.

Three distinct methods are employed to isolate proteins in L-GJ. First, L-GJ undergoes direct freeze-drying using a lyophilizer (Alpha 1-4 LSC plus – Martin Christ, Germany), after which it is ground into a powder (P-GJ) with a stainless-steel grinder. The second method utilizes microwave-assisted (MW) techniques, as outlined by Domokos-Szabolcsy et al. [5], to precipitate proteins in L-GJ through thermal methods. In this approach, L-GJ is heated to a temperature of 80-85 °C using 800 watts of power (Samsung M1711N, South Korea). The non-thermal method utilized is lactic acid fermentation (LA). In this process, green juice liquid is mixed with 1 M lactic acid at a 5% (v/v) concentration and incubated anaerobically in a controlled environment (Memmert, GmbH, Germany) for 48 hours at 36 °C. Successful fermentation is indicated by a pH dropping below 4. After cooling the coagulation to room temperature or after fermentation, vacuum filtration is conducted using a membrane filter with a 5 µm pore size to isolate the solid fraction (MW or LA wet LPC), which is then freeze-dried, while the liquid fraction (MW-BJ or LA-BJ) is stored at -20 °C for further analysis.

2.3 Analysis of Nutritional Value and Physical Characteristics

2.3.1 Protein content

Protein content analysis used the Dumas method (Rapid MAX N exceed, Elementer Analysensystem GmbH, Hesse, Germany) with a conversion factor of 6.25 [5,22].

2.3.2 Antioxidant capacity

The total phenolic content (TPC) and total flavonoid content (TFC) were described by Barna et al. [23] and Kaszás et al. [24]. For the TPC assessment, a calibration curve was constructed utilizing gallic acid at a concentration of 0.03 M, whereas rutin was employed as the standard for the TFC measurements. The TPC and TFC measurement solutions were prepared using Folin-Ciocalteu reagent, which was diluted with distilled water in a 1:10 (v/v) ratio and methanol in a 70:30 (v/v) ratio. Sample preparation involved the combination of 10 mg of finely dried plant material with 1 mL of 70% methanol. This mixture was vortexed for 2 minutes and subsequently subjected to ultrasonic treatment for 30 minutes at ambient temperature. Following incubation, the mixture was centrifuged at 10,000 rpm for 5 minutes to separate the supernatant. Subsequently, 50 µL of the supernatant was added to a tube containing 1.25 mL of Folin reagent and 200 µL of 70% methanol. After allowing the reaction to proceed for one minute, 1 mL of sodium carbonate (Na_2CO_3) was incorporated, and the resultant solution was incubated in a water bath at 50°C for 5 minutes. The blank control solution consisted of a mixture of 1.25 mL of Folin reagent, 250 µL of 70% methanol, and 1 mL of sodium carbonate. The absorbance of the prepared solutions was measured using a UV/VIS spectrophotometer (Ultrospec 2100 pro, Amersham BioSciences) at wavelengths of 760 nm for TPC and 415 nm for TFC.

The ACL and ACW photochemiluminescence (PCL) methods were employed to assess antioxidant capacity as described by Barna et al. [23] and are marketed under the name Photochem® by Analytik Jena AG (Jena, Germany). To align with the calibration range, samples were diluted with either distilled water (ACW) or methanol (ACL). This assay involves the photochemical generation of the radical superoxide anion (O2•−), coupled with sensitive detection through chemiluminescence. The process begins with the optical excitation of the photosensitizer (S), which produces O2•−. Two distinct protocols, ACW and ACL, are utilized to measure hydrophilic and lipophilic antioxidants separately. Results are reported in µg/mL ascorbic acid equivalents (AAE) for ACW and Trolox equivalents (TE) for ACL. The outcomes are expressed as µg/mL of the LPC sample.

2.3.3 Color, pH, Brix, and electrical conductivity (EC)

The color value was expressed in the CIELAB color space (L*, a*, and b*) and measured with a Croma meter (CR-410, Konica Minolta Sensing, Inc., Japan). A refractometer was used to test the soluble solids in liquids and determine the soluble sugar content (Brix, °Bx). The pH was measured using a pH meter (Mettler Toledo S20 Seven Easy, Switzerland), and the EC was determined using an EC meter (Thermo Scientific Orion Model 209A+, Germany).

2.4 Statistical Analysis

Independent replications of each experiment were performed a minimum of three times. Statistical analyses were conducted using IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL, USA), with data generated in Microsoft Excel. Graphing analysis was executed using OriginPro® 2024 software. Results were expressed as the mean ± standard deviation (SD), and ANOVA was performed with a significance level set at α = 0.05.

3. Results and Discussions

3.1 Fresh biomass and fractionation process products

The investigation into green biomass harvesting resulted in an average yield of 19.85 kg per plot, equating to 3.83 kg/m^2 of fresh biomass. Comparative analysis indicates that rye exhibits superior biomass production capabilities relative to wheat (*Triticum* spp.). Specifically, rye can generate forage biomass within the range of 2.0 to 4.6 Mg/ha, while wheat typically produces between 1.6 to 3.7 Mg/ha [25–27]. This differential in biomass yield underscores the potential of selecting rye for biomass harvesting in agricultural practices aimed at optimizing the production of green biomass resources.

