

## DEVELOPMENT OF A FOOD FORMULATION BASED ON SUSTAINABLE ANDEAN GRAINS

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### Abstract

Quinoa is of great importance in the production of extruded foods due to its functional properties and its availability in the market. Flours derived from sprouted quinoa are useful ingredients for enhancing the nutritional and functional properties of cereal-based foods. The objective of the research was to characterize the nutritional and thermal properties of the snack extruded with quinoa sprouts of varieties (white Junín, black Ccollana and pasankalla). Analyses of chemical composition, protein digestibility and exposure index were performed through the AOAC method, thermal transition enthalpy and decomposition by differential scanning calorimetry (DSC) and thermogravimetry (TGA). The germinated varieties had a protein content (11.86 to 12.48%) and carbohydrates (68.04 to 72.44%). The extruded products had a low fat content and a balanced protein profile. The expansion rate was reduced in extruded snacks with high levels of protein digestibility (92%). The extrusion presented an endothermic event with an average decomposition temperature variation of 154 °C and an endothermic enthalpy of 2,789 ΔH(J/g). The quinoa seed sprouts in the extruded ones presented high nutritional contents with potential use as a food ingredient in cereal-based products.

**Keywords:** germinated quinoa, functional property, thermal properties, snacks

## 1. Introduction

Extruded snacks are classified as second-generation snacks. Usually their bulk density is low and they are often marketed as products high in sugar, fat, salt, dyes, flavorings and are low in nutrients; leading to health problems in consumers. Extruded snacks are usually low in protein, vitamin, mineral, and dietary fiber content (1). There is currently a need to produce extruded snacks with nutritional properties and breakfast cereals and snacks are sought to be among the fastest growing segments in the world market (2).

There are studies that show that consumers are aware of the consequences of consuming unhealthy snacks, however, they look for snacks with natural ingredients, free of dyes and high in protein, fiber and whole grains (3). The need to develop new products with healthy characteristics requires the use of food processing technologies. The extrusion process has the benefits of denaturing undesirable enzymes, inactivating some anti-nutritional factors (trypsin inhibitors, hemagglutinins, tannins and phytates), improving the digestibility of starch and proteins, sterilizing the finished product and preserving the natural colors and flavors of food (4). Extrusion is a technology that helps to use traditional and novel ingredients for the manufacture of healthy snacks. During the elaboration process, cereals are used mainly for their nutritional aspects. However, legumes and oilseeds can be used effectively due to the high protein content to obtain a nutritional improvement from cereal-based extruded snacks (5). Quinoa is an Andean grain that has a high protein content of around 15%. It has an excellent content of essential amino acids wider than legumes and other cereals. Therefore, quinoa proteins are able to complement cereal or legume proteins (6). Germination is a biological process that can be easily and cheaply applied to obtain new biotechnologically processed food products. Consumption of sprouted products is increasing because numerous studies have documented their health advantages and benefits (7; 8). During the germination process, hydrolytic enzymes are activated and they are also the newest synthesized enzymes that, together with the reserve substances in the seed, are mobilized to be used in the initial growth of the seedling (9). This process causes changes in the content and composition of proteins, carbohydrates and lipids. Proteins are hydrolyzed and consequently their digestibility is improved (10). Some authors demonstrated that the germination of grains and legumes (such as quinoa, soybeans, chickpeas, beans, peas, millet, rice and corn) can decrease the content of antinutrients such as phytates, tannins and inhibitory proteases (10) However, these modifications can also affect their proximal composition and the functional properties of the sprouted grain in processed products, which are important characteristics to know for use as food ingredients.

The research aimed to characterize the nutritional and functional properties of extruded snacks based on quinoa sprouts of different varieties from the Andean region of Peru.

## 2. Materials and methods

### 2.1. Source of seeds

The quinoa seeds were acquired from the Machupichu Cooperative, with organic certification, in the province of Andahuaylas, Apurímac, Peru, Quinoa of three white Junín varieties, black Ccollana and pasankalla were used, the samples were stored in plastic bags until their subsequent germination.

