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Whey-Based Protein Powder Supplement

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Abstract

This research was carried out to take advantage of the whey from cheese production in our country, which, for many years, was considered waste and generated a negative environmental impact on our environment. Currently, the industry has focused on giving this byproduct a new opportunity. This work seeks to develop a protein supplement in powder from whey concentrate to complement the nutritional and protein needs of people, and also contribute to sustainable practices within the food industry. Ultrafiltration and spray drying techniques were used to prepare the concentrate, and maltodextrin, organic cocoa powder, and a mixture of stabilizers were added to prepare the protein supplement, to give added value to the product. This research work included a test series, which resulted in a protein content of 30.18% in the best treatment, the microbiological tests performed on this treatment comply with the requirement from INEN 2983 standard for nutritional supplements, while the results obtained through sensory analysis highlight the acceptability of treatment 1, which contains a 29.48% protein. All the treatments analyzed in this research provide more than 20% of the daily protein requirement, being considered a high protein product according to the INEN 1334:3 standard, and it has exceptional nutritional properties and attractive sensory characteristics.

Keywords: *Food Industry, Nutritional Need; Protein, Whey, Protein Supplement.*

Introduction

Whey is the main by-product of the dairy industry and has a high load of contaminants, due to its rich nutrient content, its major component, lactose, contributes to an appreciable biochemical and chemical oxygen demand (1), for this reason, it is considered one of the largest waste generated by the dairy industry and its derivatives (2); which makes it one of the most polluting and problematic by-products that exists at the environmental level. Whey is a liquid by-product derived from milk in the process of cheese production (3).

In the dairy industry, whey has been considered a waste product that is generally used for animal feed or is discharged into water sources or sewers, causing a serious pollution problem. However, recent studies have shown several alternatives for its use and animal or human consumption, within the daily diet through the elaboration of protein concentrates.

Whey proteins are considered biologically optimal and are widely used in the food industry. Due to its high content of branched-chain amino acids, it is also used in supplements for athletes (4). Whey can be concentrated by filtration and purification, transforming the residues into a

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valuable source of protein for food and dietary supplements (5).

Among the main by-products are whey powder and different by-products: demineralized whey powder, lactose powder, lactose-reduced and lactose-free whey powder, whey protein isolates, whey protein concentrates, lactalbumin and whey permeate. Thanks to their technological functionality, some of these protein concentrates are used as ingredients in the formulation of new products in various food and beverage sectors and as a replacement or alternative to other traditional ingredients (6).

Jaramillo (2021), in his research entitled "Development of a nutritional supplement from whey for adult athletes" reports the ways of taking advantage of the proteins that whey has, his study had as a purpose the elaboration of a nutritional supplement under the transformation of this derivative into a high purity powder (3).

Food fortification has been a common practice in today's societies, seeking to offer more nutritious food, especially to sectors of the population with malnutrition problems (7).

Protein supplements are products designed to increase dietary protein intake, especially for people seeking to increase muscle mass, improve sports performance or ensure adequate protein intake for a balanced diet. They come in various forms, such as protein powders, protein bars, protein-rich drinks, protein-rich beverages, and animal protein supplements (8). Overall, the results suggest that the new protein concentrates not only meet the nutritional needs of people under extreme conditions, but also offer a practical solution for long-term food storage and consumption (9).

Whey powder and whey protein concentrate are used in a wide variety of food products, such as processed meat, sausages, baby food, beverages and confectionery. They provide greater nutritional value and improve the texture of food products (10).

The objective of this research is to elaborate a powdered protein supplement from whey concentrate, highlighting its organoleptic and nutritional properties, because, in addition to all the environmental and economic qualities and advantages, its consumption is beneficial to human health, since whey protein has a high nutritional value, containing more than 50% of milk solids such as protein, lactose, minerals and vitamins (8).

The biological components of whey, specifically its proteins, have a wide range of bioactivities: antioxidant, antihypertensive, antitumor, hypolipidemic, antimicrobial, antiulcerogenic, anti-inflammatory, immunomodulatory, chelating, and muscle strength and body composition enhancers. Therefore, whey protein concentrates, whey fractions and their biologically active peptides can be used as dietary supplements as well as in pharmaceutical preparations or functional ingredients (11).

