

# Application Of Ultrasound As A Pre-Treatment For The Germination Of Cañihua (*Chenopodium Pallidicaule*) Illpa Inia 406 And Cupi Variety And Its Effects On The Properties Of Bioactive And Nutritional Compounds

Brigitte Condori<sup>1</sup>, Vianka Zapana<sup>2</sup>, Erika Pachari<sup>3</sup>, Iván Jachmanián<sup>4</sup>

<sup>1</sup>*Escuela Profesional de Ingeniería de Industrias Alimentarias, Facultad de Ingeniería de Procesos, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú*

*Email: bcondoripa@unsa.edu.pe*

<sup>2</sup>*Escuela Profesional de Ingeniería de Industrias Alimentarias, Facultad de Ingeniería de Procesos, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú*

*Email: vzapanag@unsa.edu.pe*

<sup>3</sup>*Escuela Profesional de Ingeniería de Industrias Alimentarias, Facultad de Ingeniería de Procesos, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú*

*Email: epachari@unsa.edu.pe*

<sup>4</sup>*Facultad de Química, Universidad de la República, Montevideo, Uruguay*

*Email: ijachman@fq.edu.uy*

**Abstract:** The objective of this study was to apply ultrasound as a pretreatment in the germination of cañihua (*Chenopodium pallidicaule*) of the ILLPA, INIA 406, and CUPI varieties to evaluate its effects on bioactive and nutritional compounds. The following parameters were used: 40 kHz, intensity of 0.11 W/cm<sup>2</sup>, ambient °T for 10 and 20 min, with an initial conditioning of 14 h/20°C, and germination at 72 h/20°C. The cañihua was then processed to obtain flour. Consequently, in the ILLPA variety, treatment with 20 minutes of ultrasound (US) increased the concentration of proteins 21.1% on a dry basis (BS), 0.083 g/Kg (BS) of iron, 35.7 mmol/ml TROLOX of antioxidant capacity, and reduced carbohydrates with 74. Conversely, the CUPI variety exhibited an augmented ash content of 3.97% (BS) and 0.004 g/100g BS of polyphenols, along with a 0.711% (BS) increase in fat, following a 20-minute US treatment. Statistical analyses revealed substantial disparities in the treatments of proteins, iron, polyphenols, carbohydrates, and antioxidant capacity for both varieties, as well as in ash for CUPI. Conversely, no substantial disparities were observed in ash and fat for ILLPA, and only in fats for CUPI. The present study demonstrates the potential for the application of ultrasound to sprouted foods other than those previously examined, with the objective of enhancing their nutritional and bioactive profiles.

**Key words:** Cañihua; germination; ultrasound; nutritional properties; bioactive properties

## INTRODUCTION

Germination is a low-cost and simple process, which improves the absorption of the nutrients contained in the grain, as well as its antioxidant capacity and the content of bioactive compounds, as well as enriching the taste and flavor of the food [1]. This process also allows the bioavailability of vitamins and minerals, as well as the reduction of compounds called antinutritional [2].

On the other hand, ultrasound is waves that have frequencies between 20 to 100 kHz, which allows seeds to germinate quickly and store beneficial compounds for health, has advantages such as high productivity and environmental protection [1]. This technique is applied to different processes such as extraction, homogenization, crystallization, hydration, germination, causing alterations in the functional, structural and rheological properties of the nutritional compounds of the food, reducing the hydration process which allows the speed of germination [3].

As far as Cañihua is concerned, this is a very old crop used in Latin America before the conquest by the Spaniards, so that, in the mid-twentieth century, its high nutritional contribution was recognized and it was considered a gluten-free cereal, with small seeds with 1 mm, brown color, 63.1 – 70% carbohydrates, 12 – 19% protein and 5.0 – 8.0% fat [4].

In Peru, especially in the Andean area, there is mostly the consumption of Andean grains such as Cañihua, domesticated by the culture called Tiahuanaco on the Collao plateau, at an altitude of 3,600 meters above sea level, this grain has methionine and lysine which compensates for the lack of nutrients from other foods consumed, it is also rich in fiber and lipids. it also contains 1.08% glucose, 1.7% maltose, and has an absence of saponins [2].