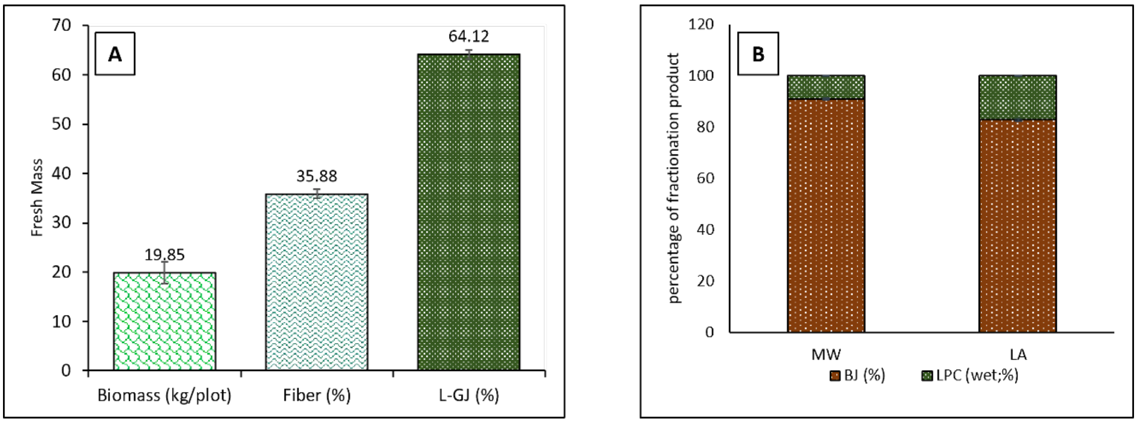


Figure 2. (A): green biomass and resulting from wet pressing; (B): Percentage of leaf protein concentrate and brown juice fractions resulting from microwave coagulation (MW) and lactic acid fermentation (LA) of rye. Data are presented as mean ± SD (n = 3).

After pressing, the biomass composition was obtained to be 64.12% green juice (L-GJ) and 35.88% fiber (Figure 2). The findings indicate that the L-GJ yield of rye is comparatively lower than those of triticale biomass, which obtained L-GJ in the range of 71-73%, alongside fiber of 18-19%. Comparative analysis revealed that the wet LPC derived using the MW technique (9.05%) exhibited a lower value when contrasted with the LA technique (17.13%). These results highlight the varying potential of different biomass sources in terms of biofuel production efficiency. Conversely, BJ from the MW technique (90.59%) was significantly higher than the LA technique (82.87%). These findings underscore the influence of fractionation methods on the yield of fresh LPC and the production of BJ as a by-product. We also observed that the vacuum filtration process associated with LA-LPC retained more BJ than the drier MW-LPC. While the wet weight of LA-LPC was higher, the drying process resulted in a lower overall weight when compared to MW-LPC (Figure 3).

3.2 Yield and Protein Content of LPC

Protein isolation techniques from rye green biomass are carried out by two methods, i.e., the thermal method by microwave (MW) and the cold method by lactic acid fermentation (LA). Dry-mass of MW-LPC and LA-LPC were lower than that of green juice (P-GJ) as a control, results that contrasted with crude protein content (Figure 3).

MW coagulation is a relatively novel technique that uses microwave radiation to promote protein coagulation. Results show that MW technique applied to green biomass can result in crude protein content 29.03% within LPC, where LA 21.42% and P-GJ 14.06%. In particular, Domokos-Szabolcsy et al. [5] found that LPC of broccoli leaves derived from microwave coagulation yielded is of 34.30% crude protein, which is significantly higher than the 27.43% found within the unprocessed green juice fraction. Furthermore, Kaszás et al. [28] found the crude protein content of the Jerusalem artichoke LPC with MW technique varied from 24.2 to 31.4% depending on clones and harvesting time, and alfalfa LPC was 32.3%. The microwave process facilitates rapid heating and minimizes nutrient degradation during coagulation, thereby preserving amino acid composition.

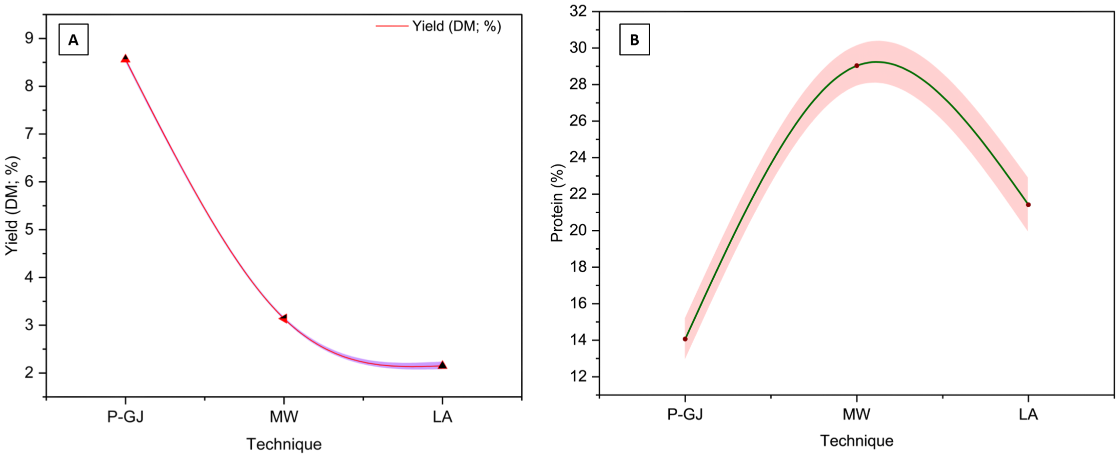


Figure 3. (A) Dry matter content and (B) protein content of LPCs from different isolation techniques of rye biomass. Data are presented as mean ± SD (n = 3).

