PHYSICAL AND CHEMICAL PROPERTIES OF GELATIN ENRICHESERLA WITH SHELL AND SAMBO SEEDS.

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Summary

Products from Andean raw materials are desired by many people, much more when they take advantage of residues such as shell and sambo seed whose nutritional properties described are very high, it becomes necessary then to define a scheme of processes to integrate these elements. For this, a gelatin was elaborated in appropriate proportions following a process and then through physicochemical analysis where pH, protein, total ash, humidity, as well as microbiological analysis were determined, seeking to guarantee safety, quality and greater nutritional value while maintaining other desirable characteristics. in similar products. All the characteristic parameters determined from gelatine fit into the ranges established in the standard to be considered as a safe and suitable product for consumption. A product is then proposed as an alternative for not only economic development, but also the use of materials considered as waste that can be a nutritional contribution for humans and not a polluting element.

Abstract

The products made with Andean raw materials are desired by many people much more so when residues such as lupine shell and sambo seed are used. Their nutritional properties are very high. It becomes necessary to define a process scheme to integrate these elements. For this, a gelatin was elaborated in adequate proportions following a process. Then, through physicochemical analysis pH, protein, total ashes, humidity were determined, as well as microbiological analysis seeking to guarantee safety, quality and greater nutritional value while maintaining other desirable characteristics in similar products. All the characteristic parameters determined from the gelatin fit into the ranges established in the standard to be considered as a safe product and suitable for consumption. A product is then proposed as an alternative for not only economic development, but also the use of materials considered as waste that can be a nutritional contribution to humans and not a polluting element.

Keywords: Lupinus mutabilis, sambo, gelatina, properties, analysis.

INTRODUCTION

Andean crops throughout the inter-Andean zone in Ecuador are focused on rural areas (Taco-Taype & Zúñiga-Dávila, 2020), due to the climatic and altitude conditions of the place, this allows the production of a series of products with a nutritional contribution of great importance for the consumerr (Thambiraj, S. R., Phillips, M., Koyyalamudi, S. R., & Reddy, N. , 2018), in addition to becoming the livelihood of many families dedicated to the camp(Velarde, C. T., Wanderley, F., Cartagena, P., Rivero, C. P., & Carrasco, C. S. , 2021) . Recipes such as (*Lupinus mutabilis*) also called lupine, the sambo from which they extract the seeds are foods rich in nutrients, vitamins, minerals, fiber which become very desirable within the diet of those people who know their properties(Hemler, E. C., & Hu, F. B. , 2019).

From agricultural products such as legumes, vegetables, fruits, cereals, certain unusable residues are generated, such as seeds-seeds(Delgado-Paredes, G. E., Rojas-Idrogo, C., Sencie-Tarazona, Á., & Vásquez-Núñez, L. , 2014), shells, and although many of them have a number of nutritional properties(Dogan, H., ERCİŞLİ, S., andt.al., 2014), they are treated as desiccations, which generate most of the time environmental pollution instead of being used as a nutritional supplement.

Most of the population does not have a balanced diet focused on the consumption of nutritional foods, on the contrary, it consumes junk food generating a series of problems such as: malnutrition, cardiovascular problems(De Oliveira Mota, J. , Boué, G. , et.al. , 2019) , obesity, cancer(Bayat, A. , et.al. , 2014); sustained by lack of time, comfort, ignorance of nutritional valuel (Pohlmann, A. , 2021) of many Andean foods as a whole much less raw materials such as and sambo that are very easily accessible especially in the Ecuadorian highlands(Cutler, H. C., & Whitaker, T. W. , 1968).