There are several studies that carry out the application of ultrasound for the germination of cereals and seeds, which produces encouraging and remarkable effects, but so far the application of these procedures has not been found to enhance the nutritional and functional properties of Cañihua. That is why the following general objective was proposed: To apply the ultrasound method as a pre-treatment to execute the germination of Cañihua (*Chenopodium pallidicaule*) of the ILLPA INIA 406 and Cupi varieties, and thus know its effects on the properties of the bioactive and nutritional compounds, and as specific objectives it was proposed: to apply the pre-treatment with ultrasound for the germination of Cañihua, to determine the effects of ultrasound on the properties of the bioactive compounds of the Cañihua and finally to carry out the determination of the nutritional effects of the pre-treatment with ultrasound in the Cañihua.

## MATERIALS AND METHODS

The acquired Cañihua was cleaned to remove foreign materials and separate damaged or broken grains. It was then disinfected with 0.3% sodium hypochlorite for 15 minutes, rinsed four times with sterile water to remove any traces of the disinfectant [5].

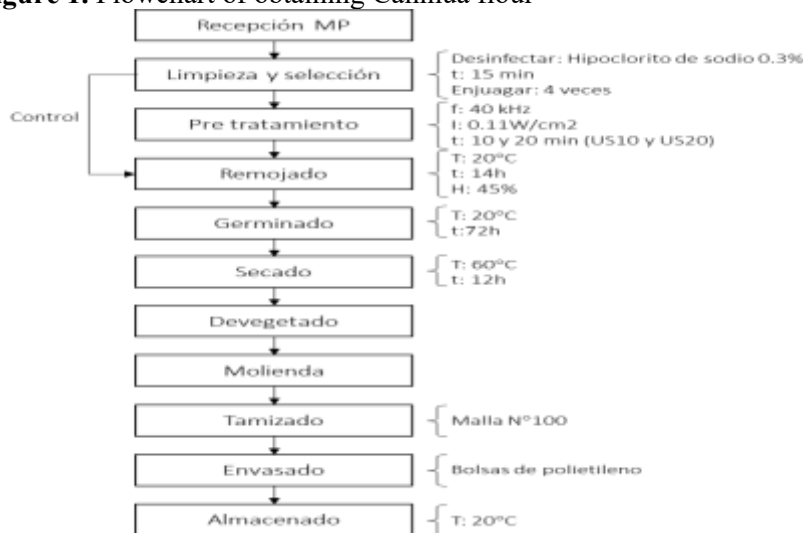
### Pre-treatment with ultrasound

A frequency of 40 kHz and an intensity of 0.11W/cm<sup>2</sup> containing 4L of distilled water at room temperature were used for two times: 10 and 20 minutes [6].

### Cañihua germination and flour preparation

The soaking process was executed, where the Cañihua grain was placed in a container with distilled water for a time of 14 hours at a temperature of 20°C in darkness. Germination was then carried out in a Memmert germination chamber for a period of 72 hours at 20°C without light [5] [7]. The germinated seeds were processed in a tray dryer at 60°C for 12h. Grinding and sieving were carried out with No. 100 mesh, then the flour was vacuum-packed in polyethylene bags and stored at -20°C until its analysis [7] [8].

**Figure 1.** Flowchart of obtaining Cañihua flour



## Determination of physicochemical properties

**Protein content:** The following materials were performed using the Kjeldahl method [9] [10] [11]: sulfuric acid, potassium sulfate, copper sulfate, NaOH, boric acid and hydrochloric acid.

**Ash content:** Executed using the gravimetric method per NTP 209.265:2013 REVISED 2018 [12] [13].

**Iron content:** The sample was prepared using AOAC 945.40 (mg/50 g) [14], and AOAC 944.02, Iron in Flour [15] [16] was used to determine iron.

**Antioxidant capacity:** It was executed using the CUPRAC method [11] [17].

**Total polyphenol content (TPC):** It was executed using the Folin – Ciocalteu colorimetric method, where the following materials were used: 95% methanol, the Folin – Ciocalteu reagent, Na<sub>2</sub>CO<sub>3</sub> and distilled water, as well as the equipment called spectrophotometer (Specord 205, Analytik Jena, United Kingdom) [18] [19], the results were presented as mg of gallic acid equivalent per 100 g of sample (mg EAG/100 g).