### 2.1.1. Germination of quinoa seeds

Quinoa seed samples were taken to a manual washing process with water to remove impurities and saponins. Once washed and without saponin they were soaked in water (1:5) for 6 h at room temperature. Water drained and wet grains were spread in a thin layer in plastic trays covered with paper filters and incubated under controlled conditions: 22-24 °C and 80-90% relative humidity in darkness, with a time of 48 h where the sprouts reached the same radical length (1 to 1.5 cm). The germination capacity was determined (11), counting the germinated grains and expressing it as a percentage of the total number of grains. The sprouted grains were dried in a forced circulation furnace at 40 °C to constant weight. The dried grains were ground in a centrifugal mill MJ-W176P, brand (Panasonic, Japan) then sieved through a 60 mm mesh sieve. The flours were packaged in polyethylene bags and stored at room temperature until later use.

### 2.2. Preparation of Snacks

For the formulation and elaboration of the extruded, yellow corn purchased from the brand P.A.N. and the sprouted quinoa flours product of the preparation in the study were used. Sprouted quinoa flour and corn were mixed in the proportions of 20:80, 60:40, 40:60 and 20:80 sprouted quinoa/corn flour. Feed mixtures were adjusted to the desired moisture content with calculated amounts of distilled water and thoroughly mixed for 15 min. The samples were packed in polyethylene bags and placed in the refrigerator overnight to balance the moisture. The moisture content of the sample and raw material was determined in a 75% furnace under a vacuum of 50 mm Hg up to constant weight. The moisture content levels of the feed were 13 % bs. Samples were brought to room temperature before extrusion cooking.

The formulations used for the production of extruded snacks are given in Table 1

Table 1. Powder formulation mixtures for extrusion.

Sample code	Sprouted quinoa flour (%)	Yellow corn flour (%)
Extruded-20	20	80
Extruded-40	60	40
Extruded-60	40	60
Extruded-80	20	80

### 2.3. Extrusion

The formulations were extruded in a double screw extruder brand LABOR PQ DRX-50 in which they worked at a speed of 800 RPM, feed speed of 22 Hz, cutter speed of 4 Hz, the temperature was 150 °C (along the sections of the extruder). The die used was rectangular in shape of dimensions 0.098 x 0.787 in. The extruded snacks were dried in a tray dryer at 50 °C for 18 hours to reduce humidity. The extruded snacks were packaged in aluminum bags with hermetic closure and stored at room temperature. The products obtained were evaluated in their proximal and functional chemical composition, being the temperature and the enthalpy of gelatinization.

### 2.4 Chemical analysis of raw and extruded materials

#### 2.4.1. Compositional analysis

The nutritional composition of all samples was analyzed using standardized techniques for raw material and processed product. The analyses were performed in triplicate comprising the following analyses: Humidity (12), Total protein: it was performed by the Kjeldahl method, the mixture resulting from digestion was neutralized with sodium hydroxide and distilled. The distillate was collected in a solution of boric acid, to then be titrated and determine the nitrogen contained in the sample. Fat: by extraction with petroleum ether in Soxhlet (20). Fiber: crude fiber was expressed as the loss of mass that is lost in the incineration of dry waste, obtained after digestion with solutions of HSO<sub>4</sub> and NaOH at 1,25 % (20). Ash: by incineration in muffle at 550 °C (20). Carbohydrates (nitrogen-free extract) by difference.

#### 2.4.2. Protein digestibility

In vitro protein digestibility was determined using the modified AOAC 971.09 method (13). Degreased in one (1 g) were digested with 150 ml of pepsin solution in HCl 0,075 M (0,0002 % v/v) and incubated in a stirring bath (45 °C, 16 h). The digested samples went into the vacuum, then filtered and washed three times with water and acetone. In the residue, the nitrogen content was determined using the Kjeldahl method (AOAC 920.87). The value was corrected by a determination of nitrogen in a pepsin-free HCl solution; then the protein was calculated using factor 6.25. The analyses were performed in triplicate.