On the other hand, LA, as an organic acid, can significantly influence the protein content LPC from green biomass, particularly through its ability to modify protein structures and solubility. Lactic acid assists in the denaturation and precipitation of proteins. This method can lead to alterations in the protein content and digestibility of the biomass. Research by Kondo et al. [29] indicated that lactic acid fermentation could enhance protein degradation, leading to improved protein digestibility as evidenced by an increase in soluble protein content during the fermentation of silage stored over time. The LA technique on broccoli leaves also increased 26.23% the protein content compared to MW [5], but in contrast to the results of this study, where the protein of LA-LPC rye was lower than MW.

Applying the lactic acid (LA) method reduces pH levels, enabling the precipitation of soluble proteins in liquid green juices (L-GJ). Comparative analysis of broccoli leaves and rye protein content indicates contrasting outcomes, attributed to various factors. These include the intrinsic properties of the leaves, particularly the pH values observed pre- and post-fermentation. Rye demonstrates a more significant decrease in pH, dropping from approximately 5.80 to 3.80, whereas broccoli leaves show a reduction from around 6.27 to 4.57. Additionally, the fermentation duration, temperature, and the inherent texture differences, where fermented broccoli leaves have a softer consistency than rye, play crucial roles. These factors contribute to a more favorable protein yield and coagulation in broccoli leaves compared to rye.

3.3 Antioxidant Potential of LPC

Two phytochemical composition analyses (TPC and TFC) and two different antioxidant assays (ACW and ACL) were used to get a comprehensive view of the antioxidant potential of LPC. Fig. 4 shows the results of different phytochemical composition analysis and antioxidant assays in the LPC of the rye, using three different protein isolation techniques. The ACL value of P-GJ exhibited an increase of 23.31% compared to MW-LPC, yet it presented a significant decrease of 31.85% compared to LA-LPC. This finding contrasts with the ACW data, indicating that P-GJ possesses a 75.01% higher ACW value than the MW and LA techniques. Furthermore, the observations regarding TPC values align with those of ACL. Specifically, LA-LPC demonstrates an increased TPC value relative to MW-LPC while remaining lower than P-GJ. In terms of TFC, applying the MW technique results in LPC with a TFC content that surpasses that of both P-GJ and LA-LPC.

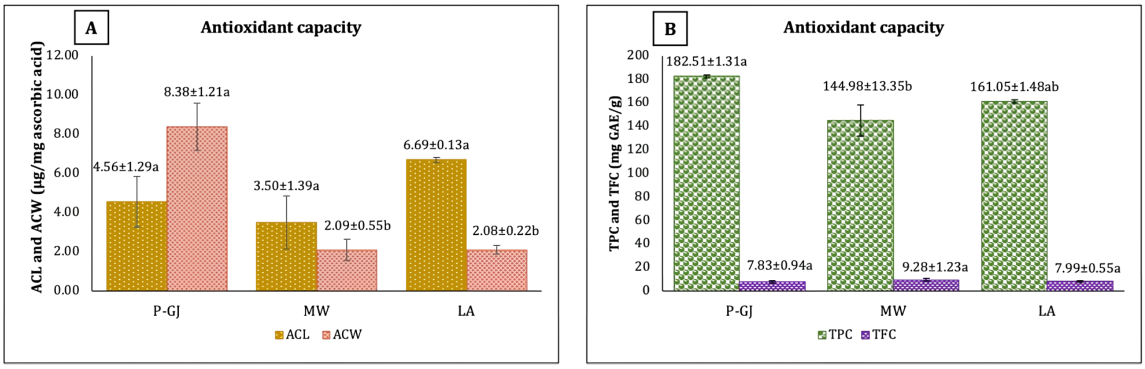


Figure 4. Antioxidant potential with (A) two antioxidant essay and (B) two phytochemical composition analyses in LPC. Data are presented as mean ± SD (n = 3)

Studies have demonstrated that the heating effect facilitated by microwaves can improve the solubility and availability of phenolic compounds, leading to higher antioxidant capacity. The microwave extraction process not only preserves vitamins and phenolic compounds but also allows the rapid breakdown of cell walls, making those antioxidants more accessible[30–33]. For instance, LPC extracted from broccoli has shown marked increases in TFC after heat processing, correlating with improved antioxidant activity as assessed by various assays [34]. On the other hand, the fermentation process tends to increase TPC effectively, driving a corresponding rise in both ACL and ACW as reported in several studies. With lactic acid fermentation, the antioxidant capacity is often enhanced due to the breakdown of complex polyphenols into simpler, more active forms that exert greater antioxidative effects [35–37]**.** This process has been particularly noted in LPC derived from various leafy greens, such as alfalfa and spinach, where fermentation leads to a substantial increase in TPC and TFC levels, contributing to enhanced radical scavenging activities.