**Fat content:** It was executed using the AOAC 963.15 method, which uses hexane extraction [20] [21].

## Analysis and interpretation of results

The determination of the physical properties which correspond to protein content, moisture content, ash content, iron content, antioxidant capacity, polyphenol content and fat content, were executed in triplicate for each sample of germinated Cañihua of the ILLPA and CUPI varieties, treated at 10 and 20 minutes with ultrasound. Likewise, the statistical tests of Dunnett, Kruskal-Wallis, Anova and Tukey, with a significance of 0.05, were used in order to determine the differences between the treatments, which is why this analysis was carried out using the SPSS program.

## RESULTS AND DISCUSSION

Through the Table 1, in the quantification of proteins, for the ILLPA and CUPI varieties, the normality test called Shapiro – Wilk was executed, resulting in a parametric behavior for the treatments of both varieties of Cañihua ( $p>0.05$ ), for this reason the ANOVA test was executed in which a  $p<0.05$  was presented, which indicated that there is evidence that some of the treatments have an effect on the determination of proteins.

**Table 1.** Statistical test of the physicochemical characterization of the Cañihua ILLPA and CUPI

ANALYSIS OUT	CARRIED OUT	ILLPA VARIETY			CUPI VARIETY		
		Estadistic	Gl	itself.	Estadistic	Gl	itself.
Protein determination		F = 5760	2	<0.001	F = 35,278	2	<0.001
Ash determination		F = 0.022	2	0.978	F = 81,284	2	<0.001
Iron determination		F = 116268	2	<0.001	F = 80377	2	<0.001
Determination of polyphenols	of	F = 29,679	2	0.001	F = 358	2	<0.001
Fat determination		H = 1.867	2	0,393	F = 2,993	2	0,125
Carbohydrate determination		H = 7.200	2	0,027	F = 94,728	2	<0.001
Determination of antioxidant capacity		H = 7.261	2	0,027	F = 214813	2	<0.001

Note: F: calculated value of the ANOVA test; H: calculated value of the Kruskal Wallis test; gl.: degree of freedom; sig. significance

The multiple comparison test for the ILLPA variety (Table 2) was developed through the statistical test called HSD Tukey, since it presented homogeneity of variances, a  $p$ -value  $< 0.05$  was obtained, evidencing that there is a difference between the treatments, so that, the best treatment, according to the difference in means, it was the sample with 20 minutes of ultrasound compared to the control; with respect to the multiple comparison of the CUPI variety (Table 3), since it did not have homogeneity of variances, the Dunnett statistical test was developed, showing a difference between the treatments ( $p > 0.05$ ).

**Table 2.** Multiple Comparison Statistical Test of the Cañihua ILLPA

<b>ILLPA VARIETY</b>			
<b>ANALYSIS CARRIED OUT</b>	<b>TREATMENTS</b>	<b>MEAN DIFFERENCE</b>	<b>itself.</b>
PROTEIN DETERMINATION	T0 - T1	-0,10578	0,003**
	T0 - T2	-1,74046	<0.001**
ASH DETERMINATION	T0 - T1	ON	ON
	T0 - T2	ON	ON
IRON DETERMINATION	T0 - T1	-0,02876	<0.001**
	T0 - T2	-0,02913	<0.001**
DETERMINATION OF POLYPHENOLS	T0 - T1	-0,00008	0,082**
	T0 - T2	-0,00023	0,001**
FAT DETERMINATION	T0 - T1	ON	ON
	T0 - T2	ON	ON
CARBOHYDRATE DETERMINATION	T0 - T1	0,13094	0,086*
	T0 - T2	1,81025	<0.001*
DETERMINATION OF ANTIOXIDANT CAPACITY	T0 - T1	-16,07000	<0.001*
	T0 - T2	-2,29000	<0.001*

Note: \*Dunnett test, \*\*Tukey, NA post hoc test: Not executed

Regarding the Table 4, in the percentage of proteins, the sample with 20 minutes of ultrasound of the ILLPA variety had the highest concentration (21.1%), followed by the sample with 10 minutes of ultrasound (19.4%) and the sample without treatment (19.3%); for the CUPI variety (

Table 5) The sample with 20 minutes of ultrasound had a higher concentration with 19.0%, the sample with 10 minutes of ultrasound with 18.9% and the sample without treatment with 17.9%.