The (Alejandrina Sotelo-Méndez, et.al., 2023), is a legume that is distinguished by its protein content, the main mineral of lupine is calcium and is present mainly in the shell of the seed hence the recommendation to consumeit without peeling, while the samb o (Mora , 2018) is a round, green, fleshy fruit called the fruit of life that occurs in the main producing regions of the Ecuadorian region very resistant to low temperaturess (Moreno-Quiroga G. , et.al. , 2023), from which the oval-shaped Sambo seed is derived, whose own color can change from light brown to dark brown depending on the fruit and its state of maturityz (Delgado-Paredes, G. E., et.al., 2014), also influencing the variety and geographical conditions where it develops, with great nutritional propertiess (Pettao, J. , 2015).

Gelatina (Teijón, J. M. , 2017), is considered a complex protein that is composed of amino acids of semisolid appearance, colorless at room temperature, but these molecular solutions with a peculiar behavior with temperature changes, being liquid in hot water, while colloidal gels solidify in cold water(Karim, A., & Bhat, R. , 2009). Its most important nutritional characteristic is the presence of a large amount of proteins: protein, mineral salts and water, depending on the degree of hydration of its precursors(Saxena, A., B.P. , et.al., 2009). Gelatin is manipulated in food production to enrich proteins, reduce carbohydrates and as a carrier of vitamins, it solidifies at room temperature, considering gelatin as a food very desired by people(Roman, J. A., et.al., 2009).

MATERIALS AND METHODS

The purpose of this research work is to obtain a product that generates in the consumer a nutritional interest for himself, from the integration of the base raw materials such as, sambo seed and gelatin, for which a scheme of processes is initially proposed as shown in figure 1, that facilitates its elaboration in addition to defining the operations and activities to be developed.

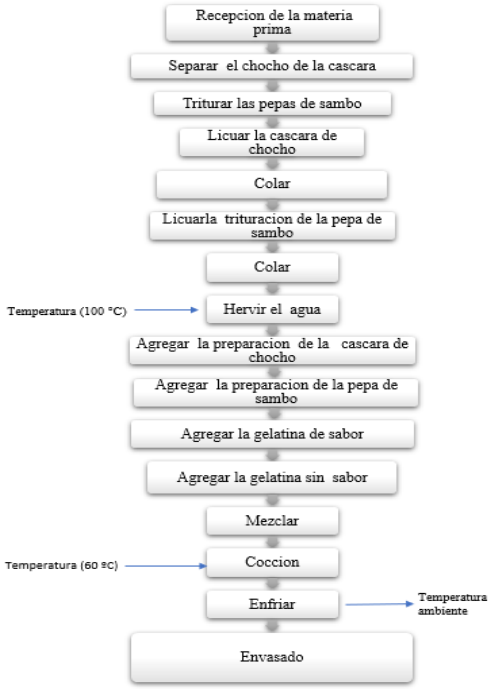

FIGURE 1. Flow diagram elaboration of gelatin enriched with shell and sambo seeds.

To establish the proportion of product to be made, it is considered as a base the amount of water will be 1.00 lt that will be considered as total volume and from it 5 portions of 61.5 ml will be obtained, defining the percentages of raw materials to be used as shown in table 1:

Table 1. Formulation of the quantities of the raw material to make the product

No.	COMPONENTS	QUANTITY (g)	PERCENTAGE %	READJUSTMENT%	QUANTITY (g)
1	Commercial Gelatin Flavor	200	15	15	150
2	Unflavored Gelatin (Moisturize after mixing)	30	2	3	30
3	Cascara De	60	4	6	60
4	Pepa De Sambo	60	4	6	60
5	Water	1000	74	70	700
	TOTAL	**1350**	**100**	**100**	**1000**

The product comes from raw materials provided by producers in the area and that through rapid sensory tests will guarantee the quality of these. The suitability of the product will be reflected in the results of the sensory analysis, hence the need to make the adjustment in terms of proportions. The analyses to be considered to guarantee the safety, quality and nutritional contribution of the product are described below:

A) PHYSICOCHEMICAL ANALYSIS

A physicochemical analysis will be carried out based on the INEN 1961:2018 number, which mentions the requirements with their respective test method as shown in table 2:

Table 2. Physical and chemical requirements for edible gelatine

Requirements	Unit	Minimal	Maximum	Test method
Humidity	%*	-	12	NTE DESCENDING 1953
Total ash	%*	-	2	NTE DESCENDING 1954
Nitrogen (N)	%*	15,5	17,9	NTE INEN-ISO 937
Protein, dry base (N x 5.55)	%*	86,02	99.3	
pH of 1 %* solution at 55 °C		3,8	7,6	NTE DESCENDING 1519
Gel strength		50°	300°	NTE DESCENDING 1955
a - Bloom: Force, expressed in grams, required to penetrate the surface of a gelatin gel by 4 mm with a cylindrical probe of standard diameter of 0,5 inches. * Fraction by mass expressed as a percentage (%).				

Source : Source: (INEN), I.E. (20 July 1993). NTE INEN 1954. Retrieved from NTE INEN 1954: https://www.normalizacion.gob.ec/buzon/normas/1954.pdf

In the case of gelatine moisture, the test method of the INEN 1953:11 engine will be used, which is suitable for edible gelatins, which tells us how to establish the moisture content and other volatile materials of edible gelatine and the test method for total ash ((INEN) I. E. (July 20, 1993) NTE INEN 1954. Retrieved from NTE INEN 1954: https://www.normalizacion.gob.ec/buzon/normas/1954.pdf)

The test method for determining total ash in edible gelatine shall be based on the standard INEN 1954:93 which gives us the appropriate guidelines for it ((INEN) I. E. (July 20, 1993) NTE INEN 1954. Retrieved from NTE INEN 1954: https://www.normalizacion.gob.ec/buzon/normas/1954.pdf).

MICROBIOLOGICAL ANALYSIS

The gelatine that will be obtained must have the following microbiological requirements according to the NTE INEN 1961: 2018 standard that mentions that, for each MO, it has a CFU / g, minimum and maximum, for which future tests will be carried out that will be based on the relevant test methods in the standard, which are described below ((INEN), I. E. (May 12, 2018). NTE INEN 1521. Retrieved from NTE INEN 1521: https://www.normalizacion.gob.ec/buzon/normas/nte_inen_1521-2.pdf).

Table 3. Microbiological requirements for edible gelatine

Requirements	Unit	Case	C	Minimal	Maximum	Test method
Staphylococcus aureus	*UFC/g	8th	1	1,0 x 10^2	1,0 x 10^4	NTE DESCENDING 1961
Molds	*UFC/g	2[b]	2	1 x 10^1	1 x 10^2	NTE INEN-ISO 21527-2
Salmonella SPP	*UFC/g	11[c]	0	Absence		NTE INEN-ISO 6579
CFU: Colony Forming Units M : limit exceeded which is rejected. c: number of admissible samples with results between m and M.						

Source: (INEN), I. E. (20 July 1993). NTE INEN 1954. Retrieved from NTE INEN 1954: https://www.normalizacion.gob.ec/buzon/normas/1954.pdf

The test methods that will be applied to the gelatin sample, when examined, must comply with the microbiological requirements as established in Table 3, in the case of *Staphylococcus aureus* and *Clostridium perfringens*, tests referring to the INEN 1961: 2018 number will be used.

The method of the test numberINEN-ISO 7937: 2013 respectively will be used , in the same way for other requirements that the standard has. From the tests that will be carried out both sensory, physical, chemical and microbiological we will have as a result a gelatin that complies with the requirements mentioned by the INEN Standards that will guarantee that the product is of quality and suitable for human consumption (Roman, J. A., De Mendonca, S. N. T. G., & Sgarbieri, V. C. , 2009).

In a total of 9 Petri dishes is preparedn 3 agar es for *Staphylococcus aureus* for this will have DNAsa agar, p ara molds will be used to gar Sabouraud Dextros and finally for salmonella SPP MacConkey agar will be used, incubation will be carried out for 48h at 45 ° C , the coloration will be identified and the colony count will be done per agar.