This result is also in line with the results of the correlation test (Figure 6). This study has established a positive correlation between the phytochemical content of rye, particularly total phenolics, and antioxidant capacity in water (ACW). The total phenolic content (TPC) within rye is pivotal in its antioxidant activity. Khairani et al. [38] highlighted that elevated concentrations of total phenols correspond to increased antioxidant activity, a phenomenon attributed to the reactivity of polyphenolic compounds with reactive oxygen species. A similar pattern was observed in a study by Uranishi et al. [39], which reported a robust correlation between total phenolic content and DPPH radical scavenging activity in chrysanthemum leaves despite these leaves containing a minimal amount of flavonoid compounds.

The observed negative correlation between TPC and total flavonoid content (TFC) suggests a complex interplay between these two categories of compounds. Although flavonoids are recognized for their antioxidant properties, their chemical structures may engender competition with phenolic compounds for identical mechanisms of action. The investigation by Mahmud et al. [40] illustrated that while flavonoids and phenolic compounds can exhibit positive correlations, their distinct antioxidant pathways may inhibit the effectiveness of one another.

3.4 Colour of LPC

The colour change, especially chlorophyll pigments in green biomass from protein isolation technique was mentioned by the hue angle value of LPC (Table 1). The colour of LPCs among the techniques were significantly different. The results indicate that the colours of LPC are dark-greenness for P-GJ, green-yellowness for MW-LPC, and brown-darkness for LA-LPC (Figure 5).

Table 1. LPC color characteristics of thermal and cold protein isolation techniques

Technique	L*	a	b	Hue Angle (°)
P-GJ	49.52 ± 0.65^{b}	5.31 ± 0.07^{c}	12.34 ± 0.10^{a}	66.96 ± 0.20^{a}
MW-BJ	54.25 ± 1.23^{a}	4.36 ± 0.31^{b}	13.86 ± 1.69^{a}	58.79 ± 0.84^{b}
LA-BJ	52.63 ± 0.21^{ab}	1.10 ± 0.28^{a}	12.35 ± 0.22^{a}	45.26 ± 0.15^{c}

Means in the same column followed by different letters are statistically at α = 0.05. Data are presented as mean ± SD (n = 3).

Protein isolation techniques had different effects on the hue angle values of LPC. MW and LA techniques showed a significant decrease (α =0.05) in the hue value compared with P-GJ, which means a decrease in the intensity of greenness. Since consumers did not well accept the dark-green colour of P-GJ [41], isolation techniques are expected to change the colour, thereby enhancing consumer acceptance of LPC.

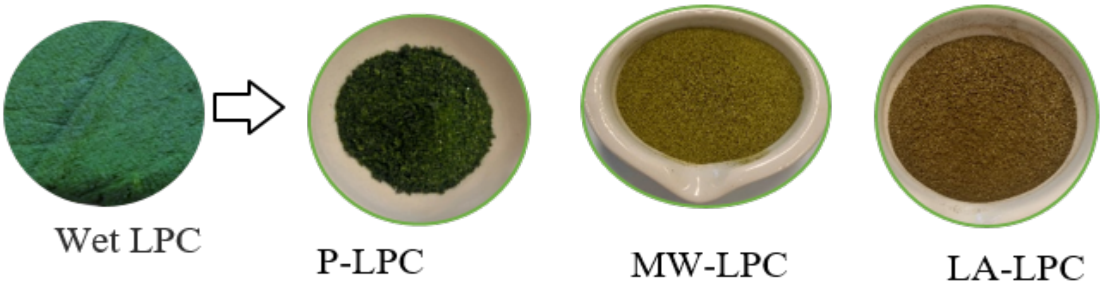
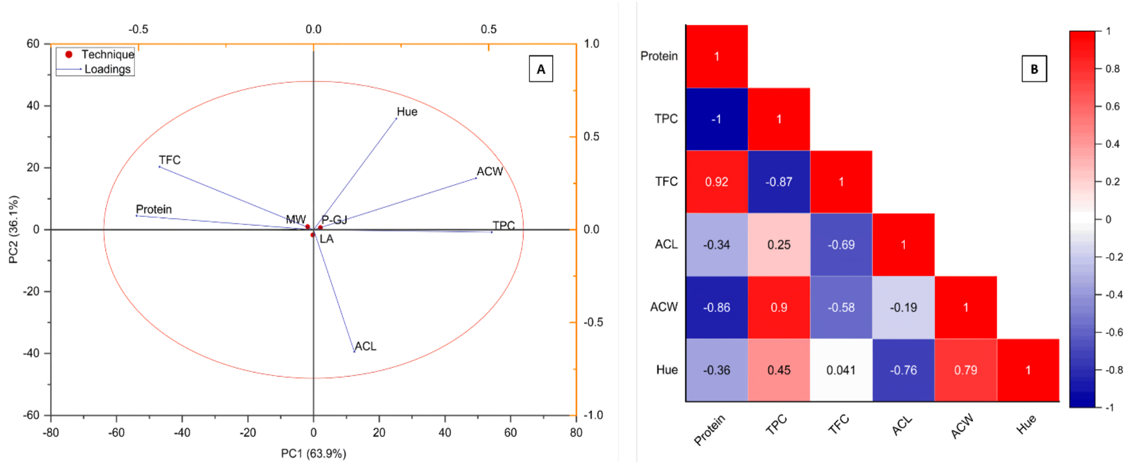


Figure 5. Characteristics of LPC color in different protein isolation techniques

MW-LPC has the highest hue angle value compared to LA-LPC, which is brighter and has more yellowness. Turkmen et al. [42] reported that the microwave technique retained the most chlorophyll a and b, which agrees with our result. On the other hand, chlorophylls can significantly degrade into pheophytins after the fermentation process by lactic acid [43], which changes the colour of green biomass to brown-darkness. In other studies, converting chlorophyll to pheophytin changes from bright green to dull olive-green or olive-yellow [44].

