



Prediction, by Mathematical Model, of the Effects of Hydration for Germination at Different Times and Temperatures on the Nutritional and Functional Characteristics of Chickpea Flour (*Cicer Arietinum* L.)

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ABSTRACT

The objective of this work was to study the effects of germination time and temperature on chickpeas by determining appropriate mathematical models corresponding to each dependent variable to predict the behaviour of the process and improve the physical, chemical, and functional properties of the flour obtained. Chickpea grains were subjected to germination processes at the laboratory, after a hydration process, to study the physical, chemical, and functional characteristics of the flour obtained from the legume. The Response Surface methodology was used by the central composite rotational layout. For the soaking process of the grains, the independent variables were considered, with their respective levels: time (6, 8, 12, 16, and 18 hours) and temperature (18, 20, 25, 30, and 32 °C), being evaluated through percent moisture as a dependent variable. During the germination process, the independent variables time (14, 24, 48, 72, and 82 hours) and germination temperatures (23, 25, 30, 35, and 37 °C) were controlled, keeping the relative humidity fixed (95%). All mathematical models determined were suitable for calculating values for each of the variables studied. The best response (R) operational R1, total protein content (TPC); R2, total lipid content (TLC); R3, total sugar content (TSC); R4, reducing sugar content (RSC) and R5, total ash content (TAC). Likewise, the following functional properties were evaluated: R6, water absorption index (WAI); R7, lipid absorption index (LAI); R8, water solubility index (WSI); R9, apparent density (APD); and finally, the presence of antinutrients was studied through R10, urease activity (UA).

Keywords: functional properties, germination effects, proximal composition, urease total, reducing sugar.

INTRODUCTION

Chickpea is a legume with a high content of proteins and fibres, and its consumption recommended due to the number of benefits it provides as a bioactive component due to the diversity of available derived products and their applications in food systems [1]. So much success is not for nothing. The legume – from the same family as lentils, peas, soybeans, and

beans – is highly nutritious and versatile, serving as the basis for several recipes. The regular consumption is relational to several health benefits: it helps control blood sugar levels, prevent heart disease, boosts digestion, and even increases satiety – a good ally for those who want to lose weight, as pointed out in a review of studies published in the American Journal of Clinical Nutrition [2]. The survey compared 21 studies and found that volunteers who consumed at least one daily portion (approximately 130g) of legumes as soon as chickpeas showed a reduction in the scale after six weeks – even without dieting! It has high protein content. It is an adequate option for those who want to reduce their consumption of protein of animal origin (such as red meat, poultry, and fish) as it provides a good supply of the nutrient – around 9 g for every 100 g of cooked grain. Food improves mood, considered by some as the “grain of happiness” it contains tryptophan, fundamental amino acid in the production of serotonin (a neurotransmitter linked to the feeling of well-being). The magnesium in legumes is a nutrient that can help relax and reduce anxiety.

The cereal is packed with iron, a mineral that helps prevent anemia and can help alleviate symptoms like fatigue and headaches. In more severe cases, these symptoms can affect cognitive function. Furthermore, it also supports brain health by effectively neutralizing the harmful effects of free radicals with its high levels of antioxidants. With this, it acts to protect the brain and stimulates its proper functioning. Legumes reduce the risk of type 2 diabetes; their low glycemic index (GI) helps control blood glucose levels and lower insulin release. Therefore, research such as that published in The American Journal of Clinical Nutrition associates the food with a reduced risk of type 2 diabetes. Its fibres are valuable. Chickpeas (especially if they are in their shells) are absorbed more slowly in the digestive system, leaving the consumer feeling fuller for longer. Fibres also improve bowel function. Strengthens the immune system like other legumes, grains are rich in zinc and vitamins A and E, fundamentals agents in combating flu and colds, ensuring reinforcement of the immune system. It maintains healthy bones and teeth; chickpeas are rich in calcium and contain generous amounts of vitamin K and phosphorus. Together, the nutrients help to strengthen bones, which can prevent fractures and diseases that affect the bones, such as osteopenia and osteoporosis. The grain acts in the prevention of cardiovascular diseases, helping to reduce triglycerides and bad cholesterol related to the obstruction of blood vessels. Furthermore, the potassium in food has a vasodilating effect, which helps to reduce blood pressure and protect heart health [3], [4], [5], [6]. India is the largest chickpea producer in the world with 11.380.000 tonnes production per year. Australia comes second with 998,231 tonnes of yearly production. With 630.000 tonnes of production per year, Turkey is the third largest producer of chickpeas [7]. Comparatively, in countries like India, the Middle East, and South American countries, chickpea consumption is not very significant. To solve the problems related to post-harvest losses of this legume, and to promote better use, numerous investigations have been carried out. Thus, for example, studies carried out showed that it is possible to develop a DIP-type product (Diversified Innovative Products), made from chickpeas and dehydrated tomatoes to promote the consumption of healthy foods, rich in vitamins, proteins, minerals, and other health benefits [8]. According to [9], the American Association of Clinical Chemistry (AACC), in 2008, defined sprouted grains as “Malted or sprouted grains containing all bran, germ and endosperm. They originate and are considered whole grains. Since the growth of the germination does not exceed the compression of the grain and the nutritional values are not lower in comparison with germinated”. To

germinate chickpeas, first wash them, soak them with double the volume of water, for 8 to 12 hours, place a damp cloth at the base of a tray, spread them out, and cover them with another damp cloth. Approximately every 8 hours, spray both the chickpeas and the cloth with water, remove them so that the humidity is uniform, and cover again [10]. The grains should be kept moist but without excess water. Repeat this operation until the 1 to 2 cm root emerges. For a seed to germinate, certain conditions must be favourable, such as an adequate amount of water, and a desirable temperature, from 10 °C the chickpea is capable of germinating, although the optimal germination temperature ranges between, 25 to 35 °C. The objective of this work was to study the effects of germination time and temperature of chickpeas by determining appropriate mathematical models corresponding to each dependent variable, to predict the behaviour of the process to improve the physical, chemical, and chemical properties functionalities of the flour obtained.

MATERIAL AND METHODS

Raw Material

Chickpea bean grains were used, produced in the city of Camaná, Peruvian origin, with good characteristics for human consumption, obtained from the *Grau Wholesale Market* of Tacna - Peru located in the enclosure of the Tacna city.

Experimental Details

Figure 1 shows the main stages and controls that were carried out during the development of the experiment.

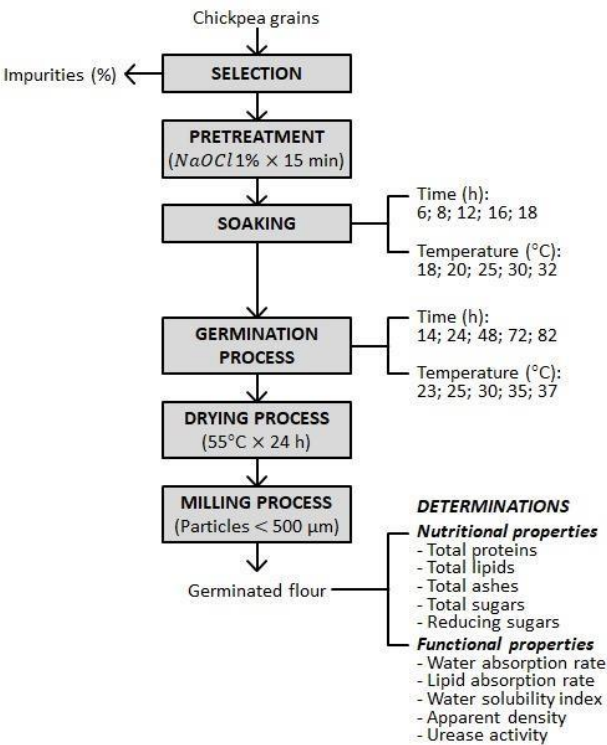


Figure 1: Main stages and controls that were carried out during the development of the experiment.

Production of Germinated Chickpea Flour in the Laboratory

Pre-treatment: Immersion of chickpea grains in 1.5% (v/v) sodium hypochlorite solution for 10 minutes to prevent the proliferation of microorganisms, mainly fungi. The grains were then washed with plenty of water to remove sodium hypochlorite residues, as suggested by [11].

Soaking or hydration: It was carried out with distilled water, for this purpose, 250 g of chickpeas were placed in a glass container with a capacity of one liter. The best hydration conditions were studied according to the experimental design described in Table 1. The variables and levels of variation were temperature and time, keeping the raw material/water ratio constant (1:3 w/v) until the grains were completely saturated.

Experimental Outline for the Soaking Process and Germination Process

Table 1 shows the variables that were studied, with the respective levels of variation. Planning 2^2 with a central point was used [12]; the variables were established at three levels coded as -1, 0, +1. The actual values were calculated according to the following equation: $x_i = (X_i - Y)/\Delta X_i$; Where: x_i = encoded value of the variable X_i ; X_i = real value of the variable; Y = real value of the variable at the centre point; ΔX_i = differential between two consecutive values. This type of planning requires two levels of axial variables coded as $-\alpha$ and $+\alpha$. The value of α is a function of the number of independent variables ($k = 2$, for the present case), which is defined by the following equation:

$$\alpha = (2^2) = x^2 + x^2 = 1.4142$$

The present statistical design requires 11 treatments, of which four are factorial (combinations between levels -1 and +1), two axial (one variable at level α and two at zero) and three central (at level zero). Which serve to estimate the experimental error and determine the precision of the polynomial equation [13]. Table 1 presents the composite central rotational experimental outline for two variables and five levels used in the soaking or hydration process of chickpea grains, and Table 2 show the effects of temperature and germination time were studied using a composite rotational central planning (star planning) of the [14] methodology.

Table 1: Levels of the independent variables to be studied during the soaking or hydration process of chickpea grains.