#### 2.4.3. Thermal properties

The thermal properties of various mixtures were determined in triplicate before and after the extrusion process. The methodology cited by Zeng et al. (14) carried out by a differential scanning calorimetry medium (TA Instruments DSC-2500), previously calibrated with indium of 99.99 % purity. The samples were analyzed in airtight aluminum capsules and the measurement was made by comparing with the heat flow of a similar, empty capsule. The mass of the sample was 2.5±0.1mg, of which 80% corresponds to water and the remaining 20% corresponds to flour. After sealing the capsule the sample was left to stand for 30 minutes to homogenize the mixture. The heating was carried out at a heating rate of 5°C/min, from room temperature to 120 °C, in the nitrogen atmosphere. The thermogram was constructed by varying from the initial temperature to

250 ° C and the melting temperature ( $T_m$ ) and melting enthalpy ( $\Delta H$ ) were calculated by dry weight of flour and expressed in J / g.

Thermogravimetry analyses were performed to determine the thermal stability of the flours. Analyses were performed taking into account standard measurement procedures TGA ASTM E1131-03. A TGA Q500 equipment from TA Instruments was used, previously calibrated with high purity nickel. The mass of the sample was  $10.0 \pm 0.1$  mg, and they were analyzed in platinum cymbals for TGA. The heating was carried out in a controlled manner from 25°C to 600°C at a constant rate of 10°C/min, in a nitrogen atmosphere. The percentage of moisture (Hm), percentage of carbohydrates (Stage 1 and Stage 2) and final amount of residues (Rs) were developed for each sample.

#### 2.4.5. Expansion rate

It was developed by measuring the extruded product cross-sectional area and the matrix. The measurements were developed using a Control Company Traceable stainless steel vernier digit calibrator and the expansion rate value was calculated from an average of 20 observations.

#### Statistical analysis

The results were expressed as a mean. The analysis of variance was used in order to determine the significance of the properties between the different samples, using the Pearson test with a level of significance ( $p < 0.01$ ). the analyses were performed with Restudio. All analyses were performed in triplicate. Data were reported as the mean  $\pm$  standard deviation (SD). The analytical data obtained were applied a unidirectional ANOVA, as well as Duncan's multiple range test, in order to establish statistically significant differences ( $p < 0.05$ ).

### 3. Results

#### 3.1. Nutritional properties of extruded snacks

Table 3 shows the nutritional composition analyzed from selected extruded snacks that correlate with the values calculated according to each selected variety. The protein content in the extruded samples showed a slight decrease of between (9.19 to 11.23 %) as shown in Table 2. The extrusion process reduced the protein content with low levels for the extruding of quinoa white variety Junín in its different tests. The extrusion process influences the composition of cereal and legume snacks (15; 16). In addition, during extrusion cooking, the aggregation of proteins from hydrophobic interactions, hydrogen bonds and disulfide bonds and/or the formation of Maillard-type complexes is reduced, causing the reduction of protein solubility. The moisture content was found from (6.75 to 7.83%), the percentage in fat did not present a variation in the snacks of each variety of germinated quinoa.

The carbohydrate content (69.18 to 73.81 %) was increased in extruded compared to their composition of raw germinated quinoa flour, in the different trials, this is consistent with what was reported in mixtures of extruded chickpeas and lentils (17). This increase has been linked to a

modification in mechanical structure induced by cell breakdown during extrusion (18). Cooking on the other hand caused an increase in carbohydrates in the three varieties of quinoa.

The moisture content is the most important factor both in the processing of the extrude and in the storage, being considered as safe a moisture content below 10 % in order to avoid any microbial growth (19). According to the evaluation processes of commercial extrudes, the protein content was in the range of 2.8 to 9.2%; fat content ranged from 3.15% to 35.7% and carbohydrate content ranged from 4.0% to 70.9% (19). Fat and carbohydrate content ranges include extruded snack values of selected optimal formulation, but the range of protein content is below the protein content of the optimal formulation.

Table 3. Nutritional composition of extruded snacks according to germinated quinoa variety