**Table 3.** Multiple Comparison Statistical Test of the Cañihua CUPI

<b>CUPI VARIETY</b>			
<b>ANALYSIS</b>	<b>TREATMENTS</b>	<b>MEAN DIFFERENCE</b>	<b>itself.</b>
PROTEIN DETERMINATION	T0 - T1	-1,06222	0,06*
	T0 - T2	-1,18788	0,051*
ASH DETERMINATION	T0 - T1	-0,14807	0,024**

		T0 - T2	-0,49878	<0.001**
IRON DETERMINATION		T0 - T1	-0,00372	<0.001**
		T0 - T2	0,00563	<0.001**
DETERMINATION OF POLYPHENOLS		T0 - T1	-0,00016	0,003**
		T0 - T2	-0,00073	<0.001**
FAT DETERMINATION		T0 - T1	ON	ON
		T0 - T2	ON	ON
CARBOHYDRATE DETERMINATION		T0 - T1	1,2229	<0.001**
		T0 - T2	1,74764	<0.001**
DETERMINATION OF ANTIOXIDANT CAPACITY		T0 - T1	-0,20667	<0.001**
		T0 - T2	10,48667	<0.001**

Note: \*Dunnett Test, \*\*Tukey Post Hoc Test, NA None

**Table 4.** Physicochemical characterization of the Cañihua ILLPA

ANALYSIS CARRIED OUT		T0 (No treatment)	T1 (Sample 10 min ultrasound)	T2 (20 min ultrasound sample)
	UNITS	AVERAGE	AVERAGE	AVERAGE
PROTEIN DETERMINATION	% B/S	19.3	19.4	21.1
ASH DETERMINATION	% B/S	3.63	3.62	3.63
IRON DETERMINATION	mg/Kg B/S	53.4	82.2	82.5
DETERMINATION OF POLYPHENOLS	mg EAG/100g B/S	2.94	3.03	3.18
FAT DETERMINATION	% B/S	0.639	0.641	0.680
CARBOHYDRATE DETERMINATION	% B/S	76.3	76.2	74.5
DETERMINATION OF ANTIOXIDANT CAPACITY	mmol/mL TROLOX BH	19.6	35.7	21.9

**Table 5.** Physicochemical characterization of the Cañihua CUPI

ANALYSIS CARRIED OUT	UNITS	T0 (No treatment)	T1 (Sample 10 min ultrasound)	T2 (20 min ultrasound sample)
		AVERAGE	AVERAGE	AVERAGE
PROTEIN DETERMINATION	% B/S	17.9	18.9	19.0
ASH DETERMINATION	% B/S	3.47	3.62	3.97
IRON DETERMINATION	mg/Kg B/S	55.7	59.4	50.1
DETERMINATION OF POLYPHENOLS	mg EAG/100g B/S	2.90	3.06	3.63
FAT DETERMINATION	% B/S	0.645	0.654	0.711
CARBOHYDRATE DETERMINATION	% B/S	78.0	76.7	76.2
DETERMINATION OF ANTIOXIDANT CAPACITY	mmol/mL TROLOX BH	31.0	31.2	20.5

### Ash determination

Through the Table 1, in the quantification of ash, for the ILLPA variety, the Shapiro Wilk normality test was executed, resulting in a parametric behavior ( $p>0.05$ ), the ANOVA test was executed, with a  $p>0.05$ , none of the treatments has an effect on the determination of ash; for the CUPÍ variety, a parametric behavior was also obtained, in the ANOVA test it was a  $p<0.05$ , some of the treatments have an effect on the determination of ash, and the HSD Tukey multiple comparison test was executed.

The HSD Tukey multiple comparison test was developed for the CUPÍ variety (Table 3), due to the homogeneity of variances, presented a significance greater than 0.05 for T0 and T1, while for T0 and T2 it presented a significance less than 0.05, with this, it was determined that the best treatment is the sample with 20 minutes of ultrasound in front of the control.