Variables			Variable levels		
Independent	$-\alpha = -1.4142$	-1	0	1	$+\alpha = +1.4142$
X_1	18	20	25	30	32
X_2	6	8	12	16	18

Note. X_1 , soaking temperature ($^{\circ}\text{C}$) and X_2 , soaking time (hours).

Table 2: Levels of the independent variables studied during the germination process of chickpea grains (star planning).

Variables			Variable levels		
Independent	$-\alpha = -1.4142$	-1	0	1	$+\alpha = +1.4142$
X_1	23	25	30	35	37
X_2	14	24	48	72	82

Note, X_1 , germination temperature ($^{\circ}$ C), and X_2 , germination time, (h).

For each experiment, the dependent variables indicated below were evaluated (R, response): R_1 = Total protein content (TPC); R_2 = Total lipid content (TLC); R_3 = Total sugar content (TSC); R_4 = Reducing sugar content (RSC); R_5 = Total ash content (TAC). R_6 = Water absorption index (WAI); R_7 = Lipid absorption index (LAI). R_8 = Water solubility index (WSI). R_9 = Apparent density (AD) and R_9 = Urease activity (UA).

Drying and milling: For drying, it was carried out in an oven at atmospheric pressure at 55 ° C for a period of 24 hours, until reaching an approximate 12% moisture. **Milling:** For experimental purposes and for comprehensive use, the unsprouted and sprouted chickpea grains were ground in a disc mill without removing the outer covering and the roots that came from germination. The samples were then subjected to a size reduction operation with an OSTER blender, at speeds: medium and maximum; they were then sieved using a 500- μ m mesh. Finally placed in glass jars and closed hermetically.

Data Processing and Analysis

For the analysis of the combined effects of the independent variables (X_1) and (X_2) in the evaluated responses (Y_i), the response surface methodology was used [14]. The averages of the data from each of the 11 experiments for each response were treated by multiple region analysis, to obtain first or second order mathematical models containing the linear, quadratic and product terms of the interactions. In the present case, significant effects were treated by ANOVA. In it, the degree of significance of the region and the lack of adjustment was observed, at 95% confidence, with the help of the F test, and the analysis of the coefficient of determination (R^2). The model, to be considered predictive to describe a certain characteristic in the analysed region, must present significant regression at the 95% confidence level, non-significant lack of adjustment at the same confidence level and a high R^2 value (very close to 1 or 100%). In order to identify the experimental area that would allow maximizing some properties studied, and minimizing others, according to the tendency of each of them, a new dependent variable was established, based on the models developed by [15]. The mathematical expression of this multi-response function (MRF) was:

$$\text{MRF} = \text{Superior response product} / \text{Inferior response product}$$

Proximal Composition

Moisture was determined according to method 44-15 of the American Association of Cereal Chemists [16]; protein, by the Kjeldahl micro distiller method 46-13 of the AACC (1990) using the factor 6.25; Lipids, according to the Soxhlet method (method 30 -10) AACC [16]; ashes, method 08-01 established by the AACC [16].

Total Sugars

The Sulfuric Phenol method, according to recommendations established by Dubois [17] in which glucose is used as a standard solution, determining the absorbance at 490 nm, carried them out.

Reducing Sugars

The methodology proposed by Summer & Sisler [18] was followed, 200 μ L of the samples to be analysed and 200 μ L of DNS solution were placed in test tubes with lids. For 5 minutes, the tubes were kept in a boiling water bath; immediately after finishing, the tubes were immersed in cold water (10 °C) for approximately 1 minute. 2 mL of distilled water was added to each test tube. Using a Vortex, the samples were shaken and absorbance readings were taken in a UV-Visible Spectrophotometer at a wavelength of 540 nm with respect to a blank.

Functional or Technological Properties of Germinated and Unsprouted Chickpea Flour

Water Absorption Index (WAI). The Anderson et al., [19] method, determined it.

Lipid Absorption Index (LAI). To 2 g of previously defatted sample, 20 mL of corn oil was added in 50 mL centrifuge tubes and shaken in a shaker for 5 min, at room temperature. Then it was centrifuged at 3000 rpm for 30 minutes, the supernatant was discarded and the difference between the mass of the sample before and after absorbing the oil was taken as the amount of oil absorbed. The results were expressed as oil bucks retained per sample buck [20].

Water Solubility Index (WSI). Was determined according to Anderson & Griffin [19].

Determination of apparent density (APD). To determine the APD, a beaker (container) of known volume (50 mL) was used, previously calibrated, in which each of the samples of germinated and ungerminated chickpea flour was placed. Then with the use of a ruler it was levelled, adjusting to the volume of the container, removing the excess material, no compaction was used, then the container was weighed with the sample and the result was recorded to the nearest decimal point. Finally, the apparent density was calculated using the formula $APD = m/v$, expressed in g/mL. The measurements were carried out in duplicate, according with Blandon [21]

Urease activity (UA). It was carried out through the urease test Standard NTC 771, ICONTEC (2000), soy cakes. Determination of urease. According to the Icontec NTC 771 Technical Standard, the method to determine the urease activity for soybeans and their by-products consists of measuring the pH difference between the sample solution and a blank.

RESULTS AND DISCUSSION

The physical-chemical composition of chickpea obtained as raw material was, moisture, 9.67, protein (factor 6.25), 22.23, lipids, 5.62, ashes, 3.19, crude fibre, 3.52, total carbohydrates, by difference, 55.7%, respectively. Regarding the protein content, was noted that the value obtained (22.23%) is consistent with the statements made by other researchers [23], [24], [25], [26], As visualized in the present experiment, the value is very similar to that reported by several researchers. It should also be noted that the protein content in chickpea varies significantly when the total mass of the dry grain is considered, from 17 to 22%; and when it is shelled the levels increase from 25.3% to 28.9%. In the work published by [27]. On the other hand, regardless of the varieties, cultural practices and soil and climate influences are factors that affect the variation of nutritional components.

Effect of the Independent Variables Temperature and Time on the Chickpea Soaking Process

The key process to trigger germination, once the specific environmental conditions necessary for each plant are given, is known as hydration and consists of the uptake or absorption of water by the dry tissues of the seed. The hydration reactivates the different chemical reactions of the seed metabolism involved in the development and growth of the embryo, allowing it to transform into a seedling. Soaking the seed prior to the germination process also has an effect on the reduction of non-nutritive substances [28]. The experimental results obtained in the hydration capacity during soaking of the chickpea, evaluated through moisture are shown in Table 3. A regression (Table 4) was statistically significant and useful for prediction purposes, when the value of the SMR/SM_R ratio is greater than the value of F_t . This condition is largely satisfactory in the present study. The ANOVA does not present evidence of lack of fit, as observed SM_{LOF}/SM_{PE} ($F_c = 1.86$ vs. $F_t = 19.16$) therefore it can be recommended that this equation can be used to determine values corresponding to the moisture content during chickpea hydration. Note also that, according to the analysis of the degree of significance, the linear effects of temperature (X_1) and time (X_2) significantly influenced the moisture content of the chickpea during soaking under the conditions carried out and also, due to the quadratic effect of time (X_1^2) showing greater influence the linear effect of time (X_2).

Table 3: Experimental results of the application of the variables time and temperature, including soaking final moisture and germination on the nutritional properties of chickpea flour.

Independents variables			Dependents Variables					
Trial	X1 (°C)	X2 (h)	Soaking Moisture (%)	TPC (%)	TLC(%)	TSC (%)	RSC (%)	TAC (%)
01	20	8	51.48	23.31	7.122	4.380	0.470	2.704
02	30	8	54.55	24.08	7.093	5.894	0.611	2.581
03	20	16	56.25	22.77	7.754	7.510	0.480	2.603
04	30	16	58.87	23.57	7.042	4.740	0.690	2.659
05	25	12	56.35	24.35	7.315	6.191	0.522	2.566
06	25	12	57.05	23.99	7.457	6.850	0.500	2.552
07	25	12	56.31	25.01	7.412	6.670	0.540	2.574
08	18	12	55.25	22.49	7.231	7.230	0.510	2.751
09	25	18	56.79	24.25	7.591	6.350	0.570	2.480
10	32	12	57.68	24.97	7.028	5.880	0.740	2.610
11	25	6	49.92	23.20	7.354	4.050	0.430	2.599
Average response			55.50	23.768	7.309	5.977	0.555	2.607
Unsprouted flour				20.870	6.612	9.351	0.428	3.129
Sprouted flour (VMR)				25.613	7.692	6.914	0.789	2.582

Note. X1, soaking and germination temperature (° C), and X2, soaking and germination time (h)

Table 4: Regression model, level of significance, and analysis of variance corresponding to the experimental results of the chickpea soaking process.

Regression model				
$Y = 56.57 + 1.14x_1 + 2.35x_2 - 0.04x_1^2 - 1.51x_2^2 - 0.11x_1x_2$				
(0.24) (0.29) (0.35) (0.29) (0.35) (0.41)				

Effect of the independent variables	Level of Significance (P < 0.05)				
Interception	0.000018*				
Line					
X1	0.016231*				
X2	0.003895*				
Quadratic					
X_1^2	0.8332251				
X_2^2	0.013133*				
Interaction					
X_1X_2	0.642913				
Analysis of variance corresponding to the quadratic model					
Average response: 56.5700 Determination Coefficient (R²): 0.9813					
Standard deviation: 0.51286 Variation Coefficient (VC = %): 0.0091					
Variation	SS	FD	MS	Fc	Ft
Regression	69.06357	5	13.81271	52.51	5.05
Residue	1.31513	5	0.263026		
Lack of fit (LOF)	0.96873	3	0.32291	1.86	19.16
Error	0.3464	2	0.1732		
Total	70.3788	10			
% of Variation Explained = 98.13 Maximum % Explainable Variation = 99.51					

Note. Y_i, regression model, errors: minor letter between (parentheses); (*), significant at 95% confidence level; X₁, soaking temperature, (°C); X₂, soaking time, (h); R², determination coefficient; VC, variation coefficient; FD, freedom degrees; SS, squares sum; MS, mean square; Fc, F calculated; Ft, F tabular.