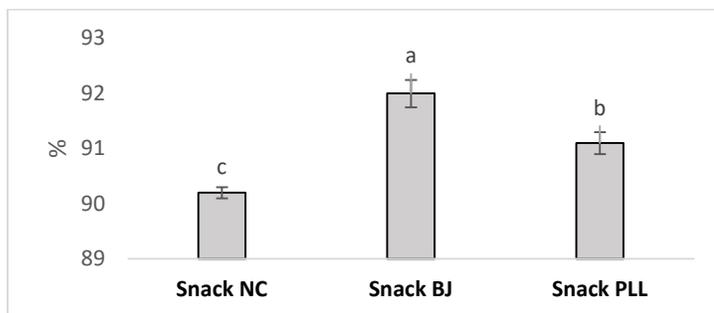
	60% VNC Snack		Snack VBJ 60%		Snack VP 60%	
Humidity	7,83	± 0.01 <sup>c</sup>	6,75	± 0.02 <sup>b</sup>	7,07	± 0.02 <sup>a</sup>
Protein	11,03	±0.04 <sup>b</sup>	9,19	± 0.03 <sup>c</sup>	11,23	± 0.06 <sup>a</sup>
Grease	3,40	± 0.02 <sup>a</sup>	3,83	± 0.02 <sup>a</sup>	3,35	± 0.03 <sup>b</sup>
Ash	1,56	± 0.03 <sup>b</sup>	1,48	± 0.04 <sup>th</sup>	2,53	± 0.04 <sup>th</sup>
Fibre	5,12	± 0.12 <sup>th</sup>	5,45	± 0.03 <sup>c</sup>	5,40	± 0.01 <sup>b</sup>
Carbohydrates	69,18	± 0.03 <sup>c</sup>	70,75	± 0.02 <sup>a</sup>	73,81	± 0.01 <sup>b</sup>
Protein digestibility	90,20	± 0.01 <sup>c</sup>	92,00	± 0.02 <sup>c</sup>	91,10	± 0.01 <sup>c</sup>

Note: VNC: Ccollana black variety; VBJ: Junin white variety; VPLL: pasankalla variety. Different lowercase letters indicate that there is a significant difference, ( $p < 0.05$ )

### 3.2. Protein digestibility in extruded snacks

Figure 1 shows protein digestibility of the extruded according to the selected tests containing a concentration level of 60 % of germinated quinoa flours according to variety. Protein digestibility was found to be greater than 90% in the extruded samples. The high digestibility of proteins is the main requirement for considering a food product as having high nutritional quality (20).

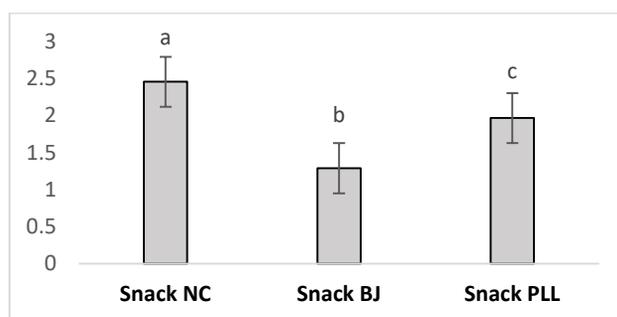
In general, the extrusion process caused an increase in digestibility of more than 90% as shown in Figure 1. This increase could be due to protein denaturation and the inactivation of thermolabile antinutritional factors (protease or lectin inhibitors) of legumes that impair protein digestibility (21; 25). The protein digestibility in greater percentage was found for the snacks extruded with the sprout of quinoa white variety Junín.



**Figure 1.** Protein digestibility of snacks extruded with different varieties of quinoa. Different lowercase letters indicate that there is a significant difference, ( $p<0.05$ )

### 3.3. Expansion rate

Table 2 shows the exposure index that are between 1.29 and 2.46 for the formulations selected according to the study, the values found are below the expected values being close to the values found in quinoa extruded (4). The low rates of expansion are attributed to the fact that quinoa flour has higher amounts of protein, fiber, and fat compared to other cereals. The decrease in the rate of expansion correlated with the quinoa protein.



**Figure 2.** Expansion rate of snacks extruded with different varieties of quinoa. Different lowercase letters indicate that there is a significant difference, ( $p<0.05$ )

### 3.5. Thermal properties

During the extrusion of the snacks, an endothermic event was observed for each formulation of each germinated quinoa variety as shown in Table 3. The start, peak, end and endothermic enthalpy temperature are shown in Table 3. Calorimetric analyses of extruded assays did not reveal the glass transition behavior that could be due to the effect of extrusion that causes partial or total loss of crystallinity due to changes in structural factors in starch and the protein-starch interaction that produces complexes of high molecular weight (22).

