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# Innovating Plant-Based Foods: Physicochemical and Protein Profile of A Vegan Dressing Made from Flaxseed (*Linum usitatissimum*), Soybeans (*Glycine max*) and Lupin (*Lupinus mutabilis*)

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**Abstract**

The purpose of the present study was to evaluate the physicochemical characteristics and protein profile of a vegan dressing made from flax (*Linum usitatissimum*), soybean (*Glycine max*), and lupin (*Lupinus mutabilis*), to promote the development of this type of vegan product with significant nutritional characteristics. For this purpose, three treatments and five repetitions of the product formulations were developed. In addition, an ANOVA study with Tukey's test at 5% significance was used to interpret the results of protein, fat, and pH. The protein values for the three proposed treatments ranged from 2.71 - 5.68 %, fat: 52.40 - 54.10 %, and pH: 4.10 - 4.37. Formulation 1 contains the highest levels of lupin flour and flaxseed meal compared to the other two formulations (Soy Beverage 22%, Lupin Flour 9%, and Flaxseed Meal 8%) stood out with the highest protein content (5.68 %), fat (54.10 %) and pH (4.37). Subsequently, the protein profile of this treatment resulting in a higher proportion of the following amino acids: glutamic acid (2.21 g), threonine (0.93 g), and aspartic acid (0.90 g). The vegan dressing's protein profile, with 5.68% total protein, highlights essential and non-essential amino acids, including Glx and Asx, derived from acid hydrolysis, showcasing the nutritional potential of lupin flour and flaxseed meal. Thus, the microbiological quality of this treatment

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was determined using as a reference the Sanitary Technical Standard (NTS), obtaining molds (20 CFU/g), yeasts (10 CFU/g), and total coliforms (< 10 CFU/g). These results showed the nutritional potential of dressings developed from plants and their safety, becoming an alternative to be considered in these products. The significance of these findings lies in their potential applications in the functional food and plant-based product industry. The high protein content and well-balanced amino acid composition position this dressing as an attractive alternative for consumers seeking plant-based, high-protein condiments. Moreover, the stability in pH and fat content indicates that these formulations could serve as a foundation for further innovation in vegan emulsions, improving their sensory and nutritional attributes.

## Introduction

The number of people turning to plant-based dietary lifestyles is steadily increasing. It is now closely linked to the evolving food consumption patterns, emphasizing the growing preference for plant-based diets and highlighting the digital transformation shaping food production and consumption as key emerging trends in the agri-food sector.[1] In recent years, there has been a growing trend toward substituting eggs with plant-based ingredients. This shift is driven by various factors, including consumers with egg allergies, dietary preferences such as veganism, and concerns about animal welfare, particularly the exploitation of hens in egg production.[2] This transition has spurred research into the functionality, processing methods, and acceptability of vegan macronutrients as substitutes for animal-derived products.[3]

In food formulation, egg replacement has gained considerable attention, with research focusing on plant-based macronutrients that serve as viable alternatives.[4] Among these, plant-based emulsions have emerged as a key area of study due to their potential applications in various food products, including dressings and sauces. Studies have demonstrated the effectiveness of plant-based emulsifiers, such as soy protein, pea protein, and hydrocolloids, in achieving the stability and textural properties necessary for successful egg replacements.[5-6] Additionally, novel protein sources like oat, chickpea and lentil proteins show promising emulsification properties and contribute to the nutritional value of plant-based dressings.[7-8]

In the modern world, consumers are increasingly seeking healthy food options that offer new and exciting flavor experiences. Within this context, dressings have garnered significant attention due to growing trends favoring healthy, spiced, and flavorful products.[9] Additionally, market tendencies highlight the importance of incorporating vegan options that deliver the same delightful taste experience as their conventional counterparts. Interest in replacing this ingredient has been extreme in the formulation of mayonnaise analogs as vegan dressings. In particular, soy-based proteins combined with some other food additives such as emulsifiers that mimic the properties of eggs are highlighted.[10] Vegan dressings are described as those products, of variable consistency, elaborated from the mixture of vegetable sources that can give rise to an emulsion together with oil, where the substance that is in the form of drops in the emulsion establishes the dispersed phase or oily phase,[11] while the substance that constitutes the surrounding liquid is known as the continuous phase or aqueous phase; to which seasonings and spices can be added or not, but it does not include the use of ingredients of animal origin.[10] The interest in substituting this ingredient has become especially relevant in the development of alternatives to traditional mayonnaise. In particular, soy-derived proteins stand out, which, combined as emulsifiers, are capable of replicating the functional properties of egg or oil.[12]