In the quadratic model, it was observed that the moisture content increases proportionally to the temperature and soaking time, reaching a maximum moisture value of 58.00% at a temperature of 28°C and a soaking time of 15 hours. In this way, the parameters studied, which showed greater efficiency during the soaking operation, were established as temperature of 28 °C and time of 15 hours (Fig. 2j). Noting that at these levels, the opening of the intercellular spaces is probably favoured, allowing a greater intake of water, necessary for the start of germination, note that, if the soaking temperature is increased to 32 °C, the time is reduced to 12 hours.

Effect of Independent Variables: Temperature and Time on Nutritional Properties during the Chickpea Germination Process

Table 3 shows the results of the experimental values corresponding to the nutritional analyses: Total protein content (TPC), Total lipid content (TLC). Total sugar content (TSC), reducing sugar content (RSC) and Total ash content (TAC). These results correspond to each of the chickpea treatments subjected to the germination process in accordance with the experimental design planned based on preliminary tests. Table 5 shows the Regression Models and significance levels of the variables in the nutritional properties of sprouted chickpea flour.

Table 5: Regression models and significance levels of the variables in the nutritional properties of sprouted chickpea flour.

Variables	Averages	Standard deviation	R ²		CV (%)
TPC	23.768	0.4432	0.9744		1.8649
TLC	7.3090	0.0985	0.9119		1.3476
TSC	5.9770	0.7814	0.9536		13.0734
SRC	0.5551	0.0218	0.9738		3.9272
TAC	2.607	0.0806	0.8826		3.0932
Responses Quadratic Regression Models					
TPC Y ₁ = 24.2767 + 0.7865x ₁ - 0.0268x ₂ - 0.5508x ₁ ² - 0.11483x ₂ ² + 0.4525x ₁ x ₂ (0.150) (0.092) (0.092) (0.109) (0.109) (0.130)					
TLC Y ₂ = 7.3948 - 0.1286x ₁ + 0.1145x ₂ - 0.1447x ₁ ² + 0.0267x ₂ ² - 0.1709x ₁ x ₂ (0.021) (0.026) (0.026) (0.030) (0.030) (0.036)					
TSC Y ₃ = 6.5703 - 0.3956x ₁ + 0.6536x ₂ - 0.0693x ₁ ² - 0.7468x ₂ ² - 1.07x ₁ x ₂ (0.196) (0.120) (0.120) (0.143) (0.143) (0.170)					
SRC Y ₄ = 0.5206 + 0.0845x ₁ + 0.0358x ₂ + 0.0522x ₁ ² - 0.0103x ₂ ² + 0.0173x ₁ x ₂ (0.012)(0.007) (0.007) (0.084) (0.084) (0.010)					
TAC Y ₅ = 2.5641 - 0.03334x ₁ - 0.0238x ₂ + 0.6492x ₁ ² - 0.0573x ₂ ² + 0.0444x ₁ x ₂ (0.006) (0.004) (0.005) (0.004) (0.005) (0.006)					
Variables Independents	Signification levels (P <0.05)				
	TPC	TLC	TSC	SRC	TAC
Intersection	0.000038*	0.000032*	0.000895*	0.000493*	0.00006*
Line					
X ₁	0.013472*	0.037376*	0.081483	0.006947*	0.013716*
X ₂	0.798675	0.046489*	0.032313*	0.036841*	0.026394*
Quadratic					
X ₁ ²	0.037488*	0.041508*	0.676551	0.025077*	0.005187*
X ₂ ²	0.309134	0.472187	0.34921*	0.347303	0.346341
Interaction					
X ₁ X ₂	0.073968	0.042014*	0.24366*	0.227187	0.015414*

Y_y = Regression models; experimental errors placed in (parentheses); TPC, total protein content; TLC, total lipid content; TSC, total sugar content; RSC, reducing sugar content; TAC, total ash content; R_2 , determination coefficient; VC, variation coefficient; P, probability; X_1 , germination temperature, ($^{\circ}$ C); X_2 , germination time (h); (*), significant at the 95% confidence level; N.S., not significant.

Numerous studies have shown that germination improves the nutritional quality of flours obtained from legumes; however, the increase or decrease of biomolecules and nutritional compounds will depend on the type of legume treated as well as the germination conditions, mainly time and temperature. Generally, germination increases protein digestibility, the bioavailability of amino acids, and reduces anti-nutritional factors such as saponins, alkaloids, polyphenols, alpha-galactosides, phytates, and trypsin inhibitors [28], [1], [30], [9].

Based on the experimental results (Table 6), the quadratic regression model was established, observing by the analysis of variance that the equation is significant at a 95% confidence level. Through the analysis of variance, it is observed that the coefficient of determination (R^2 =

0.9744) shows a good fit to the experimental data. The SMR/SMr ratio ($F_c = 38.11$ vs. $F_t = 5.05$) at the 95% confidence level. Which indicates that it is highly significant [31]; the latter pointed out that for a model to be valid and used for predictive purposes; the region must consider that the value of F_c is greater than F_t .

On the other hand, the SM_{LOF}/SM_{PE} relationship ($F_c = 1.86$ vs. $F_t = 19.16$) shows that the model does not present a lack of fit, which indicates that the quadratic model is adequate to describe the response surface in the research region.

According to the significance analysis (Table 5), it is observed that the total protein content (TPC) of the sprouted chickpea flour was significantly influenced by the linear (X_1) and quadratic (X_1^2) effects of the temperature of germination, noting that the linear effect of temperature (X_1) was the variable that had the greatest influence.

Figure 4a represents the response surface diagram showing the effect of temperature and chickpea germination time on total protein content (TPC). The highest value of total proteins (24.495%) in sprouted chickpea flour is reached at a temperature of 32 °C and a germination time of approximately 60 hours.

The protein content increased in all treatments subjected to germination, between values of 22.49 and 24.35%, compared to 20.87% corresponding to the ungerminated chickpea flour. Through the mathematical model obtained, it is indicated that, theoretically, it is possible to reach values of 24.5%, which would show that the germination process increased the level of total proteins by 17.37% in the chickpea flour.

These results agree with the majority of values reported in the literature and are consistent with the observations indicated by other researchers. Thus, [30] also found higher values in the protein content in sprouted wheat flour 21.1%; indicating that these increases are attributed to the loss of dry matter, mainly in the form of carbohydrates, due to grain respiration, and to the changes that occurred in nitrogenous substances during the germination process. Meanwhile, studies carried out by [32] reported protein values present in ungerminated seeds. For amaranth (12.52%); quinoa (14.76%); soybean (37.71%) and pigeon pea (18.83%), and that after the germination process, a significant increase in crude protein was generated in amaranth seeds (14.25%); soybeans (38.73%); and pigeon pea (20.83%); while in quinoa a slight decrease (14.68%) was observed on the third day of germination. The tendency to increase the concentration of crude protein observed in amaranth, pigeon pea and soybean seeds agrees with the findings found by other researchers, [32] studied the protein content in soybean, lupine and black bean seeds, and found an increase in protein concentration when they were subjected to the germination process. Ramos-Pacheco [28], studied three varieties of quinoa from the Andean Region of Peru, the results showed colour variations and significant increases, in proteins, fibre, minerals, phenolic compounds, flavonoids, and antioxidant capacity after 72h of germination, attributed to the activation of enzymatic pathways. Jiménez, [33] investigating the effects of germination in soybean seeds for five days. Noted an increase in the protein content of the seed throughout the germination period, stating that there are different factors that can contribute to variations in the protein content of the seed throughout

the germination period. Among these factors are, processing type temperature and germination time, method of germination, species/varieties, soaking time and germination percentage.

Table 6: Regression model, significance level, and analysis of variance corresponding to the effect of chickpea germination on total protein content.

Regression model					
$Y_1 = 24.2767 + 0.7865x_1 - 0.0268x_2 - 0.5508x_1^2 - 0.11483x_2^2 + 0.4525x_1x_2$ (0.150) (0.092) (0.092) (0.109) (0.109) (0.130)					
Effect of independent variables			Level of Significance (P < 0.05)		
Interception			0.000038*		
Line					
X ₁			0.013472*		
X ₂			0.798675		
Quadratic					
X ₁ ²			0.037488*		
X ₂ ²			0.309134		
Interaction					
X ₁ X ₂			0.073968		
Analysis of variance corresponding to the quadratic model					
Average response: 23.7664		Determination Coefficient (R ²): 0.9744			
Standard deviation: 0.4432		Variation coefficient (CV = %): 1.8649			
Variation	SS	FD	SS	Fc	Ft
Regression	7.487318	5	1.497464	38.11	5.05
Residue	0.196446	5	0.039289		
Lack of fit	0.060379	3	0.020126	0.29	19.16
Error	0.136067	2	0.068033		
Total	7.683764	10			
% Variation Explained, 97.44					
Maximum % Explainable Variation = 98.23					

Note: Y₁, region model; errors: minor letter between (parentheses); (*), significant at 95% confidence level X₁, germination temperature, (° C); X₂, germination time, (h); R², determination coefficient; VC = variation coefficient; FD, = freedom degrees; SS, sum of squares; MS = mean square; Fc = F calculated; Ft = F tabular.

In Figure 2, the response surfaces of different effects on physical and functional properties during germination process of chickpea are showed.