However, a melting point temperature transition observed in extrudeds is due to the melting of the complex between amylose and lipids that are present in the extruded mixture (23). The formation of complexes between starches and lipids is due to the ability of amylose to bind to lipids such as

fatty acids. Many researchers have studied the formation of complexes during twin-screw extrusion cooking in the past (24).

Table 3. Thermal properties of extruded snacks

Variety	Non-extruded				Extruded snack			
	T0 (°C)	TP (°C)	Tf (°C)	ΔH(J/g)	T0 (°C)	TP (°C)	Tf (°C)	ΔH(J/g)
H.Q. Pasankalla	165,80	169,97	176,29	169,97	146,93	150,38	172,11	3928,8
H.Q. Black Bcorllana	96,49	99,13	109,40	731,11	152,20	152,27	166,29	2095,6
H.Q. White Junin	94,28	95,84	104,57	1378,4	155,34	159,47	167,12	2345,4

In the thermogram of the extruded snack in Figure 1. Changes in the specific heat were detected, in a temperature range between 146 and 172 °C, a range in which the glass transition temperature is located. The process begins at an initial temperature T0 = 146.90 °C, the peak temperature (Tp) is the temperature where the highest values of heat absorption (25) are recorded, which occurs at a temperature of 150.38 °C. The results also show that the transition occurs in a temperature range (ΔT=Tf-T0) of 25.87 °C. The enthalpy, calculated with the area under the peak curve that represents the energy needed to carry out the gelatinization process is 3928.8 J/g.

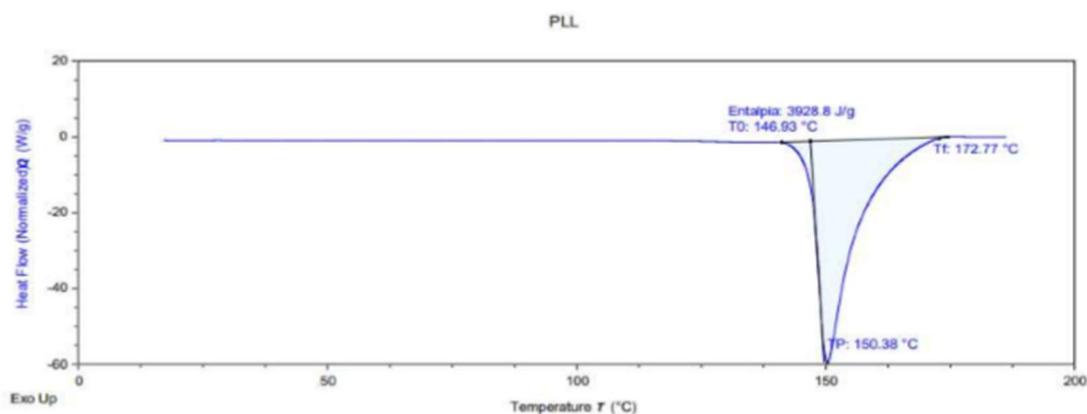


Figure 1. Quinoa variety snack thermogram pasankalla

Table 4 shows the dough losses in sprouted quinoa flours and extruded snacks.

Table 4. Percentage of mass losses in each phase

Variety	Quinoa sprouts				Extruded snack			
	Phase 1 (%)	Phase 2 (%)	Phase 3 (%)	Phase 3 (%)	Phase 1 (%)	Phase 2 (%)	Phase 3 (%)	Phase 3 (%)

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H.Q. pasankalla	9,378	63,35	6,68	4,73	63,76	7,44
H.Q. black Ccollana	4,95	59,97	7,05	5,47	63,62	6,94
H.Q. white Junin	5,12	67,33	6,31	4,75	63,73	6,22

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## Conclusions

From the research it can be concluded that it was possible to characterize the nutritional value of the extruded snack based on germinated quinoa flours of different varieties with an improved nutritional quality. The extrudes had low fat content, in addition to complex carbohydrates and, theoretically, had a balanced protein profile due to the germination of quinoa. Likewise, extrusion improved protein digestibility and soluble dietary fiber content. All extruded formulations can be considered as a good source of protein and dietary fiber, will appeal to health-conscious consumers and at the same time allow the food industry to meet the demands of consumers of functional foods. It is important to note that these extruded products can be consumed directly as a nutritious extruded product for its protein quality content, the snack of optimal formulation is a good option to be part of the school lunch boxes.

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