B) NUTRITIONAL INFORMATION

To determine the nutritional information, the bromatological analysis will be carried out considering as a basis the NTE INEN 1961: 2018 standard, which sets out the humidity and pH criteria, also establishes the specific minimum and maximum values for the determination of each component ((INEN) I. E. (May 12, 2018). NTE INEN 1521), and this can be seen in tabla 4 in a detailed and precise way.

Table 4. Chemical requirements for pure gelatine comestible (NTE INEN 1961:2018).

Requirement	Unit	Minimal	Maximum	Relevant legislation
Humidity	%	-	12	NTE DESCENDING 1953
Total Ash	%	-	2	NTE DESCENDING 1954
pH	-	3,8	7,6	NTE DESCENDING 1519

Theanalysis will be carried out with the instruments established under the standard, and individual studies of the raw materials will allow the true nutritional contribution of the final product.

RESULTS AND DISCUSSION

HUMIDITY

The determination of humidity after keeping the sample in the muffle at 110 °C for 30 min defines the P1 notation, varying the temperature inside the muffle at 105 °C for one hour and 30 minutes defines the P2 notation, and by the relationship:

% Humidity= ((P1 – P2) / m) *100

Where

P1 = Crucible weight + wet sample

P2 = Crucible weight + dry sample

m= Sample weight

It allows to define the following humidity results of the sampless as shown in Table 5:

Table 5. Humidity percentage

Sample	P1 (g)	P2 (g)	Time (g)	Humidity %
1	26,51	24,64	2,1	11
2	25,47	23,58	2,1	10

The samples analyzeds of gelatine based on husk and sambo seeds, by means of the corresponding process defines a humedad of 10% and 11%. According to the INEN 1961 standard for edible gelatin establishes that the level of moisture that must be presented in a food is a maximum of 12%, being acceptable the moisture values presented by the food.

Total ash

Thegelatin sample is placed in the muffle at a temperature of 550 ° C, for 2 h and approximately 1 h in the stove, for the calculation the INEN 1954 standard raises the relationship:

$$\%\left(\frac{m}{m}\right) = \frac{(\mathrm{m1} - \mathrm{m2})}{\mathrm{M}} * 100$$

Where,

%(m/m) = ash content as a percentage as given
m = mass of the sample, in grams
m1 = capsule mass with the final mass of the sample, in grams
m2 = mass of the empty capsule, in grams.

Table 5. Results of the calculation of % ash

Sample No.	Crucible	Samples (g)	PF+muesta	Dry matter	Ash
1	24,4	10	24,64	0,24	2,40
2	20,51	10	20,76	0,25	2,50
3	23,4	10	23,58	0,18	1,80
				Average	2,23

The average of all the treatments that were practiced was obtained 2.23% of ashes. Based on the INEN 1961 standard where it mentions that the percentage of total ash must be a maximum of 2% in gelatin, but within the analysis of the product 2.23% of ashes was obtained. This difference is due to the addition of the husk of and zambo seeds, these contain inorganic elements, which are of nutritional interest such as calcium, phosphorus, among others. Therefore, by containing very high nutritional values in the aforementioned products, a higher ash content will be obtained.

Protein

1. It should be emphasized that it was intended to obtain a product with the highest possible nutritional level, which is why the bitterness of the shell used was removed, because the in that condition raises its protein level (Dogan, H., ERCİŞLİ, S., Temim, E., Hadziabulic, A., Tosun, M., Yilmaz, S., & Zia-ul-Haq, M. , 2014), to this we add the Lupinus that has protein levels of 30 to 40% of dry weight and a large amino acid profile (Normalizacion, I. E. (May 4, 2013). NTE INEN-ISO 7937. Obtained from NTE INEN-ISO 7937:https://www.normalizacion.gob.ec/buzon/normas/nte_inen_iso_7937.pdf).