Regarding the Table 4, in the percentage of ashes, the sample with 20 minutes of ultrasound of the ILLPA variety, together with the control sample had a higher concentration with 3.63%, followed by the sample with 10-minute treatment that presented a value of 3.62%, so, according to the

Table 5 in the CUPÍ variety, the highest ash content was found in the sample with 20 minutes of ultrasound treatment with 3.97%, followed by the sample with 10 minutes of ultrasound with 3.62% and finally the sample without treatment with 3.47%.

### Iron Determination

Through the Table 1, for the ILLPA variety and the CUPÍ variety, the Shapiro – Wilk normality test was executed, resulting in a parametric behavior ( $p>0.05$ ) for the treatments of both varieties, the ANOVA test was executed presenting a  $p<0.05$ , at least one of the treatments has an effect on the

determination of iron, so the Tukey multiple comparison test was performed, since there was homogeneity of variances.

In the Tukey test of the ILLPA variety (Table 2), a  $p < 0.05$  was obtained as a result, so there is a difference between the treatments, with the difference in means it was determined that the best treatment was the sample with 20 minutes of ultrasound in front of the control; considering the Tukey test of the CUPI variety (Table 3) a  $p < 0.05$  was obtained, which means that there is a difference between the treatments, thus, from the difference in means, the sample with 10 minutes of ultrasound was the best compared to the control.

According to the Table 4, in the iron content, the sample with 20 minutes of ultrasound of the ILLPA variety had the highest content with 0.083 g/Kg, followed by the sample with 10 minutes of ultrasound which presented 0.082 g/Kg and the untreated sample had 0.053 g/Kg; on the other hand in the CUPI variety, from the

Table 5, the sample with 10 minutes of ultrasound had the highest concentration with 0.059 g/Kg, followed by the sample without ultrasound which had 0.056 g/Kg, and the sample with 10-minute treatment had 0.050 g/Kg.

### **Determination of total polyphenols**

Through the Table 1, for the ILLPA and CUPI varieties, the Shapiro – Wilk normality test presented a parametric behavior, the ANOVA test was executed obtaining a  $p < 0.05$  for both varieties, where at least one of the treatments has an effect on the determination of polyphenols, with this, the Tukey multiple comparison test was executed.

In the Tukey test, thanks to the homogeneity of variances, for the ILLPA variety (Table 2), there was no difference between T0 and T1 ( $p > 0.05$ ), but there was a statistically significant difference between T0 and T2 ( $p < 0.05$ ), where the sample with 20 minutes of ultrasound was better compared to the control; as soon as, to the Tukey test of the CUPI variety (Table 3) there was a statistically significant difference between treatments ( $p < 0.05$ ), and with the mean difference, the best treatment was the sample with 20 minutes of ultrasound in front of the control.

Likewise, according to the Table 4, in the content of total polyphenols, the samples of the ILLPA variety had the same concentration 0.003 g EAG/100g; on the other hand, in the CUPI variety, from the

Table 5, The highest concentration was in the sample with 20 minutes of ultrasound with 0.004 g EAG/100g, followed by the sample with 10 minutes of ultrasound and the sample without treatment which had 0.003 g EAG/100g.

### **Fat Determination**

Through the Table 1, for the ILLPA variety, the Shapiro – Wilk normality test presented a non-parametric behavior ( $p < 0.05$ ), the Kruskal – Wallis test was executed resulting in a  $p > 0.05$ , that is, none of the treatments has an effect on the determination of fats; for the CUPI variety, a parametric behavior ( $p > 0.05$ ) was obtained, so the ANOVA test was developed resulting in a significance greater than 0.05, which indicated that no treatment has an effect on the determination of fats.

In the Table 4, regarding the percentage of fat, the sample with 20 min of ultrasound of the ILLPA variety had the highest concentration (0.680%), followed by the sample with 10 min of ultrasound (0.641%) and finally the control sample (0.639%); for the CUPI variety (

Table 5), treatment with 20 min of ultrasound had the highest concentration (0.711%), followed by the sample with 10 min of ultrasound (0.654%) and the sample without treatment (0.645%).