Recent research highlights the nutritional benefits of using flaxseed powder in vegan dressings, as it enriches the product with omega-3 fatty acids, such as alpha-linolenic acid, which has been associated with reduced cardiovascular disease risk.[13] Similarly, lupin, a leguminous rich in high-quality protein, offers a well-balanced amino acid profile, though it is relatively low in cysteine, arginine, and tyrosine, necessitating its combination with complementary protein sources.[14-15] Studies have also demonstrated that using alternative emulsifiers, such as pea and lentil proteins, can enhance the texture and stability of vegan dressings, providing additional options for plant-based formulation strategies.[16,17,3]

It is of vital importance to know the nutritional potential of vegetable proteins through the identification of amino acids.[18] That indicates that soy in the diet is limited to tofu, soy beverage, soy sauce, and miso; flaxseed, on the other hand, was used in flours, and lupin is most often marketed as a nutritious snack.[19] Therefore, using flaxseed, soybean and lupin to prepare a vegan dressing becomes relevant in the difficulty of resorting to new food sources of plant origin that can provide significant nutritional characteristics.[20]

The present study aims to develop a vegan dressing using flaxseed, soybean, and lupin proteins, considering the increasing interest in sustainable and functional plant-based foods. Unlike previous studies that have focused primarily on single-ingredient emulsifiers, this research integrates multiple plant proteins to enhance the nutritional profile and emulsification properties of the final product. By evaluating the amino acid composition, physicochemical stability, and microbiological safety of the formulation, this study provides novel insights into the optimization of plant-based emulsions for commercial applications. Furthermore, this research contributes to the growing of knowledge on plant-based food innovation by demonstrating the potential of underutilized protein sources in functional food applications.[21,22]

## Materials and Methods

### Raw Materials

The lupin, flaxseed flour, and beverage used in the research complied with quality standards, a local supplier in Guayaquil.

### Dressing Development

Based on the formulation established in the study of the elaboration of mayonnaise from soybeans carried out by another author,[23] the different ingredients to be used were established, incorporating flaxseed and lupin flour. The soy beverage was mixed with the flaxseed meal and the lupin flour to form the aqueous phase of the emulsion. With this, the flaxseed was left to stand for 10 minutes in the soy beverage to activate the mucilage present, and then it was mixed with the lupin flour. The oil was slowly incorporated into the previous mixture while it was homogenized at a controlled speed of 4000-5000 rpm to facilitate emulsification without destabilizing the system. Once the emulsion was formed, it was homogenized at a controlled speed of 8000-10000 rpm for 2-3 minutes to ensure stability and uniformity, and the remaining ingredients, such as sodium chloride and potassium sorbate were added. Throughout the mixing and homogenization process, the temperature was maintained within a 20-25°C range to prevent protein denaturation and ensure emulsion stability.

To minimize potential bias, sample preparation and data collection followed standardized procedures to ensure consistency across formulations.[24] While blinding was not fully applied, efforts were made to standardize handling and testing conditions.[25] Future studies should implement double-blind protocols to enhance objectivity. This study focused on physicochemical and microbiological stability, without assessing sensory attributes like taste, texture, and acceptability. Sensory perception is crucial for consumer acceptance of plant-based emulsions,[26] as emulsifier type and oil composition impact mouthfeel.[27,28] Future studies should integrate standardized sensory evaluations to align stability with consumer preferences.[29]

In the experimental development of the product, a Completely Randomized Design (CRD) was implemented through three formulations and five replicates of each one (Table 1).