   For 1g dry sample (solid) or 2-5 g of fresh sample (liquid), the amount of copper sulphate, sodium sulphate, concentrated sulphuric acid, distilled water, 40% NaOH was introduced into the Kjeldahl digestion balloon until obtaining in the distillation balloon , with 100 ml of H3BO3 at 2.5% and 3 to 4 drops of the mixed indicator red methyl and green bromocresol, was obtained approximately 100 ml of distillate, with the formulato determine the total protein:

$$\%\boldsymbol{P} = \frac{\boldsymbol{V} * \boldsymbol{N} * \boldsymbol{F} * \mathbf{0.014}}{\boldsymbol{m}} * \mathbf{100}$$

Dwhere:

%P: protein content as % by mass

f: factor to transform %N2 into protein, and which is specific to each food.

V: volume of HCl (hydrochloric acid) or H2SO4N/10 (sulphuric acid) used to titrate the sample in ml

N1: normality of HCl (hydrochloric acid)

M: mass of the sample analysed.

The value for g elatina according to the reference table of factors for the calculation of protein from nitrogen determined analytically in food is 5.55, which, as a final result a total percentage of 88.38 of protein present in the food.

The volume of HCLat the time of titration we have 450ml applied to the distillation of the process, the normality of HCL indicates the 0.1N referential to the percentage of hydrochloric acid then the nitrogen factor of each food should be considered taken as a reference of the standard table of foods that for gelatin is 5.55, and finally the weight of the initial sample of the product was 4g giving us as a total result 88.38% of protein contained in the food.

According to the INEN 1961 standard for edible gelatin establishes that the level of protein present in foods such as gelatin, indicates that the minimum range is 86.02% and the maximum range is 99.3%, so gelatin based on husk and sambo seeds is within the range established by Ecuadorian standardization standards which is 88.38% being a level of Acceptable range of protein you should have in your food composition.

pH

Fulfilled the procedure and comparing with the scale it can be said that it is within a pH of 6, as established in NTE INEN 1519 where an optimal pH for gelatin is in the range of 3.8 -7.6, so the gelatin would be meeting the necessary requirements to be acceptable to the market.

Gelatin requires compliance with certain requirements, according to the NTE INEN 1961: 2018 standard mentions that Staphylococcus aureus, for each microorganism has a CFU / g must comply with a minimum of 1.0×10^{2} to a maximum of 1.0×10^{4} on the contrary according to (Navarro, 2015), this microorganism can survive for long periods of time in environments without humidity, its growth occurs between 35 ºC, in an optimal pH between 7.0 and 7.5 being a bacterium causing a large number of food poisonings and as a result of this nausea, diarrhea, vomiting intensely.

The weighted values of Staphylococcus aureus obtained with DNAsa Agar (PCA1) show values allowed by the NTE INEN 1961 standard in the plates labeled A, B that have $1.0 * 10^{4}$ CFU / g as a maximum value in our gelatin, therefore it is considered a safe food, however plate C does not meet requirements of The maximum and minimum values due to direct handling for its preparation, to the contamination of gelatine when it comes into contact with utensils, to storage containers that were not sterilized correctly, in addition, for not complying with cleaning and disinfection plans and protocols, therefore it is not suitable for consumer consumption.

Regarding the number of mold colonies of the variety Clostridium perfringens is a spore-forming microorganism that is located in the soil, in the water and is one of the main contaminants of food, whose consumption can cause a disease characterized by diarrhea and pain abdominal (INEN, I. E. (April 12, 2018). NTE INEN 1961. Obtained from NTE INEN 1961: https://www.normalizacion.gob.ec/buzon/normas/nte_inen_1961.pdf), they also grow abundantly at 43 – 47 °C, being resistant to heat, cold(Pettao, J. , 2015). The samples of the plates labeled B, C in a culture medium Sabouraud Dextrose Agar (PCA2) if they comply with the number of CFU / ml of Clostridium perfringens with $1.0 * 10^{4}$ CFU / g performing according to the standard NTE INEN 1961 requirements of edible gelatin, on the other hand, plate A does not meet because the moment we make our product is contaminatedor possibly by the direct contact between a raw product and a cooked product, dirty hands of the manipulators by the materials of the kitchen, by not using cooking temperatures according to the product.