For the preparation of the powdered protein supplement, several formulations were established, paying special attention to taste, texture and solubility. The percentage of protein concentrate, maltodextrin, stabilizers and cocoa powder was combined, taking into account compliance with the requirements of the NTE INEN 2983 standard for food supplements. A panel of tasters conducted a sensory analysis to determine the acceptability of the product. Then a nutritional analysis of all the treatments was carried out. Finally, a microbiological analysis of the treatment with the highest protein content was carried out to determine compliance with INEN 2983 standard for nutritional supplements.

Materials and Methods

Elaboration Process

- Reception of the powdered protein concentrate: Once the powdered concentrate is obtained, prior to the membrane ultrafiltration process and drying in a spray dryer, it is sieved to obtain a homogeneous powder, separating some granules.

- Addition of ingredients and inputs: After the sieving process, the whey protein concentrate is weighed according to the formulation developed and the other ingredients, maltodextrin, stabilizers (xanthan gum and CMC) and organic cocoa powder are added.

- Homogenization: The mixture is homogenized to obtain a lump-free product.

- Physicochemical and microbiological analysis of the protein powder supplement: Based on the Design Expert 8.0.6 software, thirteen optimal runs were obtained to compare the formulations, the protein content of each sample was determined, the microbiological analysis according to NTE INEN 2983 for nutritional supplements, and the undissolved solids content, crude density and compacted density of the powder obtained were determined.

- Packaging: The powdered protein supplement was packed in an airtight container and stored at room temperature.

Experimental Design

For the optimization of the manufacturing process of this protein powder supplement, a simplex mixture design was used with the help of the statistical program Design Expert 8.0.6, which allowed obtaining different formulations. Using the software, thirteen experimental runs were obtained to evaluate the effect of the addition of whey protein concentrate and maltodextrin content, considering the percentage of protein in each formulation and the percentage of total dissolved solids as the response variable.

Levels of Experimental Design

Level	Description	Content
A	Whey protein concentrate powder	Max. 90% Mín. 85%
B	Maltodextrin	Max. 11% Mín. 6%
C	Organic cocoa powder	3%
D	Mixture of stabilizers	1%

Table 1. Levels of Experimental Design

Source: Authors

Analysis and Discussion of Results

Protein Content

Run	Component 1A	Component 2 B	Component 3 C	Component 4D	Response Variable:

	Protein concentrate	**Maltodextrin**	**Organic cocoa powder**	**Mixture of stabilizers**	**Protein in the mixture (%)**
1	875%	85%	3%	1%	29.48 %
2	87.5%	8.5%	3%	1%	29.48 %
3	90%	6%	3%	1%	30.18 %
4	86.7%	9.3%	3%	1%	29.27 %
5	88.3%	7.7%	3%	1%	29.88 %
6	85%	11%	3%	1%	28.61 %
7	90%	6%	3%	1%	30.18 %
8	90%	6%	3%	1%	30.18 %
9	85%	11%	3%	1%	28.61 %
10	88.7%	7.3%	3%	1%	29.49 %
11	87.5%	8.5%	3%	1%	29.48 %
12	85%	11%	3%	1%	28.61 %
13	86.3%	9.7%	3%	1%	28.99 %

Table 2. Protein Content by Formulation

Source: *Authors*

According to the table above, the protein content of each formulation is proportional to the amount of protein concentrate in each of them. It is established that the best treatments are number 3, 7 and 8, with 30.18% protein, a value very close to the results of the characterization of the fermented concentrate from cheese whey reported by Campoverde (2023) with a value of 29.83% protein (12); and which has correspondence with the value obtained by González (2016), who found values of fat percentage of whey protein powder concentrates obtained by isoelectric thermocoagulation and dehydration at different precipitation pH were in the range of 40.23% to 44.65% (13), close to the value obtained in proximal analysis of whey protein concentrates contain 42% protein (14).

Fat-soluble proteins are unique nutritional supplements and functionally valuable food ingredients from the biological, physical and chemical point of view (15).

Whey proteins can positively influence various health conditions, such as cardiovascular health, immune function, and metabolic disorders. In addition, whey proteins can help reduce cholesterol levels and improve insulin sensitivity. They can support gut health and liver function, showing promise in managing conditions such as non-alcoholic fatty liver disease and improving intestinal permeability. (11)

In previous studies carried out by Jaramillo (2021), he demonstrated the importance of factors such as temperature and atomizer feeding flow during the drying process, obtaining a protein concentrate with 80% protein in the best treatment, and 10% in the lowest one after the ultrafiltration process by membranes (3).