**Table 1: Percentages of ingredients used in vegan dressing formulations.**

| Ingredients | Formulations (%) | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| Soy beverage | 22 | 29 | 34 |
| Lupin fluor | 9 | 6 | 3 |
| Flaxseed meal | 8 | 4 | 2 |
| Sunflower oil | 50 | 50 | 50 |
| Sodium chloride | 2.5 | 2.5 | 2.5 |
| Apple cider vinegar | 8.4 | 8.4 | 8.4 |
| Potassium sorbate | 0.1 | 0.1 | 0.1 |
| Total | 100 | 100 | 100 |

**Physicochemical Characterization of the Dressing**

The physicochemical evaluation of the vegan dressing was established through protein, fat and pH parameters. The protein analysis was based on the method described in the AOAC 21st 2001, using a digester block and Kjeldahl TE-0364 distillation equipment. The fat analysis was based on the method described in AOAC 21st 950.04.[30] A Mojonnier fat extraction system was used; this test is based on extracting the total fat from the emulsion, including the free and bound fat. The resulting fat residue was determined gravimetrically after drying, for which an ED 115 drying oven model was used; finally, the result is reflected as a percentage of total fat. The determination of pH was based on the method described in AOAC 21st 981.12,[31] this variable is measured through an electric potential, in this case a potentiometer was used with S400 glass electrodes; the electrodes of the potentiometer must be submerged in the sample, allowing the meter to stabilize for 1 min.

**Identification of Amino Acids**

This methodology traditionally consists of hydrolysis, derivatization, and chromatographic separation to identify amino acids by high-performance liquid chromatography (HPLC).[32] An L-amino acid kit (Sigma) was used as a standard solution along with an OPA Reagent (o-phthalaldehyde), and a Shimadzu HPLC system with LC-10 AD VP117 pumps.

**Microbiological Evaluation**

Microbiological parameters such as total coliforms, molds, and yeasts were evaluated during the treatment with the highest protein content; the quality criteria for this evaluation were determined from the sanitary standard for industrialized dressings Sanitary Technical Standard (NTS).[33]

For the determination of molds and yeasts, the AOAC 21st 997.02[34] method was used, incubating the samples at a temperature of 21°C to 25°C for 5 days, following the established standards for accurate quantification. The determination of total coliforms was performed according to the AOAC 21st 991.14[35] method, incubating the samples at 35°C ± 1°C for 24 hours ± 2 hours. These conditions ensured an optimal environment for microbial growth, allowing for a precise and reproducible assessment of their presence in the analyzed samples.

**Statistical Analysis**

The statistical analysis employed a Completely Randomized Design (CRD) with three treatments and five replicates. The data was analyzed using inferential statistics, employing an Analysis of Variance (ANOVA) combined with Tukey's test at a 5% significance level to identify the treatment with the highest protein content and to assess significant differences in fat content and pH values. Data management of the results obtained for each variables studied done in Microsoft Excel, while statistical calculations were performed in InfoStat software for Windows. To enhance transparency and robustness in the statistical analysis, confidence intervals (95%) and effect sizes were included alongside p-values to provide a clearer interpretation of the magnitude and practical significance of the observed differences.

## Results

**Physicochemical Characterization of the Vegan Dressing**

Concerning the quantification of protein and the physicochemical characterization in parameters of fat and pH, it is highlighted in the formulations of the proposed vegan dressing, the content of soy beverage, lupin flour and flaxseed flour varied, presenting formulation 1 (F1): 22 %, 9 %, 8 %; formulation 2 (F2): 29 %, 6 %, 4 %; and formulation 3 (F3): 34 %, 3 % and 2 %, respectively. Results varied between 2.71 and 5.68 % protein among the proposed treatments, while fat content values ranged from 52.40 to 54.10 % and pH values ranged from 4.10 to 4.37, as shown in Table 2.

The lower percentages obtained in the present study regarding fat content can be explained by the use of different raw materials and formulations in the experimental.

The results about pH reflected in the present investigation indicate that F1 obtained a higher average pH of 4.37 compared to F2 and F3 with a pH of 4.28 and 4.10, respectively.

These results indicate that incorporating a higher concentration of flaxseed in the dressings samples leads to an increase in fat, protein, and pH values, as demonstrated in treatment F1, with values ranging from 5.55 % to 5.77 %.