3.5 Principal Component Analysis (PCA) and correlation of physicochemical of LPC

Figure 6A revealed that the principal component analysis (PCA) effectively captured the full spectrum of the LPC data, accounting for 100% representation, with 63.9% attributed to principal component 1 (PC1) and 36.1% to principal component 2 (PC2). The variables P-GJ, MW, and LA were observed in distinct quadrants yet remained relatively close to the centre, indicating that these techniques of protein analyses share similar LPC characteristics while displaying unique advantages. L-GJ exhibited elevated hue, ACW, and TPC values, whereas its high protein content and TFC distinguished MW-LPC. Conversely, LA-LPC demonstrated superior 6.69 µg/mg ACL content.



	Protein	TPC	TFC	ACL	ACW	Hue
Protein	1					
TPC	-1	1				
TFC	0.92	-0.87	1			
ACL	-0.34	0.25	-0.69	1		
ACW	-0.86	0.9	-0.58	-0.19	1	
Hue	-0.36	0.45	0.041	-0.76	0.79	1

Figure 6. (A) Principal Component Analysis and (B) correlation of physicochemical characterization of LPC

A Pearson correlation analysis of the LPC characteristics derived from rye was also conducted (Figure 6B). The findings indicated a strong positive correlation ($r^2 > 0.90$) between protein content and TFC, while a significant negative correlation ($r^2 < -0.90$) was found between protein and TPC. Furthermore, TPC was positively correlated with ACW, and the protein analysis results showed a negative correlation ($r^2 < -0.86$). TPC also negatively correlated ($r^2 < -0.87$) with TFC. Lastly, the hue value was found to be positively correlated ($r^2 > 0.79$) with ACW but demonstrated a negative correlation ($r^2 < -0.76$) with ACL.

Research results demonstrate that protein content variations indicate differences in phytochemical concentrations. Czubinski and Feder [45] elucidated that while the functionality of proteins can be modified, flavonoid bioactivity may also be affected by complex formation. One of the primary mechanisms of interaction between proteins and phenolic compounds occurs through hydrogen bonding. Studies indicate that phenolic compounds possessing o-dihydroxyl groups exhibit a greater propensity to interact with proteins. This interaction arises from the hydroxyl groups in phenolic compounds' ability to form hydrogen bonds with peptide carbonyl groups in proteins [46,47].

3.6 Characterization of BJ

The investigation of how different isolation protein techniques, i.e., MW and LA, impacted the characterization of BJ or deproteinized plant juice (DPJ), also called phytoserum as LPC by-products in terms of pH, brix, and EC. The pH BJ of the LA was significantly 37.08% lower than that of the MW and L-GJ, and the EC increased significantly after protein isolation using both the MW and LA techniques. On the other hand, the brix value did not show different results between L-GJ, MW-BJ, and LA-BJ (Figure 7).

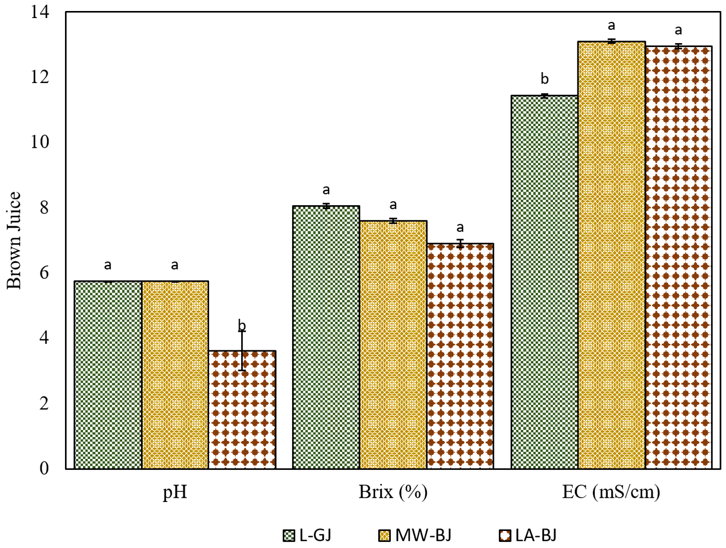


Figure 7. Characteristics of pH, sugar content (%Brix), and electrical conductivity (EC) of BJ in different techniques; liquid green biomass (L-GJ), brown juice from microwave coagulation (MW-BJ), and brown juice from lactic acid fermentation process (LA-BJ)

The pH of BJ is a crucial factor that impacts flavor, stability, and preservation qualities. Fermentation results in a decrease in pH. This is due to the production of organic acids, which are produced during the fermentation of green biomass juices. Lower pH values were beneficial for the stability of polyphenols in fermented juices, which can prevent oxidation processes and extend shelf life [48,49]. Similarly, the fermentation of various juices can lead to a pH range typically between 3.5 to 4.0 [5,50,51]. In contrast, microwave treatment does not result in a pronounced decrease in pH, as the process tends to maintain existing pH levels unless significant degradation of cellular structures occurs that promotes the release of acidic compounds [52].