In a research conducted by Camacho (2010), applying membrane technology and different pressures, a protein content of 3.05% was achieved (16). From which it can be deduced that the protein content depends on the method of elaboration of the protein concentrate.

One of the most common uses of whey is its application as the main ingredient in energy drinks, since it provides energy, regulates body temperature, prevents dehydration and quenches thirst,

in addition to being an economical source of protein, which makes it possible to manufacture products based on it on a commercial scale and provides broad economic advantages (10).

The following table illustrates the parameters of the codified model for the percentage value of protein content.

Indicator	**Protein (%)**
A B	28.65* 30.19*
R^2 R^2 adjusted R^2 predicted	0.9589 0.9552 0.9482
F model	256.90*
F lack of fit Adequate accuracy	0.00 31.52

Table 3. Parameters of the Coded Model for Protein Content (%)

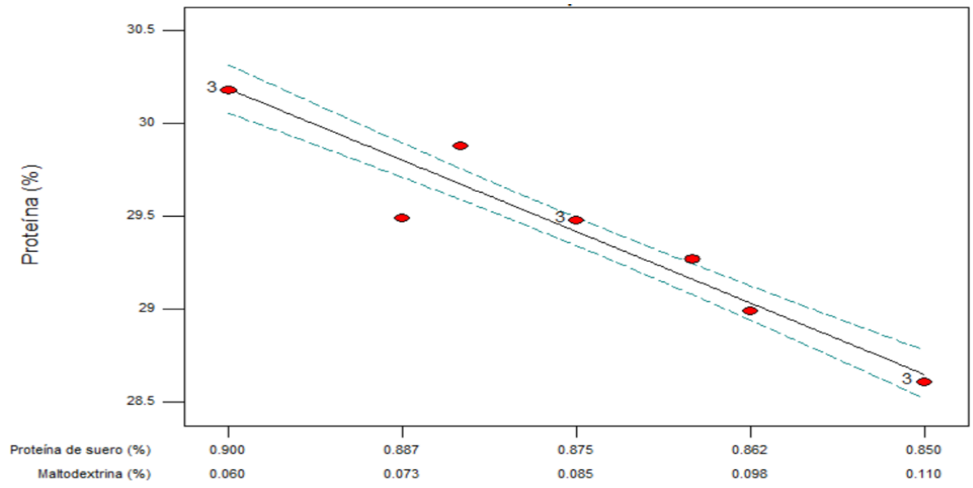
A: Whey protein

B: Maltodextrin

* Significant value for $p \leq 0{,}01$.

According to the data expressed in the table above, taking into account that A, B and F models represent significant values for $p \leq 0.01$ it is assumed that the values 28.65 and 30.19 significantly influence the formulations made. It is observed that the values of the correlation coefficient (R) are close to one, concluding that the relationship between A and B is linear, and is within the allowed range.

According to the quality indicators of the model, there is a high level of fit, since the coefficient of determination (R^2) has a value of 0.9589, which implies that the model explains about 96% of the variability in protein content. This fit is maintained, even after adjusting for model complexity (adjusted $R^2 = 0.9552$).



Graph 1. Percentage of Protein According to Formulation.

Source: Authors

The graph shows that the percentage of protein in each formulation is directly proportional to each of them, since the main ingredient represents a crucial factor that determines the amount of protein contained in the final product, and no other ingredient significantly contributes nutrients such as proteins, fats or carbohydrates, because its content is minimal.

Dissolved Solids Analysis

This research aims to develop a powder that dissolves easily in water by adding maltodextrin, due to its stabilizing properties and ability to retain fats, which are then released upon ingestion, preventing the formation of lumps when reconstituting the product.

The use of maltodextrin improves the texture and flavor of products such as protein shakes and sports drinks. It is added to many products such as protein shakes, carbohydrate drinks, and energy bars. It also promotes protein absorption and muscle glycogen recovery. Maltodextrin can help replenish muscle glycogen levels quickly, essential for recovery and physical performance (8).

For The most part, the protein content, lactose, lactic acid, and citric acid represent the whey solids.