**Table 2: Physicochemical properties of vegan dressing treatments.**

| Parameters | Treatments | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| Protein % | $5.68^{A} \pm 0.09$ | $4.13^{B} \pm 0.09$ | 2.71C ± 0.09 |
| Fat % | $54.10^{A} \pm 0.71$ | $52.76^{A} \pm 0.71$ | $52.40^{A} \pm 0.71$ |
| pH | $4.37^{A} \pm 0.03$ | $4.28^{A} \pm 0.03$ | $4.10^{B} \pm 0.03$ |

a-b Different letters between columns indicate significant statistical difference ($p<0.05$).

Content of soy beverage, lupin flour and flaxseed flour varied, presenting formulation 1 (F1): 22 %, 9 %, 8 %; formulation 2 (F2): 29 %, 6 %, 4 %; and formulation 3 (F3): 34 %, 3 % and 2 %, respectively.

**Amino Acid Profile**

To assess the nutritional potential of vegetable proteins, this study utilized Formulation 1, selected based on the physicochemical properties. The amino acid profile of Dressing 1 is summarized in Table 3. This results offering detailed insights it's into amino acid composition and highlighting its contribution to the nutritional value of the formulated dressing.

**Table 3: Amino acid profile of vegan flaxseed, soy and lupin dressing with higher protein content.**

| Amino acids | g AA/100 g sample Moisturize base |
|---|---|
| Aspartic acid (Asp) | 0.90 ±0.003 |
| Glutamic acid (Glu) | 2.21 ±0.001 |
| Serine (Ser) | 0.17 ±0.025 |
| Histidine (His) [a] | 0.62 ± 0.001 |
| Threonine (Thr) [a] | 0.93 ± 0.001 |
| Glycine (Gly) | 0.50 ± 0.004 |
| Arginine (Arg) | 0.44 ±0.002 |
| Alanine (Ala) | 0.42 ± 0.011 |
| Tyrosine (Tyr) | 0.35 ±0.031 |
| Valine (Val) [a] | 0.35 ± 0.001 |
| Methionine (Met) [a] | 0.11 ± 0.002 |
| Phenylalanine (Phe) [a] | 0.39 ± 0.001 |
| Isoleucine (Ile) [a] | 0.41 ± 0.136 |
| Leucine (Leu) [a] | 0.63 ± 0.001 |
| Lysine (Lys) [a] | 0.38 ± 0.001 |
| Amino acids Totals | 8.81 |

a: essential amino acids present in vegan dressing.

The amino acid profile analysis of Formulation 1, conducted using a 100 g sample of vegan dressing, revealed a considerable percentage of essential amino acids, including threonine (0.93 %), leucine (0.63 %), and histidine (0.62 %), along with notable amounts of non-essential amino acids such as glycine (0.50 %), glutamic acid (2.21 %), and aspartic acid (0.90 %). These results highlight this formulation as a promising nutritional alternative in the category of vegan dressings.

**Microbiological Analysis**

Regarding the microbial colony count to determine the microbiological quality of the treatment with the highest protein content of the vegan dressing, the following results were obtained: molds (20 CFU/g), yeasts (10 CFU/g) and total coliforms (< 10 CFU/g). These values obtained were lower than the microbiological criteria for industrialized dressings defined by the Sanitary Technical Standard (NTS),[18] which indicates that for a good quality level, the maximum permissible limit per gram or mL should be 102 CFU/g for molds, yeasts and total coliforms, while for an acceptable quality level it corresponds to 103 CFU/g for each microbial agent.

**Discussion**

The Physicochemical characterization of the vegan dressing show a certain similarity with other research that evaluated the protein percentage in vegan mayonnaises made from chickpea, broad bean, and lupin flour. Their findings reported protein levels ranging from 2.43 % to 2.67 % across all dressings, with one of the highest values being 2.58

% in the vegan mayonnaise made from lupin flour.[36] In another study, the authors used chickpea flour to enhance the protein content in their formulation, achieving a protein content of 4.92 % compared to 2.20 % in the control mayonnaise.[37] These findings indicate that, when properly utilized, vegan ingredients can significantly increase protein content and positively impact the sensory profile of the final product.[38]