Both microwave processing and fermentation are expected to elevate EC levels due to the increased presence of electrolytes like nitrates, potassium, and other minerals. Fermentation processes that involve the conversion of sugars to organic acids naturally lead to higher ionic concentrations. Fermented brown juice still held significant concentrations of essential elements like nitrogen and potassium, which would contribute to higher EC values [50]. The increase in EC indicates better nutrient availability, making the fermented brown juice a viable growth stimulant in agricultural practices. Microwave processing might likewise elevate EC levels through the disruption of plant cellular structures, releasing ionic contents.

4. Conclusions

To achieve optimal production of LPC from rye green biomass, harvesting rye at the appropriate phenological stage, specifically at BBCH growth stage 39, is crucial. This timing is essential as it ensures that the rye retains the necessary moisture content. Prompt pressing immediately after harvest is equally important due to the rye's propensity to harden, which can impact the extraction efficiency and quality of the resulting LPC. This highlights MW as an effective method for protein isolation from rye biomass with the highest in TFC. While P-GJ exhibited the highest ACW and yield. The LA enhanced ACL and TPC. Regarding characterization of colour, MW's brighter colour may improve consumer acceptance compared to the dark-green P-GJ. The findings from this study suggest that the MW method is the most effective approach for preserving overall quality. Based on this evidence, we strongly advocate for utilizing the MW method in the production of LPC for food and feed. However, the harvest time and plant development stage, as well as pressing techniques, need to be optimized in order to exploit the methods.

5. Acknowledgments

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References

[1] Anon 2022 OECD-FAO Agricultural Outlook

[2] Tjahyo A S, Wu J Y, Smith G, Acuin C, Maier A B, Sim S Y J, Taneja R, Haldar S and Henry C J 2024 Shifting Trend of Protein Consumption in Southeast Asia: Toward Health, Innovation, and Sustainability *Current Developments in Nutrition* **8** 104443

[3] Drewnowski A 2024 Alternative Proteins in Low- and Middle-Income Countries Face a Questionable Future: Will Technology Negate Bennett's Law? *Current Developments in Nutrition* **8** 101994

[4] Azmi A A bin, Chew K W, Chia W Y, Mubashir M, Sankaran R, Lam M K, Lim J W, Ho Y-C and Show P L 2021 Green bioprocessing of protein from *Chlorella vulgaris* microalgae towards circular bioeconomy *Bioresource Technology* **333** 125197

[5] Domokos-Szabolcsy É, Elhawat N, Domingos G J, Kovács Z, Koroknai J, Bodó E, Fári M G, Alshaal T and Bákonyi N 2022 Comparison of Wet Fractionation Methods for Processing Broccoli Agricultural Wastes and Evaluation of the Nutri-Chemical Values of Obtained Products *Foods* **11** 2418

[6] Domokos-Szabolcsy É, Yavuz S R, Picoli E, Fári M G, Kovács Z, Tóth C, Kaszás L, Alshaal T and Elhawat N 2023 Green Biomass-Based Protein for Sustainable Feed and Food Supply: An Overview of Current and Future Prospective *Life* **13** 307

[7] Kaszás L, Alshaal T, Kovács Z, Koroknai J, Elhawat N, Nagy É, El-Ramady H, Fári M and Domokos-Szabolcsy É 2022 Refining high-quality leaf protein and valuable co-products from green biomass of Jerusalem artichoke (Helianthus tuberosus L.) for sustainable protein supply *Biomass Conv. Bioref.* **12** 2149–64

[8] Nynäs A-L, Berndtsson E, Newson W R, Hovmalm H P and Johansson E 2024 Protein Fractionation of Leafy Green Biomass at the Pilot Scale: Partitioning and Type of Nitrogen in the Fractions and Their Usefulness for Food and Feed *ACS Food Sci. Technol.* **4** 126–38

[9] Gaffey J, Rajauria G, McMahon H, Ravindran R, Dominguez C, Ambye-Jensen M, Souza M F, Meers E, Aragonés M M, Skunca D and Sanders J P M 2023 Green Biorefinery systems for the production of climate-smart sustainable products from grasses, legumes, and green crop residues *Biotechnology Advances* **66** 108168

[10] Pearce F G and Brunke J E 2023 Is now the time for a Rubiscuit or Ruburger? Increased interest in Rubisco as a food protein *Journal of Experimental Botany* **74** 627–37

[11] Erb T J and Zarzycki J 2018 A short history of RubisCO: the rise and fall (?) of Nature's predominant CO2 fixing enzyme *Current Opinion in Biotechnology* **49** 100–7

[12] Feller U, Anders I and Mae T 2008 Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated *Journal of Experimental Botany* **59** 1615–24

[13] Nieuwland M, Geerdink P, Engelen-Smit N P E, van der Meer I M, America A H P, Mes J J, Kootstra A M J, Henket J T M M and Mulder W J 2021 Isolation and Gelling Properties of Duckweed Protein Concentrate *ACS Food Sci. Technol.* **1** 908–16