Run	**Component 1A Protein concentrate**	**Component 2 B Maltodextrin**	**Component 3 C Organic cocoa powder**	**Component 4 D Mixture of stabilizers**	**Total dissolved solids (%)**
1	87.5%	8.5%	3%	1%	93.65%
2	87.5%	8.5%	3%	1%	93.70%
3	90%	6%	3%	1%	91.22%
4	86.7%	9.3%	3%	1%	94.69%
5	88.3%	7.7%	3%	1%	92.79%

6	85%	11%	3%	1%	96.49%
7	90%	6%	3%	1%	91.25%
8	90%	6%	3%	1%	91.24%
9	85%	11%	3%	1%	96.38%
10	88.7%	7.3%	3%	1%	92.16%
11	87.5%	8.5%	3%	1%	93.70%
12	85%	11%	3%	1%	96.26%
13	86.3%	9.7%	3%	1%	95.73%

Tabla 4. Percentage of Dissolved Solids.

Source: Authors

According to Table 4, the percentage of total dissolved solids depends on the protein and maltodextrin content, from which their functionality within this powdered product can be deduced. It is known that, due to the thermal process undergone, protein concentrates have a lower dissolution capacity compared to dairy products in their natural state. This is explained, according to several authors, by the denaturation of a protein, by the rupture of the bonds that maintain its quaternary, tertiary, and secondary structures, leaving only the primary structure. The proteins are transformed into thin, linear filaments that intertwine to form fibrous, water-insoluble compounds.

Indicator	**Total dissolved solids (%)**
A	96.39*
B AB A * B * (A-B)	91.25* -0.26* 3.75*
R^2 R^2 adjusted R^2 predicted	0.9954 0.9939 0.9887
F model	655.17*
F lack of adjustment Adequate Accuracy	13.28 59.56

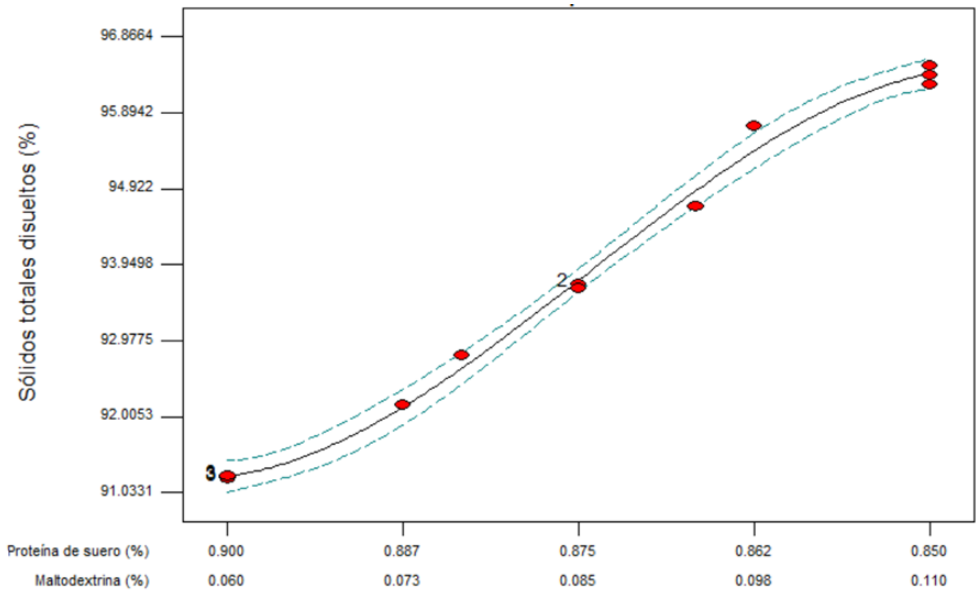
Table 5. Coded Model for Total Dissolved Solids Content (%)

A: Whey protein

B: Maltodextrin

*Significant value for $p \leq 0{,}01$.

The values obtained using the mathematical model for the analysis of dissolved solids are different; the values of A, B, AB, A*B*(A-B), and F are significant at $p \leq 0.01$; therefore, it is assumed that an important factor in the product's solubility is whey protein.



Graph 2. Percentage of Total Dissolved Solids

Source: Software Design Expert; (2024)

The results show that the sample with the best solubility is the one containing 85% protein and 11% maltodextrin; while the sample with the lowest solubility contains 90% protein and 6% maltodextrin.

Optimization

Using Design Expert software, data predictions were obtained for optimization in the production of a product with the characteristics evaluated in this research, which allowed estimating the percentage of protein content and the percentage of total dissolved solids.