Regarding the presence of Salmonella SPP ((INEN), I. E. (May 12, 2018). NTE INEN 1521. Obtained from NTE INEN 1521: https://www.normalizacion.gob.ec/buzon/normas/nte_inen_1521-2.pdf), themost frequent form of infection in humans is through contaminated water or food, causing symptoms of diarrhea, fever and abdominal cramps. For the MacConkey agar in the 3 plates there was no presence of Salmonella SPP in the gelatin, according to the NTE INEN-ISO 6579 standard tells us that it should not have the presence of Salmonella SPP therefore this product is within the appropriate parameters for subsequent consumption.

Finally, the pH of our elaborated product is 6 therefore this value indicates that it is within the requirements of the NTE INEN 1519 standard that establishes to determine the concentration of hydrogen ion (pH) in the gelatin that we rely on to evaluate the microbiological characteristics.

Under the microbiological analysis allows us to take care of the health of food consumers since we guarantee that the products they receive do not contain bacteria or microorganisms that affect their health, produce infections or poisoning.

MICROBIOLOGICAL ANALYSIS

After performing the procedure for performing the microbiological analyses, the results described in Table 6 were obtained as shown below:

Table 6. Microbiological analysis results

In order to	Color	Colonies			UFC/g		
		A	B	C	A	B	C
DNAsa	White	100	100	67	$1{,}0 * 10^4$	$1{,}0 * 10^4$	-
Sabouraud Dextrose	White	23	100	100	-	$1{,}0 * 10^4$	$1{,}0 * 10^4$
MacConkey	Yellowish white	13	10	13	-	-	-

For DNAsa Agar (PCA1) meets according to the standard LA NTE INEN 1961 plates labeled A, B that have 1.0 * 10^4 CFU / g of *Staphylococcus aureus* as a maximum value in our gelatin and plate C does not meet the number of colonies necessary for counting this may be that perhaps it did not have enough heat or was contaminated, which is normal since two correct plates were obtained.

For the agar complies according to the standard NTE INEN 1961 plates labeled B, C comply with the number of CFU / ml of *Clostridium perfringens* with 1.0 * 1 0^4 CFU / g complying with the maximum for our gelatin and plate A does not meet the number of colonies necessary for counting this may be that perhaps it did not have enough heat or was contaminated or, which is normal since two correct plates were obtained.

For MacConkey agar in the 3 plates you do not have enough colonies since all are less than 25 and you can not do the calculations saying that there is no presence of Salmonella SPP in our gelatin, so we can confirm with the INEN standard that according to the NTE INEN-ISO 6579 standard tells us that it should not have the presence of Salmonella SPP.

CONCLUSIONS

A gelatin with a high nutritional value was obtained this by the addition of the husk of, the seeds of sambo and the gelatin, raw materials that when fusedin a single element allows to have an enriched product and favorable for human consumption.

In the evaluation of the physical chemical analyzes in terms of moisture, ash, protein, pH are within the ranges established in the NTE INEN 1961 Standard, while in the microbiological analyzes the parameters established in terms of *Staphylococcus aureus* and molds were met, while in the detection of salmonella the parameters established in the standard were not met, This is possibly due to the existence of contamination in the product or the sampling was carried out incorrectly.

The importance of Andean products is reflected in the knowledge of the nutritional qualities of each of them and in the ability to take advantage of all their compositions not only from the economic aspect but also in the possibility of taking advantage of everything that could be considered as waste generating pollution.

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