### **Carbohydrate Determination**

Through the Table 1, in the ILLPA variety, with the Shapiro Wilk test a non-parametric behavior ( $p < 0.05$ ) was obtained, the Kruskal-Wallis test was executed obtaining  $p < 0.05$ , therefore there is an effect of at least one of the treatments in the determination of carbohydrates, for this reason the Dunnett multiple comparison test was performed; for the CUPI variety, a parametric behavior ( $p > 0.05$ ) was

obtained, the ANOVA test was executed, in which it was found that at least one treatment has an effect on the determination of carbohydrates, to verify the above the HSD Tukey test was performed.

In Dunnett's multiple comparison test, for the ILLPA variety (Table 2), there was no statistically significant difference ( $p>0.05$ ) for T0 and T1, but there was a statistically significant difference for T0 and T2 ( $p<0.05$ ), as a result the best sample was the 20-minute ultrasound treatment in front of the control; with respect to the CUPI variety using the Tukey test (Table 3), by the homogeneity of variances, where All treatments had a statistically significant difference ( $p<0.05$ ), with the mean difference, the best sample was treatment with 20 minutes of ultrasound in front of the control.

With the Table 4, in the percentage of carbohydrates, for the ILLPA variety, the control sample had the highest concentration (76.3%), followed by the sample with 10 min of ultrasound (76.2%) and finally the sample with 20 min of ultrasound (74.5%); for the CUPI variety (

Table 5), the control treatment had the highest concentration (78.0%), followed by treatment with 10 min of ultrasound (76.7%) and finally treatment with 20 min of ultrasound (76.2%).

### Determination of antioxidant capacity

Through the Table 1, for the ILLPA variety, the Shapiro – Wilk normality test showed a non-parametric behavior ( $p<0.05$ ), the Kruskal-Wallis test was executed, it was pointed out that at least one of the treatments has an effect on the determination of antioxidant capacity, with this the Dunnett test was developed; for the CUPI variety, parametric behavior ( $p>0.05$ ) was presented, the ANOVA test was developed, with a  $p<0.05$ , which indicates that at least one of the treatments has an effect on the determination of antioxidant capacity, to verify the aforementioned, the HSD Tukey test was performed.

Considering the Dunnett test for the ILLPA variety (Table 2), it was obtained that all treatments have a significant difference ( $p<0.05$ ), and with the mean difference, the best sample was the treatment with 10 minutes of ultrasound; the Tukey test, as a result of the homogeneity of variances, for the CUPI variety (Table 3) It was obtained that all treatments had a statistically significant difference ( $p<0.05$ ), with the difference in means, the best sample was the treatment with 10 minutes of ultrasound in front of the control.

In the Table 4, regarding the antioxidant capacity, the sample with 10 min of ultrasound of the ILLPA variety had the highest concentration (35.7 mmol/mL TROLOX), followed by the sample with 20 min of ultrasound (21.9 mmol/mL TROLOX) and the witness (19.6 mmol/mL TROLOX); in the CUPI variety (

Table 5), the highest concentration was in the sample with 10 min of ultrasound (31.2 mmol/mL TROLOX), followed by the sample with 20 min of ultrasound (31.1 mmol/mL TROLOX) and finally the witness (31.0 mmol/mL TROLOX).

## DISCUSSION

Authors point out that protein content is linked to the presence of so-called essential amino acids and also to sulfur amino acids, including lysine, histidine, methionine, cysteine or isoleucine [22]. Ultrasound improves the volume of free amino acids, changing the permeability of the cell membrane, increasing the speed of mass transfer, and encouraging enzymatic reactions [23]. In the present study, the percentage of proteins in the sample with 20 min at 40 kHz of the ILLPA variety had a higher concentration with 21.1% (BS), for the CUPI variety it had 19.0% (BS), compared to the control; there are reports that a combination of rice flour (90%), with Cañihua ILLPA (10%) has a higher protein content (8.80% dry weight) and with Cañihua CUPI (10%) a value of 8.27% dry weight [24], Chen et al. (2023) reported that the degradation of sugars caused energy in order to execute protein synthesis at the time of germination, In addition, compounds such as amylase, protease, and lipase improved significantly after performing the treatment with double frequency ultrasound [25]. The data presented coincide with Chatchavanthatri et al. (2020) who in their study of sprouted brown rice used 35 kHz for 30 min, perceived an increase in protein content of 8.41% based on dry weight for brown rice, reaching values of 8.77% based on dry weight for sprouted brown rice [26]. Ultrasound of pseudocereals transforms weak interactions leading to conformational changes of both secondary and tertiary



structures, causing proteins to unravel as well as hydrophobic nuclei, which are abundant in tyrosine, phenylalanine, and tryptophan; exposing them, increasing surface hydrophobicity [27].