Run	**Protein concentrate (%)**	**Maltodextrin (%)**	**Organic cocoa powder (%)**	**Mixture of stabilizers (%)**	**Total dissolved solids (%)**	**Total protein (%)**	**Desirability**
1	87.10	8.90	3.00	1.00	94.31	29.30	0.8903

Tabla 6. Formula Optimization.

Source: *Software Design Expert; (2024)*

The proposed formulation has a desirability value of 0.8903, which represents the geometric mean of the individual desirability values, which in turn are transformations that convert the predicted values of each response to numbers in the interval [0, 1]. If di is equal to one, it means that the corresponding predicted response Ybi takes its maximum desirable value (17), so the formula established in the optimization model demonstrates a possible maximization of the protein content of the product.

Density Analysis

The density of a powder represents the consistency of the bulk solid particles in powder form, including the spaces between them. It is essential data that determines the required size of equipment and containers within any industrial process and can also affect the functional properties of the powders themselves, especially their hydration properties.

Run	**Component 1A Protein concentrate**	**Component 2 B Maltodextrin**	**Component 3 C Organic cocoa powder**	**Component 4 D Mixture of stabilizers**	**Gross density g/cm³**	**Compacted density g/cm³**
1	87.5%	8.5%	3%	1%	3.59	4.73
2	87.5%	8.5%	3%	1%	3.59	4.73
3	90%	6%	3%	1%	3.5	4.71
4	86.7%	9.3%	3%	1%	3.68	4.86
5	88.3%	7.7%	3%	1%	3.57	4.76
6	85%	11%	3%	1%	3.81	4.86
7	90%	6%	3%	1%	3.5	4.71
8	90%	6%	3%	1%	3.5	4.71
9	85%	11%	3%	1%	3.81	4.86
10	88.7%	7,3%	3%	1%	3.97	4.89
11	87.5%	8.5%	3%	1%	3.59	4.73
12	85%	11%	3%	1%	3.81	4.86
13	86.3%	9.7%	3%	1%	3.62	4.81

Table 7. Analysis of the Bulk Density and Compacted Density of the Powder Mixture

Source: Authors

It is observed that the compacted density exceeds the raw density by just over one percentage point. Ecuadorian regulations do not consider density as a requirement for nutritional supplements, as it is a specific specification for each manufacturer. According to the table analyzed, there are no significant changes in the density of the established formulations; this is an indicator of the fluidity of this type of powder.

Microbiological Analysis

According to its classification, the Ecuadorian technical standard INEN 2983 for nutritional

supplements specifies different microbiological requirements. The product developed in this research is a type II supplement due to its content of botanical ingredients (extracts) and other nutritional ingredients.

After performing the microbiological analysis on the treatment with the highest protein content and in accordance with the requirements for nutritional supplements, the following results were obtained:

Requirement	**Unit**	**Results**	**INEN Requirements**	**Complies**	**Reference method**
Total aerobes	UFC/g	5.2 x 10^2	1 x 10^4	Yes	USP 2021, 35-NF30,2012 / REP
Molds and yeasts	UFC/g	<10	1 x 10^3	Yes	USP 2021, 35-NF30, 2012 / Petrifilm
Enterobacteriaceae	UFC/g	<10	1 x 10^2	Yes	USP 2021, 35-NF30, 2012 / Petrifilm
Salmonella spp.	UFC/g	ND	ND	Yes	USP 2022, 35-NF30, 2012 / Cualitative detection
Escherichia coli	UFC/g	ND	ND	Yes	USP 2022, 35-NF30, 2012 / Cualitative detection
Staphylococcus aureus	UFC/g	ND	ND	Yes	USP 2022, 35-NF30, 2012 / Cualitative detection

Table 8. Results And Microbiological Requirements of the Treatment with the Highest Protein Content.

Source: (*NTE INEN 2983; 2022) and (Multi Analytical Laboratory; 2024)*

When comparing the results obtained with current regulations, it was determined that the powdered protein supplement met established parameters, which constitutes a requirement for compliance with food safety regulations. It also coincides with the total aerobic and Escherichia coli values reported by Campoverde (2023) (12). This type of pathogenic microorganism represents one of the most common causes of foodborne illness worldwide and is the cause of gastrointestinal illnesses.