[14] Zira S, Salomon E, Åkerfeldt M and Röös E 2023 Environmental consequences of pig production scenarios using biomass from rotational grass-clover leys as feed *Environmental Technology & Innovation* **30** 103068

[15] Damborg V K, Jensen S K, Weisbjerg M R, Adamsen A P and Stødkilde L 2020 Screw-pressed fractions from green forages as animal feed: Chemical composition and mass balances *Animal Feed Science and Technology* **261** 114401

[16] Nynäs A-L, Newson W R and Johansson E 2021 Protein Fractionation of Green Leaves as an Underutilized Food Source—Protein Yield and the Effect of Process Parameters *Foods* **10** 2533

[17] Prade T, Muneer F, Berndtsson E, Nynäs A-L, Svensson S-E, Newson W R and Johansson E 2021 Protein fractionation of broccoli (*Brassica oleracea*, var. Italica) and kale (*Brassica oleracea*, var. Sabellica) residual leaves — A pre-feasibility assessment and evaluation of fraction phenol and fibre content *Food and Bioproducts Processing* **130** 229–43

[18] Stødkilde L, Lashkari S, Eriksen J and Jensen S K 2021 Enhancing protein recovery in green biorefineries through selection of plant species and time of harvest *Animal Feed Science and Technology* **278** 115016

[19] Miedaner T, Lauenstein S and Lieberherr B 2025 Comparison of Hybrid Rye and Wheat for Grain Yield and Other Agronomic Traits Under Less Favourable Environmental Conditions and Two Input Levels *Agriculture* **15** 163

[20] Ponomareva M, Gorshkov V, Ponomarev S, Mannapova G, Askhadullin D, Askhadullin D, Gogoleva O, Meshcherov A and Korzun V 2022 Resistance to Snow Mold as a Target Trait for Rye Breeding *Plants* **11** 2516

[21] Møller A H, Hammershøj M, dos Passos N H M, Tanambell H, Stødkilde L, Ambye-Jensen M, Danielsen M, Jensen S K and Dalsgaard T K 2021 Biorefinery of Green Biomass─How to Extract and Evaluate High Quality Leaf Protein for Food? *J. Agric. Food Chem.* **69** 14341–57

[22] Mariotti F, Tomé D and Mirand P P 2008 Converting Nitrogen into Protein—Beyond 6.25 and Jones' Factors *Critical Reviews in Food Science and Nutrition* **48** 177–84

[23] Barna D, Alshaal T, O. Tóth I, Cziáky Z, Fári M G, Domokos-Szabolcsy É and Bákonyi N 2022 Bioactive metabolite profile and antioxidant properties of brown juice, a processed alfalfa (Medicago sativa) by-product *Heliyon* **8** e11655

[24] Kaszás L, Kovács Z, Koroknai J, Elhawat N, Fári M, Cziáky Z, Alshaal T and Domokos-Szabolcsy É 2024 Identification of key parameters and phytochemical composition of fiber and brown juice by-products from green biomass of Jerusalem artichoke (Helianthus tuberosus L.) *Biomass and Bioenergy* **188** 107332

[25] Islam M A, Obour A K, Nachtman J J, Baumgartner R E and Saha M C 2013 Small Grains Have Forage Production Potential and Nutritive Value in Central High Plains of Wyoming *Forage & Grazinglands* **11** 1–10

[26] Kantar M, Sheaffer C, Porter P, Krueger E and Ochsner T E 2011 Growth Stage Influences Forage Yield and Quality of Winter Rye *Forage & Grazinglands* **9** 1–7

[27] Phillips H N, Heins B J, Delate K and Turnbull R 2021 Biomass Yield and Nutritive Value of Rye (Secale cereale L.) and Wheat (Triticum aestivum L.) Forages While Grazed by Cattle *Crops* **1** 42–53

[28] Kaszás L, Alshaal T, Kovács Z, Koroknai J, Elhawat N, Nagy É, El-Ramady H, Fári M and Domokos-Szabolcsy É 2022 Refining high-quality leaf protein and valuable co-products from green biomass of Jerusalem artichoke (Helianthus tuberosus L.) for sustainable protein supply *Biomass Conv. Bioref.* **12** 2149–64

[29] Kondo M, Shimizu K, Jayanegara A, Mishima T, Matsui H, Karita S, Goto M and Fujihara T 2015 Changes in nutrient composition and *in vitro* ruminal fermentation of total mixed ration silage stored at different temperatures and periods *J Sci Food Agric* **96** 1175–80

[30] Álvarez-Romero M, Ruíz-Rodríguez A, Barbero G F, Vázquez-Espinosa M, El-Mansouri F, Brigui J and Palma M 2023 Comparison between Ultrasound- and Microwave-Assisted Extraction Methods to Determine Phenolic Compounds in Barley (Hordeum vulgare L.) *Foods* **12** 2638

[31] Chy Md W R, Ahmed T, Iftekhar J, Islam Md Z and Rana Md R 2024 Optimization of microwave-assisted polyphenol extraction and antioxidant activity from papaya peel using response surface methodology and artificial neural network *Applied Food Research* **4** 100591

[32] Elmas E, Şen F B, Kublay İ Z, Baş Y, Tüfekci F, Derman H, Bekdeşer B, Aşçı Y S, Capanoglu E, Bener M and Apak R 2025 Green Extraction of Antioxidants from Hazelnut By-products Using Microwave-Assisted Extraction, Ultrasound-Assisted Extraction, and Pressurized Liquid Extraction *Food Bioprocess Technol* **18** 5388–406