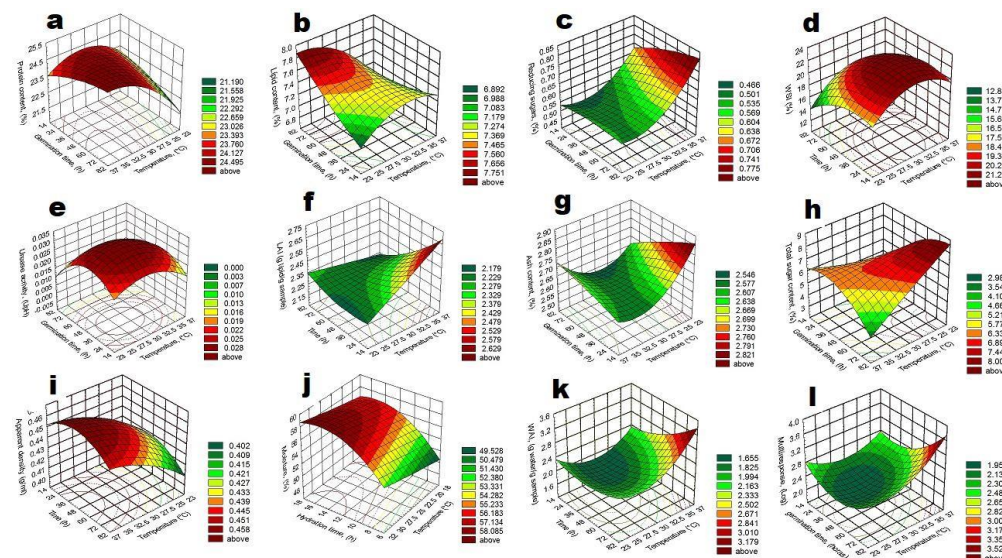


Figure 2: Response surface diagram showing the different effect on physical and functional properties during germination process of chickpea. a, effect of germination time and temperature on protein content; b, on lipids content; c, on of reducing sugars; d, on water solubility index; e, on urease activity; f, on lipid absorption index; g, on ash content; h, on total sugar content; i, on apparent density; j, on moisture content; k, on water absorption index; l, on multi-response, respectively.

Figure 3 shows photographs of the germinated grains under the different hydration time and temperature conditions established in the experiment. It was possible to observe in the different photographs the differences in the degree of growth of integument part of the grains during germination. Each figure indicated with the time and temperature to which they were subjected.

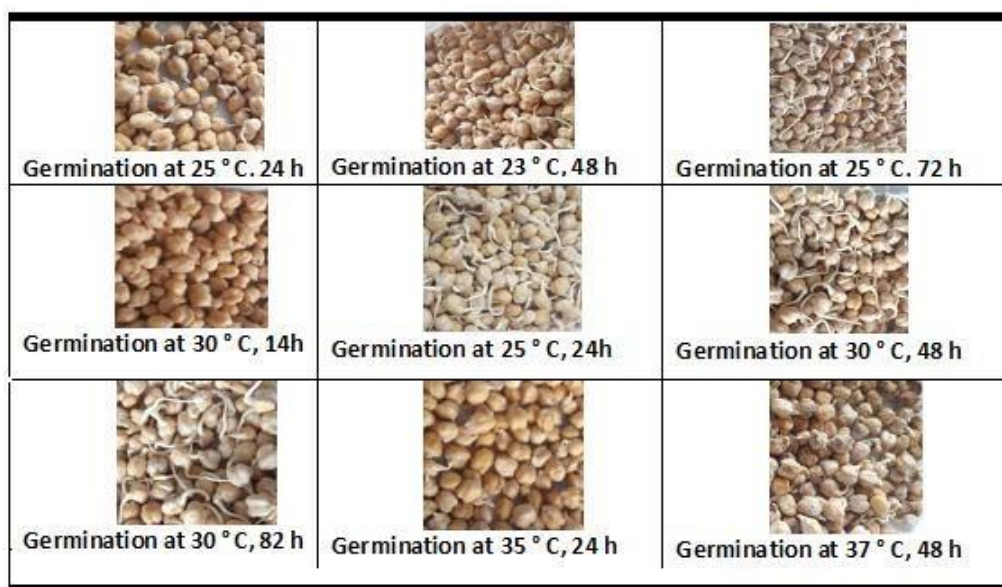


Figure 3: Germination process showing pictures the effect at different temperature, and hydration time of the chickpea grains.

Regarding the improvement of quality in relation to legume antinutrients, properly germinated chickpeas imply a decrease in the content of phytates and phytic acids during the germination of various legume seeds may result from a significant increase in phytase activity. This can be explained by the fact that sprouting is mainly a catabolic process that provides important nutrients to the growing plant through the hydrolysis of reserve nutrients [1], the same author concluded that the research results prove that the traditional methods of processing beans can be beneficial in improving the nutritional values of chickpeas. The best seed treatment method is germination and boiling. Compared to other traditional processing methods, these methods are more effective in suppressing the presence of antinutrients in chickpeas.

According with [34] treated the chickpea as follows: roasted, soaked, [soaked + boiled], [soaked + steamed], and germinated. Looking at the gain in protein values, they did not show great differences between the treatments. However, when verifying with reference to the anti-nutritional compounds, mainly phytic acid and trypsin inhibitor, differences were noted between the different treatments; those germinated for 24 h reached 7.87 and those germinated for 48 h reached 10.40 mg/g, respectively. Regarding the values of phytic acid, this work found 14.66 for the raw material and 9.18 and 9.26 mg/g for [soaked + boiled] and [soaked + steamed], respectively.

In relation to lipids (Fig. 2b), it was possible to verify that the level found in the present research work is 5.62%, which is closely related to what was stated by [35] [24], [27]; and [36], the same ones that reported values of 3.0%, 5.0%, 3.40%, and 6.30%, respectively. Chickpea has a greater amount of lipids than other legumes; the total lipid concentration ranges between 6.04% and 6.04%. relation to lipids (Fig. 2b), it was possible to verify that the level found in the present research work is 5.62%, which is closely related to what was stated by [35]); [24]; [27] and [26], the same ones that reported values of 3.0%, 5.0%, 3.40%, and 6.30%, respectively. However, it is superior to lentils and beans; although less than soy, 66% are polyunsaturated fatty acids, 19% are monounsaturated, and 15% are saturated. Thus, because it is a legume, it constitutes a good source of polyunsaturated fatty acids, such as linoleic and oleic acids, with a higher content than other legumes.

Regarding the 55.77% carbohydrate content obtained in the present work, it shows a lot of similarity with the values reported by many researchers. Thus, for example, songs that vary have been found in literature. According to the Organization of Consumers and Users [37] the carbohydrate level in chickpeas is 50.70%; while [38] reported a value of 57.8%; On the other hand, [27] published a value of 66.0%. All this shows us that the value obtained in the present investigation is no different and is found in the values published by other authors.

Regarding the content of ash, (Fig. 2g) the value of 3.19% obtained is similar to that shown in the work by [39] on the composition of ash using commercial chickpea varieties, *costa-2004* and *blanoro*, values statistically significant differences between both varieties. The results were 3.0% for the *costa-2004* variety and 2.8% for the *blanoro* variety. However, these results can be attributed to the interaction of genotypes and the environment itself, it is said, the cultivar type, soil where it grew and the management conditions for its development, and climatic conditions as expressed by [40]

Just as other researchers such as [41] mention a value of 3.0%, [27] reported a rate of 2.91%, while [41] observe rates of 2.5% to 3.1%. Which indicates the existing agreement with the value obtained.

Regarding the content of raw or raw fiber and the value of 3.52 observed in the present investigation, it could be inferred that this is related to the references of other authors. The report values between 3.9 and 4.5% [41]; while indicate a value of 3.0%. All values are within the limits of variation allowed for chickpeas. The differentiation observed by each of the authors when referring to the physical-chemical or proximal composition of this legume shows that there may not always be precise agreements between researchers. The possible sources of variations are the chosen analysis method, the product variation, the edaphic-climatic situation, the maturity level, and the part of the product chosen to perform the analysis.

Figure 2c shows the response surface diagram showing the effect of temperature and germination time on the chickpea on the total sugar content (TSC). The highest value of total sugars in flour chickpea germinated corresponds to 8.006%. This value was reached at temperature (25°C) for a germination time (72 hours), approximately.

The total sugar content decreased in all treatments subjected to germination, between values: 4.05% and 7.510% compared to 9.351% corresponding to flour chickpea without germinating. The value of 9.351% of total sugars is close to that reported by [43],[44] which will find a value of $6.98\% \pm 0.9$; other authors USDA Food data (2019) showed a content of 10.7% of total sugars for chickpea. Using the mathematical model obtained, it is possible to reach values of 8.006%, as shown in figure 2h, and this would indicate that the germination process caused a decrease of 14.38% in the level of total sugars, when compared to the product without germinating. Our results are in full agreement with results reported in other research works. An experiment carried out by [44], indicates that during the germination of legumes, there is a marked decrease in the grain content of grains, due to their degradation in sugar; the ones that don't accumulate; bell that are used throughout the breathing process for the production of energy and for the synthesis of other complex molecules. If the vegetables remain submerged in water for several hours, the skin membranes disintegrate and release oligosaccharides. It was observed by [45] that, during the germination of wheat, there is a degradation of the starch in maltooligosaccharides and that the total sugars in the flour samples decreased after the germination period of four days (96 hours), due to the activity of alpha amylase.