The pH of mayonnaise or dressings is a critical parameter that indicates product stability and shelf life.[39] Consistent with this premise, various studies have highlighted pH as the most significant intrinsic characteristic of mayonnaise, dressings, and sauces, as it directly affects the growth and survival of pathogenic bacteria.[40] For optimal safety and quality, the pH of mayonnaise should be acidic, ideally ranging between 3.0 and 4.5, to prevent spoilage by harmful microorganisms.[41]

In this study, the authors reported that Formulation 1 (F1) exhibited a higher average pH of 4.37 compared to Formulations 2 (F2) and 3 (F3), which recorded pH levels of 4.28 and 4.10, respectively. The interaction between the formulation's ingredients and the recorded pH aligns with findings from other studies, where ingredient interactions and storage time significantly influenced the structure of the dressing, resulting in an observable increase in pH levels.[42]

Regarding fat content, this study demonstrates similarities with previous research that evaluated the impact of incorporating flaxseed meal at different concentrations (4 %, 8 %, 12 %, and 16 %) on the physicochemical characteristics of reduced-fat mayonnaise. The reported fat content in that study ranged from 36.99 % to 37.72 %, while protein content ranged from 1.74% to 3.71 %, and pH values varied between 3.7 and 4.2.[43] Another study on vegan dressings analyzed the relationship between fat content and viscosity properties. It was observed that the addition of vegetable proteins, such as chickpea protein, modified the consistency of the dressing.[44] Similarly, the inclusion of vegan thickeners like passion fruit juice increased the viscosity of a pseudo plastic dressing sample.[45] Both of cases presents increase to the fat content, alight the results with this research. Concluding that incorporating a higher concentration of flaxseed in the mayonnaise samples will provide an increase in fat, protein, and pH values.

Regarding the amino acid profile, the protein profile of the vegan dressing formulation highlights the presence of essential and non-essential amino acids, underscoring its nutritional potential. During acid hydrolysis, amide groups in glutamine (Gln) and asparagine (Asn) are hydrolyzed, resulting in the formation of glutamic acid (Glx = Glu + Gln) and aspartic acid (Asx = Asp + Asn). This process is critical for accurately quantifying the amino acid content, as it ensures the inclusion of all forms of these amino acids in the final calculation.[46] Consequently, the reported values for Glx and Asx provide a comprehensive understanding of the amino acid composition, which is essential for evaluating the nutritional quality of plant-based foods.

Furthermore, the total protein content of the formulation (5.68%) served as a reference point for calculating the concentrations of individual amino acids. This methodological approach aligns with established practices in amino acid analysis, ensuring the validity of the reported data.[47] The high levels of glutamic acid (2.21 g/100 g sample) and aspartic acid (0.90 g/100 g sample) reflect the significant contribution of plant-based ingredients such as lupin flour and flaxseed meal, which are known for their amino acid richness.[48]

The inclusion of Glx and Asx in amino acid profiling is particularly relevant when evaluating the nutritional value of plant-based proteins. Studies have shown that Glx and Asx play a crucial role in metabolic processes, such as nitrogen metabolism and the biosynthesis of other amino acids.[49] Additionally, these amino acids contribute to the sensory properties of food products, enhancing umami flavors and improving overall palatability.[50]

This study aligns with the amino acid profile of a quinoa dressing identified by high-performance liquid chromatography. Both studies agree that the amino acids present in lower proportions in the developed dressings were methionine and serine. However, they differ in the essential amino acid present in the greatest quantity. In the vegan dressing of this study, threonine is the most abundant, while in the quinoa dressing, it is histidine. The differences between the results of the two studies can be explained by the combination of ingredients and the amino acid content in each. This research used three ingredients soy beverage, flaxseed meal, and lupin flour while the aforementioned study used un-moisturized quinoa seeds.[51]