[33] Gil-Martín E, Forbes-Hernández T, Romero A, Cianciosi D, Giampieri F and Battino M 2022 Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products *Food Chemistry* **378** 131918

[34] Kim H Y, Ediriweera M K, Boo K-H, Kim C S and Cho S K 2021 Effects of Cooking and Processing Methods on Phenolic Contents and Antioxidant and Anti-Proliferative Activities of Broccoli Florets *Antioxidants* **10** 641

[35] Adebo O A and Gabriela Medina-Meza I 2020 Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review *Molecules* **25** 927

[36] Liang Z, Huang Y, Zhang P and Fang Z 2023 Impact of fermentation on the structure and antioxidant activity of selective phenolic compounds *Food Bioscience* **56** 103147

[37] Zhao Y-S, Eweys A S, Zhang J-Y, Zhu Y, Bai J, Darwesh O M, Zhang H-B and Xiao X 2021 Fermentation Affects the Antioxidant Activity of Plant-Based Food Material through the Release and Production of Bioactive Components *Antioxidants* **10** 2004

[38] Afiqa Khairani I, Arliani Nur Anisa H, Siswitasari Mulyana J and Nurma Riana E 2024 The Comparison Of Bioactive Compounds Between Brewed and Extracted Robusta Coffee From West Lampung ed I E Buot and A Luqman *BIO Web Conf.* **101** 02005

[39] Uranishi R, Aedla R, Alsaadi D H M, Wang D, Kusakari K, Osaki H, Sugimura K and Watanabe T 2024 Evaluation of Environmental Factor Effects on the Polyphenol and Flavonoid Content in the Leaves of Chrysanthemum indicum L. and Its Habitat Suitability Prediction Mapping *Molecules* **29** 927

[40] Mahmud S, Didarul Islam A M, Billah M, Rahman M, Biswas R and Emdadul Islam Md 2022 Evaluation of Antioxidant and Anticoagulation Activity of Piper chaba Hunter Stem *PBR*

[41] Santamaría-Fernández M and Lübeck M 2020 Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals *Animal Feed Science and Technology* **268** 114605

[42] Turkmen N, Poyrazoglu E S, Sari F and Sedat Velioglu Y 2006 Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables *Int J of Food Sci Tech* **41** 281–8

[43] Janiszewska-Turak E, Witrowa-Rajchert D, Rybak K, Rolof J, Pobiega K, Woźniak Ł and Gramza-Michałowska A 2022 The Influence of Lactic Acid Fermentation on Selected Properties of Pickled Red, Yellow, and Green Bell Peppers *Molecules* **27** 8637

[44] Koca N, Karadeniz F and Burdurlu H S 2007 Effect of pH on chlorophyll degradation and colour loss in blanched green peas *Food Chemistry* **100** 609–15

[45] Czubinski J and Feder S 2019 Lupin seeds storage protein composition and their interactions with native flavonoids *J Sci Food Agric* **99** 4011–8

[46] Peyrot Des Gachons C, O'Keefe A J, Slade L and Beauchamp G K 2021 Protein suppresses both bitterness and oleocanthal-elicited pungency of extra virgin olive oil *Sci Rep* **11** 11851

[47] Quintero-Flórez A, Sánchez-Ortiz A, Gaforio Martínez J J, Jiménez Márquez A and Beltrán Maza G 2015 Interaction between extra virgin olive oil phenolic compounds and mucin *Euro J Lipid Sci & Tech* **117** 1569–77

[48] Bákonyi N, Barna D, Suhartini W, Cziáky Z, Péter M, Alshaal T, Fári M G and Domokos-Szabolcsy É 2025 Brown juice processed from alfalfa green biomass as a source of phytohormones and saponins *Sci Rep* **15** 18653

[49] Jin X, Chen W, Chen H, Chen W and Zhong Q 2019 Combination of Lactobacillus plantarum and Saccharomyces cerevisiae DV10 as Starter Culture to Produce Mango Slurry: Microbiological, Chemical Parameters and Antioxidant Activity *Molecules* **24** 4349

[50] Bákonyi N, Kisvarga S, Barna D, O. Tóth I, El-Ramady H, Abdalla N, Kovács S, Rozbach M, Fehér C, Elhawat N, Alshaal T and Fári M G 2020 Chemical Traits of Fermented Alfalfa Brown Juice: Its Implications on Physiological, Biochemical, Anatomical, and Growth Parameters of Celosia *Agronomy* **10** 247

[51] Chwastek A, Klewicka E, Klewicki R and Sójka M 2016 Lactic Acid Fermentation of Red Beet Juice Supplemented with Waste Highbush Blueberry-Sucrose Osmotic Syrup as a Method of Probiotic Beverage Production: Red Beet Juice in Probiotic Beverage Production *Journal of Food Processing and Preservation* **40** 780–9

[52] Wang J, Wei B, Xu J, Jiang H, Xu Y and Wang C 2024 Influence of lactic acid fermentation on the phenolic profile, antioxidant activities, and volatile compounds of black chokeberry (*Aronia melanocarpa*) juice *Journal of Food Science* **89** 834–50