Figure 2g presents the response surface diagram, which shows the effect of temperature and chickpea germination time on the total ash content (TAC). The value corresponds to 2.821%. Which is reached with a temperature of 23 °C, for a germination time of approximately 24 hours. The total ash content was lower in all treatments subjected to germination processes, fluctuating between values, 2.480% and 2.750% compared to 3.129% corresponding to ungerminated chickpea flour. If to consider that theoretically using the mathematical model a value of 2.821% is obtained, compared to 3.129 in ungerminated product, can be concluded that the ash content, due to the effect of germination, suffered a decrease of 9.84%. According to [46], during wheat germination, a decrease in total ash content was evident, which can be attributed to losses during maceration and washing. These researchers also report that the

quantitative changes of an individual mineral and its chemical form during germination can be important and contribute to the variation of total ash content. In legumes such as chickpeas, ashes represent an important source of essential minerals for the human body, among them, and can be mention the Ca, Fe and Zn. [47], found a decrease in Fe (30-60%) and Ca (8-12%) in wheat germinated for 48 and 96 hours. During the evaluation of minerals in germinated wheat, found a decrease in Fe and Mg until the third day of germination, increasing again from the fourth day of germination [48]. The authors do not explain the reason for this behaviour. Cowpea germination led to a decrease in Ca (5.1%) and Fe (3.5%), however, they were not statistically significant. The decreases that occurred in Ca and Fe may be due to their use for shoot growth or losses in water during maceration [49]. The studied the ash content and concluded that it varies depending on the sources consulted, due to the influence of the variety, particle size and the method of obtaining the flour, since the minerals are found mainly in the outer layers of the grain [50]. Legumes contain digestible and indigestible sugars. In the raw chickpea, free reducing sugars (monosaccharides) such as glucose and galactose have been reported at levels of 700 and 50 mg/100g, respectively. In the determination of the monosaccharides quantified for the hull chickpea, the majority of components are arabinose and galactose, which indicates the presence of arabinogalactans and rhamnogalacturonans, which are components of pectin. Figure 2c presents the response surface diagram showing the effect of temperature and germination time of the chickpea on the reducing sugar content (RSC). It is noted that the highest value of reducing sugars in germinated chickpea flour corresponds to 0.775%. This value is reached at a temperature of 37°C for a germination time of approximately 60 hours.

The content of reducing sugars was increased in all treatments carried out during germination processes, between 0.430% and 0.740%, compared to 0.428% corresponding to chickpea flour without germinating. After reaching a concentration of 0.775% with the application of the model and with 0.428% of the value contained in the chickpea without germinating, it is concluded that, in general terms, the reducing sugar content increased by 81.07% in relation to the chickpea flour not germinated. These results are in agreement with those reported in other research works. Every time it was demonstrated by [51], that the grains germination and cereals cause an increase in enzyme activity, causing losses in the total dry matter, changes in the amino acid's composition, a decrease in starch and an increase in sugars, which are hydrolysed into simpler sugars, generally reducing sugars. On the other hand, [45] carried out studies on the hydrolysis of starch in malt oligosaccharides during the germination process, noting that the reducing sugars increased in the flours, during the germination process, which was attributed to the increase in the rate of movement of soluble carbohydrates in the endosperm of legumes. Was observed considerable increases in free sugars [52]. Reducing sugars during wheat germination, postulating that starch grains are gradually degraded to levels of reducing sugars. Likewise, [46] also reported increases in reducing sugar levels during wheat germination in the laboratory, saying the effect is due to the degradation of the starch.

Effect of Independent Variables (Temperature and Time) On the Functional and Antinutritional Properties of Sprouted Chickpea Flour

Table 7 shows the results of the functional and anti-nutritional characteristics, corresponding to each of the treatments, of two chickpea grains subjected to germination. It is understood that

the term functional property refers to any nonnutritional property of an ingredient that mainly affects the sensory character of a food, according to [53]. The functional characteristics analysed were water absorption index (WAI), lipid absorption index (LAI), water solubility index (WSI), and apparent density (APD). Regarding anti-nutritional factors, the presence of trypsin inhibitors was investigated, with the latter evaluated through the urease test. Table 8 shows the regional models and significance levels of the variables and functional and anti-nutritional properties of sprouted chickpea flour.

Table 7: Effect of variables, temperature, and germination time on the functional and anti-nutritional properties of chickpea flour.

Trial	Variables		WAI	LAI	Variables		UA
	Independents				Dependents		
	X ₁	X ₂	WAI	LAI	WAI	APD	UA
	(°C)	(hours)	(gH ₂ O/g)	(g lip/g)	(%)	(g/mL)	(ΔpH)
01	-1	-1	1.640	2.179	20.240	0.424	0.030
02	+1	-1	1.650	2.496	20.920	0.459	0.020
03	-1	+1	2.070	2.215	16.350	0.412	0.020
04	+1	+1	2.639	2.212	18.210	0.452	0.010
05	0	0	1.710	2.252	22.540	0.450	0.030
06	0	0	1.541	2.223	20.890	0.455	0.030
07	0	0	1.620	2.270	21.430	0.446	0.030
08	-1,4142	0	1.854	2.181	16.010	0.425	0.020
09	0	+1,4142	2.097	2.172	17.660	0.433	0.010
10	+1,4142	0	2.583	2.329	19.740	0.460	0.020
11	0	-1,4142	1.666	2.455	20.840	0.435	0.020
Averages			1.915	2.271	19.530	0.360	0.022
Unsprouted chickpea flour			1.492	2.059	27.778	0.494	0.070
Sprouted flour (MRV)			2.719	2.582	21.212	0.456	0.000

X₁, germination temperature (°C); X₂, germination time (hours); WAI, water absorption index (g H₂O/g flour); LAI, lipid absorption index (g oil/g flour); WSI, water solubility index (%); APD, Apparent density (g/mL); UA, Urease activity (ΔpH); MRV, multi-response variable.

Table 8: Regression Models and significance levels of the independent variables on the functional and antinutritional properties of sprouted chickpea flour.

Variables	Averages	Standard deviation	R ²	VC (%)
WAI	1.9150	0.3561	0.9159	18.5931
LAI	2.2713	0.0333	0.9551	1.4661
WSI	19.5300	1.7814	0.9317	9.1213
APD	0.3605	0.0324	0.9000	8.9931
UA	0.0220	0.0084	0.8842	38.1818
Answers Quadratic Regression Models				
WAI	Y ₆ = 1.6237 + 0.2011x ₁ + 0.2535x ₂ + 0.2849x ₁ ² + 0.1162x ₂ ² + 0.1400x ₁ x ₂ (0.048) (0.029) (0.029) (0.035) (0.035) (0.042)			
LAI	Y ₇ = 2.2483 + 0.0654x ₁ - 0.0810x ₂ + 0.0011x ₁ ² + 0.0304x ₂ ² - 0.0800x ₁ x ₂ (0.014) (0.008) (0.008) (0.010) (0.010) (0.011)			
WSI	Y ₈ = 21.6200 + 0.9769x ₁ - 1.3872x ₂ - 1.7807x ₁ ² - 1.0931x ₂ ² + 0.2950x ₁ x ₂			

		(0.486)	(0.297)	(0.297)	(0.354)	(0.354) (0.421)
APD	$Y_9 =$	$0.4503 + 0.0156x_1 + 0.0027x_2 + 0.0043x_1^2 - 0.0854x_2^2 + 0.01250x_1x_2$	(0.0026)	(0.0016)	(0.0016)	(0.0019) (0.0019) (0.0022)
UA	$Y_{10} =$	$0.0367 - 0.0025x_1 - 0.0043x_2 - 0.0047x_1^2 - 0.0072x_2^2 + 0.0000x_1x_2$	(0.0066)	(0.0004)	(0.0004)	(0.0005) (0.0005) (0.0006)
Independents	Levels of significance (P < 0.05)					
Variables		WAI	LAI	WSI	APD	UA
Interception		0.000897*	0.000037*	0.000504*	0.000033*	0.000422*
Linear						
X_1		0.013541*	0.016040*	0.081525	0.010332*	0.025645*
X_2		0.003895*	0.010541*	0.043028*	0.229121	0.009027*
Quadratic						
X_1^2		0.015155*	0.918650	0.037327*	0.0115214	0.010484*
X_2^2		0.081901	0.092959	0.090819	0.045975*	0.004513*
Interaction						
X_1X_2		0.079931	0.021255*	0.555712	0.635014	1.000000

Y_6 , Region models; experimental errors placed in (parentheses); WAI, water absorption index; LAI, lipid absorption index; WSI, water solubility index; APD, apparent density; UA, ureases activity; R_2 , determination coefficient; VC, variation coefficient; P, probability; X_1 = germination temperature (°C); X_2 = germination time (hours); (*) = significant at the 95% confidence level; N.S. = not significant.

All the treatments tested during the germination process caused increases in the WAI values (1,541–2,583 g H₂O/g germinated chickpea flour) in comparison with the ungerminated chickpea flour, which presented a value of 1,492 g H₂O/g ungerminated chickpea flour. As already indicated in Figure 23, using the quadratic model, it is possible to reach WAI values of 3.179%. Which would show that, during the germination process, the water absorption rate was increased by 113.04%.

It was postulated that the water absorption rate of flours in general is influenced by two main factors. The constituents of the flour and the particle size. Reducing particle size improves water absorption by increasing the surface area of particles in contact with liquid, [54]. The most important constituents of legume flours that affect water absorption are starch grains. Intact grains can absorb up to 35% of their weight. During the germination and milling process, the breakdown of starch grains occurs, and at the same time, a considerable hydrolysis of the constituents of the chickpea grain, mainly starch, transforms into dextrin and other simple constituents with a greater number of OH- groups available that confer greater water absorption capacity [55].

Some researchers [56], have indicated that it is very difficult to compare WAI values in different types of flour since they depend on the experimental conditions, such as temperature, time, and centrifugation speed, as well as the way of preparing the sample, especially the milling type and drying process. Depending on the type of mill and its handling, there may be a greater or lesser number of modified starch particles; this fragile starchy structure allows a greater degree of water absorption. Certain mills, with loads slightly higher than the nominal value, produce excessive heating of the material during grinding, thus modifying the structure, mainly of the starch.

Water absorption capacity is important in products that require instant hydration and a rise in viscosity, such as preserving freshness in baked foods or meat products. Therefore, a possible inclusion of sprouted chickpea flour would not only improve the nutritional values but could also impart important technological properties, for example, in baking products, maintaining the soft texture. The hydration of flours is reflected in their capacity to absorb water until they form a viscoelastic mass, which is dependent on protein content, the presence of damaged starch granules, and particle size [57].

On the other hand, in reference to the lipid absorption index (LAI), it is important in the formulation of bakery products, soups, meat products, meat substitutes, which is also related to the ability to retain the flavor characteristics and the softness of the product to which it is added [32].