Sensory Analysis

The results of the sensory analysis of the reconstituted product in water are as follows:

Parameter	Percentage of Acceptability
Odor	66.92%
Color	67.11%
Taste	64.23%
Texture	66.44%
Acceptability	66.63%

Tabla 9. Acceptability of Each Parameter.

Source: Authors

According to the results in Table 9, the parameter with the highest acceptance rate was color (67.11%). This is likely due to the very important visual appearance of products, as well as their smell, which are evaluated before consumption.

Variability was found in the flavor, texture, and acceptability parameters, as these depend directly on the consumer's taste and the influence of other habits prior to the evaluation, such as smoking and alcohol consumption, among other factors.

Formula	Percentage of Acceptability
1	76.5
2	73.25
3	68.25
4	69.25
5	62.75
6	62.25
7	68
8	68.5
9	62
10	56.25
11	69.75
12	61.5

13	63.25

Tabla 10. Percentage of Acceptability of Each Sample.

Source: Authors

The results in Table 10 reveal variations in participants' acceptability of the product, with values ranging from 56.25% to 76.5%. The formulation containing 29.48% protein is the most widely accepted, reaching 76.5% acceptability.

Protein Intake

The following table shows the protein intake of each of the formulas prepared, as well as the percentage of the recommended daily value (RDV) covered by consuming 40 grams of the protein supplement. This is a reference value for commercial products with similar characteristics available on the market, since there are no regulations in Ecuador for determining it. This information is important for consumers, as it will allow them to know the dosage they should consume according to their needs (8).

Furthermore, in terms of nutritional quality, the protein concentrate has an adequate nutritional load, making it suitable for use in various food products and dietary supplements. This aspect is crucial to meet the demands of health-conscious consumers and the growing market for nutritional supplements (18).

Formula	**Protein intake in 100 g**	**Protein intake per serving (40 g)**	**Percentage VDR (50 g)**
1	29.48 g	11.80 g	23.6%
2	29.48 g	11.80 g	23.6%
3	30.18 g	12.07 g	24.14%
4	29.27 g	11.71 g	23.42%
5	29.88 g	11.95 g	23.9%
6	28.61 g	11.44 g	22.88%
7	30.18 g	12.07 g	24.14%
8	30.18 g	12.07 g	24.14%
9	28.61 g	11.44 g	22.88%
10	29.49 g	11.80 g	23.6%
11	29.48 g	11.80 g	23.6%
12	28.61 g	11.44 g	22.88%

13	28.99 g	11.60 g	23.2%

Tabla 11. Protein Intake of Each Formulation.

Source: Authors

According to Table 11, all formulations developed in this research meet the requirement of regulation 1334:3 to be considered a high-protein product, as they exceed 20% of the recommended daily value. Formulations with 12.07 grams of protein per serving and a contribution of 24.14% of the RDI constitute formulas with the highest protein content.

Similarly, it is observed that treatments with a protein content of 30.18% are close to the values reported by Campoverde (2023), who obtained a protein content of 29.8% during the physicochemical characterization of a fermented protein concentrate from cheese whey (12). Whey protein concentrate is essential in the production of bioactive peptides with significant health benefits (19).

Finally, it is recognized that whey protein concentrate (WPC) is a unique source of protein with numerous nutritional and functional values due to its high content of branched-chain amino acids (20).

Conclusions

- The whey protein powder supplement formulation with the highest protein content was obtained from 90% whey protein concentrate, 6% maltodextrin, 3% organic cocoa powder, and 1% stabilizer blend, which has 30.18% protein content and contributes 24.14% to the RDI.

- It is demonstrated that the whey protein concentrate and maltodextrin content significantly impacts the product's protein content, solubility, and acceptability.

- The density and percentage of total dissolved solids directly depend on the protein content of the formulation. Due to the molecular weight and solubility of proteins, a higher density and higher total dissolved solids result in a lower protein formulation.

- The formulation with a protein content of 29.48% had the highest acceptability rate, reaching 76.5%.

- The formulation obtained with 87.5% powdered protein concentrate, with a protein content of 29.48%, had the highest acceptability rate, at 76.5%, according to the results of the sensory analysis.

- The results of the microbiological analysis were within the range established by Standard NTE INEN 2983, which establishes the absence of pathogenic microorganisms and guarantees the safety of the finished product, since pathogenic microorganisms represent one of the main causes of foodborne illnesses and cause gastrointestinal illnesses and food poisoning.

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