The quality of macronutrient content in these innovative products has not received enough attention, particularly regarding their amino acid profiles.[52] It is especially important to highlight the content of essential amino acids, which are crucial for vegan consumers due to the potential deficiencies in their diet. A plant-based diet rich in these nutrients can help maintain homeostasis in the body.[53] The amino acid profile reveals significant results regarding essential amino acids, attributed to the presence of soybean and lupin, these components can help maintain homeostasis in the body.[54] This last ingredient, in particular, is notable for its favorable chemical composition and high protein content, comparable to soy (32.2 %), positioning it as a valuable ingredient for enriching food formulations.[55] Additionally, the glutamic acid present in the amino acid profile contributes to the proper functionality and biosynthesis of essential components.[56]

Focusing on the importance of food safety measures, the microbiological analysis represents a critical step in ensuring the quality, safety, and compliance of dressings with regulatory standards, as well as protecting consumer health.[57] In this context, other authors emphasize the need to establish guidelines for evaluating the microbiological state to prevent adverse health effects and ensure food safety.[58] Dressings have specific conditions, such as pH control (acidity), water activity (Aw) regulation, the use of chemical preservatives, and safety management through packaging. These measures help control safety parameters and reduce microbiological risks.[59,60] These practices are particularly important, as they minimize microbiological risks and help maintain a low pH.[61] This aligns with the parameters evaluated in this study, where pathogens were found to be within permissible limits.

The increasing demand for plant-based and allergen-free dressings presents a significant market opportunity, with projections estimating the global vegan dressing market will grow from USD 1,450 million in 2022 to USD 2,453.4 million by 2032 at a 5.4% CAGR.[62] Consumer acceptance is key, as sensory attributes such as taste, texture, and palatability influence purchasing decisions, making optimized formulations crucial for market penetration.[63] From an industrial perspective, ensuring formulation stability and extended shelf life is essential for large-scale production, as the global vegan sauces and dressings market is projected to reach USD 365.8 million by 2027.[64] Future research should focus on alternative plant-based proteins and emulsifying agents to improve nutritional quality, stability, and sensory attributes, broadening product diversity and enhancing consumer satisfaction.[65]

## Conclusion

The study evaluated the protein content and physicochemical characteristics (fat and pH) of vegan dressings, highlighting formulation F1, which included 9 % lupin flour and 8% flaxseed flour. Formulation F1 exhibited significant differences, with an average protein content of 5.68 % compared to other treatments. While no significant contrasts were obtained between the fat and pH variables, F1 achieved higher average values of 54.10 % fat and a pH of 4.37% relative to other food matrices studied.

The vegan dressing formulation demonstrated a rich protein profile, including essential and non-essential amino acids, with a total protein content of 5.68%. The inclusion of Glx (Glu + Gln) and Asx (Asp + Asn) values, derived from acid hydrolysis, provided a comprehensive understanding of the amino acid composition, reflecting the significant contribution of plant-based ingredients like lupin flour and flaxseed meal, known for their nutritional and functional properties.

Microbiological analyses of molds, yeasts, and total coliforms in formulation F1 confirmed that the product meets the quality and safety standards established for industrialized dressings, aligning with current sanitary regulations.

From a practical and industry perspective, the results highlight the potential for commercial-scale production of high-protein vegan dressings, catering to health-conscious consumers seeking plant-based, allergen-free alternatives. Future research should focus on optimizing formulation stability, extending shelf life, and enhancing sensory attributes through advanced emulsification techniques and novel plant-based ingredients. Additionally, exploring functional health benefit such as bioactive compounds, digestibility, and gut microbiota interactions could further position these dressings as functional food products. Sustainability considerations, including alternative protein sources, clean-label formulations, and eco-friendly packaging, should also be assessed to align with global trends in sustainable food production.

Expanding this research to market potential analysis, consumer perception studies, and industrial process scalability will provide valuable insights for manufacturers, food technologists, and policymakers looking to develop innovative, nutritionally enhanced plant-based condiments for broader commercializatio

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials.

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**Author Contributions**

Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication.

- **Carolina Paz-Yépez:** Investigation, Conceptualization, Methodology, Formal analysis, Data curation, Writing—original draft, Writing – Review & Editing.
- **Hjalmar Lino-Cortez:** Investigation, Methodology, Validation, Writing—original draft, Resources.
- **Grace Medina-Galarza:** Data curation, Writing & Editing.
- **Ahmed El Salous:** Investigation, Resources, Supervision.

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