After establishing the mathematical model and corresponding ANOVA with R^2 , 0.9551, it is observed that the LAI of sprouted chickpea flour was significantly influenced by the linear effects of temperature (X_1), germination time (X_2), and the effect of the product of the interactions (X_1X_2), with germination time (X_2) being the most influential factor. Figure 2f depicts the response surface diagram for chickpea germination temperature and time on the lipid absorption index (LAI). It can be clearly seen that the highest LAI value corresponds to 2.629 g lipid/g sprouted chickpea flour, which is reached at a temperature of 36 °C and a germination time of 14 hours.

Figure 2d shows the response surface diagram corresponding to the quadratic model, which establishes the variations of the water solubility index (ISA) of sprouted chickpea flour as a function of temperature (X_1) and germination time (X_2). The highest WSI value (21.22%) was reached with average temperature (31°C) and germination time (36 hours). All the WSI results corresponding to the sprouted flour were lower than those observed in the non-sprouted chickpea, which presented a value of 27.8%. This fact probably occurs because of the soaking, which would end up extracting most of the soluble material, such as sugars, proteins, especially albumin, and amino acids. The results of WSI agree with the results presented by [58], indicating that during the analysis of sangurache flour (*Amarantus hybridus* L.) germinated for 48 hours, lower values of 15.56% were obtained compared to 17.70% for non-germinated amaranth. However, the results do not show a statistically significant difference between the treatments. The results are consistent with the observations made by other researchers regarding WSI; for example, was reported results [59] that show increases of 200% in WSI of ungerminated green bean (*Macrotyloma uniflorum*) flour. However, after the germination process lasted between 24 and 48 hours, these values increased from 210% to 216%, respectively. Studies carried out by [46] reported that the WSI of sprouted wheat flours increased significantly compared to non-germinated wheat flours. According to this researcher, this fact is attributed to the hydrolysis of peptide bonds and the consequent increase in free amino groups due to the increase in proteolytic activity and the degradation of starch by hydrolytic enzymes.

Regarding the apparent density (APD), all determinations were lower in the sprouted chickpea flours. Varying between values of 0.460 g/mL and 0.412 g/mL when compared to the

ungerminated product, whose value was 0.494 g/mL. This probably occurred due to alterations in the chemical composition during germination, resulting in particles with different densities. The explanation for this fact is attributed to the reduction in size of the individual particles that make up the flour.

Using the quadratic model and as observed in Figure 2i, a value of 0.458 g/mL is reached, which leads to the conclusion that, during chickpea germination, the apparent density decreased by 7.287%. The effect of independent variables on urease activity (UA) during the chickpea germination process is considered an important aspect that raises the possibility that legumes contain anti-nutritional factors, among them trypsin inhibitors. Similarly, the determination of urea activity can indicate values that are satisfactory for human consumption. According to the significance analysis ANOVA, it was noted that the urease activity (UA) in the sprouted chickpea flour was significantly influenced by the linear effects of temperature (X_1) and germination time (X_2), as well as the quadratic effects of temperature (X_1^2) and germination time (X_2^2), with the latter being the variable with the greatest influence.

Figure 2e shows the response surface diagram corresponding to the quadratic model, which establishes the variations in urease activity (Δ UA) of chickpea flour as a function of temperature (X_1) and germination time (X_2). It can be noted that the highest value of urease activity (Δ pH = 0.028) was reached with a temperature of 28°C and germination time of 42 hours. Urease activity decreased in all germinated chickpea flours from Δ pH = 0.030; up to Δ pH 0.010, in relation to the ungerminated chickpea flour that obtained a value of Δ pH = 0.070, this probably occurred due to alterations in the chemical composition during the germination process. In all cases, the variations in pH units were less than 0.03, so it is considered that there is no presence of urease. Since this enzyme is not present, it is likely that other antinutrients are not present either. This occurs because these antinutrients are probably used as a source of energy for the development of the seedling during the germination process [60]. Furthermore, as can be seen in the results, the presence of urease in chickpea is very low (0.07). According to [61], the reduction levels of trypsin inhibitors published for chickpea germinated for 3 - 6 days were between 24 to 83% respectively.

Effect of the Variable's Temperature and Time on The Multi-Responses Observed During the Chickpea Germination Process

In order for all the responses to be placed in the same order of greatness, it was necessary to work with the logarithm of each of the responses. The representation of this function was established as follows,

$$MRV = \frac{(TPC) (TLC) (WAI) (LAI) (RSC)}{(TAC) (WSI) (TSC) (APD) (UA)}$$

Were, MRV, multi-response variable; X_1 , germination temperature (°C); X_2 , germination time (hours); TPC, total protein content; TLC, total lipid content; TSC, total sugar content; SRC, reducing sugar content; TAC, total ash content; WAI, water absorption index; LAI, lipid absorption index; WSI, water solubility index; APD, apparent density; UA, urease activity.

In accordance with the ANOVA, it can be seen that the coefficient of determination ($R^2 = 0.9542$) shows the goodness of the fit of the model to the variable (multi-response), showing a good fit to the experimental data. The MQR/MQr ratio ($F_c = 20.86$ vs. $F_t = 5.05$) at the 95% confidence level. Indicates that the regression is significant. Observing that the value of F_c is greater than F_t In accordance with the ANOVA, it can be seen that the coefficient of determination ($R^2 = 0.9542$) shows the goodness of the fit of the model to the variable (multi-response), showing a good fit to the experimental data. Guarantees the use of the model for predictive purposes.

The SSlof/SMpe relationship ($F_c = 2.81$ vs. $F_t = 19.16$) shows that there is no lack of fit. The small errors in parentheses below the regression coefficients confirm that the quadratic model is adequate to describe the response surface in the studied region.

According to the significance analysis, it was observed that the linear effects of temperature (X_1) and germination time (X_2) significantly influenced the multi-response variable in sprouted chickpea flour by the quadratic effects of temperature (X_1^2) and germination time (X_2^2). The most influential variable in the process is the quadratic effect of the germination time (X_2^2). Figure 2l shows the response surface diagram corresponding to the quadratic model, which establishes the variations of the multi-responses corresponding to chickpea flour as a function of temperature (X_1) and germination time (X_2). The value for determining the region that simultaneously represents all the dependent variables studied is determined with a temperature of 35°C and a germination time of approximately 82 hours. Regression model for MRV

$$Y_{11} = 1.8460 + 0.2035x_1 + 0.1983x_2 + 0.22192x_1^2 + 0.22668x_2^2 + 1.11569x_1x_2$$

(0.044) (0.027) (0.027) (0.032) (0.032) (0.038)

The proximate composition (% , d. b.), resulting from the germination treatment at 35 °C for 82 hours, of the samples without germination and germinated, respectively, were: protein (N x 5.70), 22.07 and 26.51; lipids 6.50 and 7.51; ash, 3.31 and 2.94; raw fiber, 3.72 and 4.95; carbohydrates by difference, 64.39 and 58.09%, respectively. Results that corroborate those described by different works cited. Therefore, showing that there is a significant improvement in the composition values in chickpeas.

CONCLUSIONS

All mathematical models determined in the present study, both for nutritional properties and for technical-functional properties, resulted in being suitable for calculating the corresponding values for each of the variables in the regions studied.

The best operational conditions during the chickpea removal process to begin the germination process were established at a temperature of 28 °C and a time of 15 hours. Conditions in which the grain reaches around 58% moisture. From the observations made and the knowledge of the photos shown because of the present study, was concluded that the best conditions for chickpea germination correspond to a temperature of 30 °C and a time of 48 hours.

The chickpea germination process promoted a reordering of the grain's main nutritional constituents, as the components underwent variations during the process. The experimental area made it possible to increase, in percentage terms, the total protein content by 20.12%, the lipid content by 17.22%, reducing sugars by 81.07%, and crude fibre by 33.06%. However, at the same time, a decrease in carbohydrate content totalling 9.78%, sugars totalling 9.9%, sugars totalling 14.38%, yields totalling 9.84%, urease activity, water solubility index and apparent density were noted, depending on the multiple responses, established at 35 °C temperature and time of 80 hours.

With regard to techno-functional properties, when comparing the harvest of chickpeas without germinating, and the harvest of germinated chickpeas, they show an increase of 113.04% in the water absorption index, an increase of 27.68% in the lipid absorption index. Meanwhile, decreases of 23.61% for the WSI and 7.29% for the APD, respectively. Regarding urease activity, it has been demonstrated that ungerminated chickpeas contain very little urea, the same thing that is completely inactivated during the germination process, consequently, there was no manifestation when the inhibition of trypsin and chymotrypsin, enzymes are very important in the digestion of proteins, we can rule out changes in the digestive process because of this enzyme.

Finally, the chickpea germination process constitutes a technique capable of promoting important changes in the structure of the grain. All of them aimed at improving the nutritional and functional properties of the harvest obtained from this legume, converting it into an ingredient with potential for use in different areas of the food industry. Mainly, as suggestion in bakery, pasta, meat products, canned goods, and extruded products, among other alternatives, have significant benefits for human health.

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Authors' Contributions

Miguel Angel Larrea Céspedes, conducted field and laboratory experiments and supervisory support, planning of experiments, statistical analysis and preparing the draft and editing the manuscript. José Luis Ramirez Ascheri contributed to planning, statistical analysis and preparing the draft manuscript and final redaction. Both authors contributed to the final preparation of manuscript.

References

- [1] Kaur, R., & Prasad, K. (2022). Elucidation of chickpea hydration, effect of soaking temperature, and extent of germination on characteristics of malted flour. *Journal of Food Science*, 87, 2197–2210. <https://doi.org/10.1111/17503841.16147>.
- [2] Cora M Best, Nancy F Krebs, Christopher P Duggan. (2024). A new series: Nutrition for the Clinician. *The American Journal of Clinical Nutrition*. 2024. V.119, n. 2, p. 237-238. doi.org/10.1016/j.ajcnut.2023.10.029.
- [3] Balazs H. Bajka; Ana M. Pinto; Natalia Perez-Moral; Shikha Saha, Peter Ryden, Jennifer Ahn-Jarvis. (2023) Enhanced secretion of satiety-promoting gut hormones in healthy humans after consumption of white bread enriched with cellular chickpea flour: A randomized crossover study. *The American Journal of Clinical Nutrition*. v. 117 n.3, p. 477–489. doi.org/10.1016/j.ajcnut.2022.12.008.
- [4] Mamoudou Hamadou, Mune Mune Martin Alain, Fookalbo Vagai Obadias, Muhammad Zaffar Hashmi, Bahri Başaran, Bayang Jean Paul, Minka Samuel René, (2022). Consumption of underutilised grain legumes and the prevention of type II diabetes and cardiometabolic diseases: Evidence from field investigation and physicochemical analyses. *Environmental Challenges*, v.9, doi: 10.1016/j.envc.2022.100621.
- [5] Hongyan Mao, Shuo Yuan, Qin Li, Xiaoyan Zhao, Xiaowei Zhang, Hongkai Liu, Ming Yu, Meng Wang. (2024). Influence of germination on the bioactivity, structural, functional and volatile characteristics of different chickpea flours, *Food Chemistry: X*, v. 21, doi: 10.1016/j.fochx.2024.101195.
- [6] Virginia Messina. (2014). Nutritional and health benefits of dried beans. *Am J Clin Nutr*;100(suppl):437S–42S [7] Atlas Big – Ireland. From: <https://www.atlasbig.com/en-ie/countries-by-chickpea-production>; at-09-01-2024
- [8] Arteaga, M. (2015). Dip de garbanzo (*Cicer arietinum*), con tomate (*Lycopersicon esculentum* Mill) deshidratado [Tesis de pregrado, Universidad de Guayaquil]. <http://repositorio.ug.edu.ec/handle/redug/12721>
- [9] Fernández, Marión Elizabeth Aguilar; Stamford, Thayza Christina Montenegro; Viana, Raiane Duarte, Santos; Cristina Alexandra Correia dos; Costa, Antônio Felix da; Stamford, Tânia Lúcia Montenegro. (2021). Efeitos da Germinação em Leguminosas e Cereais: Propriedades Nutricionais e Tecnológicas. In: *AVANÇOS EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS – v. 3*. Chap. 26, p. 351-366, doi: 10.37885/210203230.
- [10] Nobel Mesfin, Abera Belay, Endale Amare (2021). Effect of germination, roasting, and variety on physicochemical, techno-functional, and antioxidant properties of chickpea (*Cicer arietinum* L.) protein isolate powder, *Heliyon*, v.7, n. 9. doi.org/10.1016/j.heliyon. 2021.e08081.
- [11] Gao, Yue; Yao, Yang; Zhu, Yingying; Ren, Guixing (2015). Isoflavone Content and Composition in Chickpea (*Cicer arietinum* L.) Sprouts Germinated under Different Conditions. *Journal of Agricultural and Food Chemistry*, 63(10), 2701–2707. Doi:10.1021/jf5057524.
- [12] Box, G.E.P.; Wilson, K.B. On the experimental attainment of optimum condition. *Journal Royal Statistics*, n.3, p.1-45, 1951. https://link.springer.com/chapter/10.1007/978-1-4612-4380-9_23
- [13] Cochran, B.Y.; Cox, G.M.: *JOH-n Wiley*, (1992) *Experimental designs*. 2 ed. New York <https://www.wiley.com/en-us/Experimental+Designs%2C+2nd+Edition-p-9780471545675>
- [14] Box, G., & Draper, N. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society*, 13(1), 1–45. <https://www.jstor.org/stable/2983966>.

- [15] Silva, R. & Borsato, D. (1985). Análisis de biosistemas a través de delineamiento factorial: Optimización pelo método super simplex modificado. *Arq. Biol. Tecnol. SP*, 28(4); 521–533.
- [16] AACC. (1990). Approved methods of analysis. American Association of Cereal Chemists; 11 edit. AACC.; <https://www.cerealsgains.org/resources/methods/Pages/default.aspx>
- [17] Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P.; Smith, F. "Colorimetric Method for Determination of Sugars and Related Substances". *Anal Chem.* 1956, 28 (3), 350–356. <https://pubs.acs.org/doi/10.1021/ac60111a017>
- [18] Sumner, J. and Sisler E., (1944). "A Simple Method for Blood Sugar"; *Arch. Biochem.* 1944; 4: 333. *Arch. Biochem.*, v. 4, p. 333-336. [https://www.scirp.org/\(S\(351jmbntvnsjt1aadkozje\)\)/reference/referencespapers.aspx?referenceid=555804](https://www.scirp.org/(S(351jmbntvnsjt1aadkozje))/reference/referencespapers.aspx?referenceid=555804)
- [19] R.A. Anderson, H.F. Conway, V.F. Pfeifer, E. Griffin. (1969). Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today*, 14 (4–7), pp. 11-12.
- [20] Sosulski, F. W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem.*, 39, 344-350.
- [21] Blandón, S. L.; Larios, X.J.; (2019); Evaluación de sustitución parcial de harina de trigo por harina de frijol *phaseolus vulgaris* l. en la formulación de tortas; Universidad Nacional de Ingeniería Sede Regional Norte <http://www.revistas.uni.edu.ni/index.php/higo>; Vol. 09. No. 01, pp.35-44
- [22] Icontec; Norma técnica Colombiana NTC; 771; (2000); Frijol soya y sus sub productos; Determinación de la actividad de la ureasa; Bogotá - Colombia; Instituto de Normas Técnicas; <https://es.scribd.com/document/399661895/NTC-771-Determinacion-Ureasa-Frijol-Soya>
- [23] Sajad Ahmad Sofi, Jagmohan Singh, Khalid Muzafar, Shabir Ahmad Mir. (2020), Effect of germination time on physico-chemical, functional, pasting, rheology and electrophoretic characteristics of chickpea four; *Journal of Food Measurement and Characterization*, v. 14: p. 2380–2392. <https://doi.org/10.1007/s11694-020-00485-2>.
- [24] Moreiros, et al., 2013. (GARBANZOS); Tablas de Composición de Alimentos. Garbanzos; Chick pea; (*Cicer arietinum*); Objetivos nutricionales/día. Consenso de la Sociedad Española de Nutrición Comunitaria <https://fen.org.es/MercadoAlimentosFEN/pdfs/garbanzos.pdf>
- [25] Peralta, R., & Veas, R. (2014). Garbanzo: Usos alternativos para generar valor agogado al descarte. 1. <https://rdu.unc.edu.ar/handle/11086/1808>
- [26] Polo, CH. I. A, (2012). Determinación proximal de los principales componentes nutricionales de seis variedades de leguminosas: arveja, garbanzo, haba, lenteja, maní y soya; Pontificia Universidad Católica del Ecuador <http://repositorio.puce.edu.ec/handle/22000/7111>
- [27] Aguilar-Raymundo, V. & Vélez-Ruiz, J. (2013). Propiedades nutricionales y funcionales del garbanzo ((*Cicer arietinum*) L) *Temas selectos de Ingeniería de Alimentos*, 7(2)25-34 https://www.researchgate.net/publication/319185894_Propiedades_nutricionales_y_funcionales_del_garbanzo_Cice_r_arietinum_L
- [28] Ramos-Pacheco, B.S.; Choque-Quispe, D.; Ligarda-Samanez, C.A.; Solano-Reynoso, A.M.; Palomino-Rincón, H.; Choque-Quispe, Y.; Peralta-Guevara, D.E.; MoscosoMoscoso, E.; Aiquipa-Pillaca, Á.S. (2024). Effect of Germination on the Physicochemical Properties, Functional Groups, Content of Bioactive Compounds, and

- Antioxidant Capacity of Different Varieties of Quinoa (*Chenopodium quinoa* Willd.) Grown in the High Andean Zone of Peru. *Foods*, v.13, p. 417. <https://doi.org/10.3390/foods13030417>.
- [29] Sajad Ahmad Sofi, Jagmohan Singh, Khalid Muzafar, Shabir Ahmad Mir. (2020), Effect of germination time on physico-chemical, functional, pasting, rheology and electrophoretic characteristics of chickpea flour; *Journal of Food Measurement and Characterization*, v. 14: p. 2380–2392. <https://doi.org/10.1007/s11694-020-00485-2>
- [30] Atudorei, D.; Atudorei, O.; Codina, G.G. (2022). The Impact of Germinated Chickpea Flour Addition on Dough Rheology and Bread Quality. *Plants* v.11, p.1225. <https://doi.org/10.3390/plants11091225>.
- [31] Kaur, R., & Prasad, K. (2022). Elucidation of chickpea hydration, effect of soaking temperature, and extent of germination on characteristics of malted flour. *Journal of Food Science*, 87, 2197–2210. <https://doi.org/10.1111/17503841.16147>.
- [32] Draper, N.R.; The (1984) The Box-Wetzel Criterion Versus R^2 *Journal of the Royal Statistical Society. Series A (General)*; v. 147, n 1, pp. 100-103; Published By: Wiley <https://www.jstor.org/stable/2981740?origin=crossref>.
- [33] Chaparro-Rojas, D., Piasmag-Portilla, R., Elizalde-Correa, A., Vivas.Quila, N., & Erazo-Caicedo, C. (2010). Effect of the germination on protein content and digestibility in amaranth, quinoa, soy bean and guandul seeds. *Facultad de Ciencias Agropecuarias*, 8(1), 35–42. <http://www.scielo.org.co/pdf/bsaa/v8n1/v8n1a05.pdf>.
- [34] Ravneet Kaur, Kamlesh Prasad. (2021). Technological Processing and nutritional aspects of chickpea (*Cicer arietinum*) - A review. *Trends in Food Science & Technology*. v,109. p, 448-463. <https://doi.org/10.1016/j.tifs.2021.01.044>.
- [35] Meriem Ouaziba, Nadia Moussoua, B. Dave Oomahb, Farid Zaidia, and Janitha P. D. Wanasundara (2015) Effect of heat processing and germination on nutritional parameters and functional properties of chickpea (*Cicer arietinum* L.) from Algeria. *Journal of Food Legumes* 28(2): 1-8, 2015.
- [36] Gil Hernandez, A. (2010). Legumbres, verduras y productos hortícolas.; leguminosas; Información del libro Tratado de nutrición. 2: Composición y calidad nutritiva de los alimentos; Legumbres, verduras y productos hortícolas. <https://dialnet.unirioja.es/servlet/libro?codigo=561427>.
- [37] Polo, CH. I. A, (2012). Determinación proximal de los principales componentes nutricionales de seis variedades de leguminosas: arveja, garbanzo, haba, lenteja, maní y soya; Pontificia Universidad Católica del Ecuador <http://repositorio.puce.edu.ec:80/handle/22000/7111>.
- [38] OCU. (2017). Harina de garbanzo: propiedades y recetas. Organización de Consumidores y Usuarios - OCU. <https://www.ocu.org/alimentacion/alimentos/informe/harina-de-garbanzo-propiedades-recetas>.
- [39] Ferram, J. InfoAgo. (2021). El cultivo de garbanzo. Artículos técnicos; Toda la agricultura en Internet; https://www.infoago.com/documentos/el_cultivo_del_garbanzo.asp.
- [40] Herrera, S. M.; Savón, L.; Lon-wo, E.; Gutiérrez, O.; Herrera, M., (2014). Inclusion of *Morus alba* leaf meal: its effect on apparent retention of nutrient, productive performance and quality of the carcass of naked neck fowls. *Cuban J. Agric. Sci.*, 48 (3): 259-264; <https://www.feedipedia.org/node/25094>.
- [41] Silva-Cristobal, L., Osorio-Díaz, P., Tovar, J., & Bello-Pérez, L. (2010). *Chemical composition, carbohydrate digestibility, and antioxidant capacity of cooked black bean, chickpea, and lentil Mexican varieties*. *CyTa-Journal of Food*, 8(1), 7–14. <https://doi.org/10.1080/19476330903119218>.

- [42] Gallegos, R. G. & Polo, I. Ch.; (2013); Determinación proximal de los principales componentes de seis leguminosas; Info-ANALITICA; file:///C:/Users/DOCENTE/Downloads/Dialnet-DeterminacionProximalDeLosPrincipalesComponentesDe-8382681%20(4).pdf.
- [43] Aguilera, Y., Díaz, M. F., Jiménez, T., Benítez, V., Herrera, T., Cuadrado, C., Martín-Pedrosa, M., & Martín-Cabrejas, M. A. (2013). Changes in nonnutritional factors and antioxidant activity during germination of nonconventional legumes. *Journal of Agricultural and Food Chemistry*, 61(34), 8120– 8125. <https://doi.org/10.1021/JF4022652>.
- [44] Saura-Calixto F, Garcia-Alonso A, Goni I, Bravo L. (2007); Caracterización de los alimentos tradicionales de la dieta española: alegaciones nutricionales y alegaciones en salud; Capítulo 4. Legumbres; Madrid - España. <https://www.ucm.es/data/cont/docs/429-2016-04-27-capitulo-4-legumbres-2007.pdf>
- [45] Popinigis, F., 1985. *Fisiologia da Semente*. 2 Ed, Brasilia D.F. 289 pp. Pesquisas; Fisiologia Sementes, Manuais, Projetos, Pesquisas de Engenharia Florestal <https://www.doccity.com/pt/fisiologia-sementes-popinigis/4861354/>.
- [46] Marero, L. M.; Payumo, E. M.; Aguinaldo, A. R.; Homma, S.; (1990); Maltooligosaccharide composition of flours, weaning foods, and guels prepared from germinated rice, corn, mungbean, and cowpea. *Journal of Nutritional Science and Vitaminology*, v. 36, n. 1, p. 55-64; <https://pubmed.ncbi.nlm.nih.gov/1694542/>.
- [47] Miranda, M. Z; (2006) Germinação e posterior extrusão para obtenção de farinha integral extrusada de trigo germinado. *Passo Fundo: Embrapa trigo*, 2006. 12 p. html. Embrapa Trigo. Documentos Online, 74). http://www.cnpt.embrapa.br/biblio/do/p_do74.htm.
- [48] Danisova, C.; Holotnatova, E.; Hozova, B.; Buchtova, V. (1994) Effect of germination on a range of nutrients of selected gains and legumes. *Acta Alimentaria*, v. 23, n. 3, p. 287-298, <https://www.semanticscholar.org/paper/Effectof-germination-on-a-range-of-nutrients-of-Dani%C5%A1ov%C3%A1-Holotnakova/34f7059a4cfe523d1ae5eb364208cafb9bd33fbb>.
- [49] Ranhotra, G.S.; Loewe, R.J.; Lehmann, T.A. (1977); *Breadmaking quality and nutritive value of sprouted wheat*. *Journal of Food Science*, v.42, n.5, p. 1373-1375; <http://www.sciepub.com/reference/326950>.
- [50] Akinlosotu, A., & Akinyele, I. O. (1991). The effect of germination on the oligosaccharide and nutrient content of cowpeas (*Vigna unguiculata*). *Food Chemistry*, 39(2), 157–165. [https://doi.org/10.1016/03088146\(91\)90156-L](https://doi.org/10.1016/03088146(91)90156-L).
- [51] Valdez-Arana, J. del C.; Steffolani, M. E.; Repo-Carrasco-Valencia, R.; Pérez, G. T.; CondezoHoyos, L. (2020). Physicochemical and functional properties of isolated starch and their correlation with flour from the Andean Peruvian quinoa varieties. *International Journal of Biological Macromolecules*, 147:997-1007; https://scholar.google.com/citations?view_op=view_citation&hl=es&user=E0s_.
- [52] Asiedu, M., Nilsen, R., Lie, Ø., & Lied, E. (1993). Effect of processing (sprouting and/or fermentation) on sorghum and maize. Proximate composition, minerals and fatty acids. *Food Chemistry*, 46(4), 351–353. [https://doi.org/10.1016/0308-8146\(93\)90003-X](https://doi.org/10.1016/0308-8146(93)90003-X).
- [53] Dronzek, B., Hwang, P., & Bushuk, W. (1972). Scanning electron microscopy of starch from sprouted wheat. *Cereal Chemistry*, 49, 232–239. <https://www.cerealsgrains.org/publications/cc/backissues/1972/Documents/CC1972a30.html>
- [54] Cheftel, J.C; D. Lorent, D.; CUQ, J.L.; *Proteínas alimentarias: Bioquímica. Propiedades funcionales. Valor nutritivo. Modificaciones químicas*; Editorial Acirbia, S.A. <http://alimentos.web.unq.edu.ar/wpcontent/uploads/sites/57/2016/03/Cap%C3%ADtulo-6.1-Prote%C3%ADnas-alimentarias-Cheftel.pdf>.

-
- [55] López, G., G. Ros, F. Rincón, M. Periago, M. Martínez and J. Ortuño. (1997). Propiedades funcionales de la fibra dietética. Mecanismo de acción en el tracto gastrointestinal. Archivos Latinoamericanos de Nutrición 47(3): 203-207; https://pesquisa.bvsalud.org/portal/resource/pt/lil_228279.
- [56] Leelavathi, K., Vetrimani, R., & Rao, P. (1990). Changes in the functional characteristics of wheat during soaking and subsequent germination. Journal of Food Science and Technology (Mysore), 27(6), 349– 354. <https://eurekamag.com/research/007/094/007094875.php>.
- [57] Nazik N., Geenfield, H. & Ron B. H. Wills; (2000); Water holding capacity of selected soluble and insoluble dietary fibre; International Journal of Food Properties, 3:2, 217-231, DOI: 10.1080/10942910009524629; <https://doi.org/10.1080/10942910009524629>
- [58] Aritonang, A.; Julianti, E.; Nurminah, M; (2020); SP - 012113 N2 WF and 40% PSPF. Physicochemical and sensory characteristics of sweet bun produced from wheat and purple sweet potato flour; VL - 454; DOI - 10.1088/1755-1315/454/1/012113; JO - IOP Conference Series: Earth and Environmental Science.
- [59] Gamarra, T. N.; 2021; Valores nutricionales y propiedades funcionales de la harina de sangaroché; (*Amaranthus hybridus* L.); a distintos tiempos de germinado; Universidad Nacional Micaela Bastidas de Apurímac facultad de ingeniería escuela académico profesional de ingeniería agroindustrial; Tesis para optar el título de ingeniero agroindustrial, Abancay - Perú. file:///C:/Users/DOCENTE/Desktop/T_0623.pdf.
- [59] Vilcanqui-Pérez, F.; Chaquilla-Quilca, G. Sarmiento Casavilca, V.H., Céspedes Orosco C.N, Yeni Ventura Saldívar. Y.; Paola Cortés-Avendaño, P.C.; (2021); *Efecto del germinado sobre las características nutricionales, propiedades bioactivas y funcionales de basul (Erythrina edulis)*; Archivos Latinoamericanos de Nutrición; Revista de la Sociedad Latinoamericana de Nutrición; Revista de la Sociedad Latinoamericana de Nutrición; Vol. 71 N° 4, <https://doi.org/10.37527/2021.71.4.001>.
- [60] Vidal-Valverde, C., Frias J. 1992. Changes in carbohydrates during germination of lentil. Z. Lebensm. F A. 194 (5): 461-464; <https://digital.csic.es/handle/10261/64371>.
- [61] Guardado-Félix, D.; Serna-Saldivar, S. O.; Cuevas-Rodríguez, E. O.; Daniel A. Jacobo-Velázquez, D. A.; Gutiérrez-Urbe, J. A.; (2017); Effect of sodium selenite on isoflavonoid contents and antioxidant capacity of chickpea (*Cicer arietinum*) L.) sprouts; Food Chemistry volume 226; Pages 69-74; <https://www.sciencedirect.com/science/article/abs/pii/S0308814617300468>