### Ashes

In the percentage of ashes, the samples of the ILLPA variety did not present statistically significant differences, even so the control sample and the sample with 10 minutes of ultrasound had a higher concentration with 3.63% (BS), on the other hand for the CUPI variety there was a statistically significant difference, where the treatment of 20 minutes of ultrasound, presented a higher value with 3.97% (BS) compared to the control; Levent & Aktaş (2024) in black lentils germinated with ultrasound and ultraviolet (UV) radiation did not determine significant difference in ash content, but had a value of 4.26% (dry matter) for germinated black lentil and a value of 4.35% (dry matter) for black lentil germinated with ultrasound, being higher than what was reported for the control, thus the ash content increased slightly in the germination and ultrasound process [28]. On the other hand, Chatchavanthatri et al. (2020) in their study of sprouted brown rice used 35 kHz for 30 minutes, the ash content decreased to 0.48% (dry weight) compared to brown rice which had 0.85% (dry weight), germination had an effect on the ash content [26].

### Iron

Xia et al. (2020) reported that they used 28 kHz at 5, 10, 15 and 30 min, the treatment considerably improved amino acids and the in vitro bioaccessibility of calcium and iron, so the soluble iron after germination could be attributed to the high affinity that iron cations have with polyphenols to form the iron-polyphenol complex; in the same way, This increased, thanks to the transformation of the insoluble polyphenols bound to water during pregermination, they estimated that pregermination by high-intensity ultrasound favors the decomposition of mineral-bound molecules [29], in the results presented it was observed that at 40 kHz at 20 minutes, the ILLPA variety had a higher iron content with 82.5 mg/Kg (BS); in the case of the CUPI variety it had a higher value of 59.4 mg/Kg (BS) with the 10-minute treatment at 40kHz, in front of the control sample. Similarly, Levent & Aktaş (2024) in their study pointed out the existence of an increase in iron of 11.0% in germinated black lentils and 21.4% in black lentils germinated with ultrasound and UV, so the application of US and UV caused an additional increase in iron content in black lentils [28].

### Total polyphenols

In the results presented, there was no significant difference between the treatments of the ILLPA variety, but the highest value was presented by the 20-minute ultrasound treatment with an amount of 3.18 mg EAG/100g (BS), while for the CUPI variety there was a statistically significant difference, there was a higher polyphenol content with 20 minutes of ultrasound with a value of 3.63 mg EAG/100g (BS) compared to the control. likewise, the results presented coincide with Levent & Aktaş (2024), who pointed out that the lentil germination process with ultrasound and UV significantly affected the phenolic content, in this way, said content was increased, where the lowest values were presented in the control sample, followed by the lentil germination treatment and finally the lentil sample germinated with 35 kHz ultrasound for 10 minutes, in addition, treatment with US allows cereals to become a potent source of bioactive compounds [28], according to Altıkardeş & Güzel (2024) in their study where buckwheat and quinoa were used in a pregermination by ultrasound at 30 min with 37 kHz for a time of 72 h, thus indicating the existence of significant changes in polyphenols, therefore, they obtained values of mg EAG/100 g expressed in dry weight; for buckwheat a value of 458.24 compared to the control and 615.42 for wheat sprouted with ultrasound, with an increase of 34%; in the case of quinoa, it had a value of 204.22 for control and 216.45 for quinoa germinated with ultrasound, with an increase of 8% [30].

### Fats

There was no effect of the treatments on the fat content for the ILLPA and CUPI varieties, coinciding with what was pointed out by Levent & Aktaş (2024) that the fat content for sprouted lentils with ultrasound was not determined significant difference [28], on the other hand in the results presented the samples with treatment had a higher content compared to the control, with values of 0.680% (BS) with

20 min treatment, 0.641% (BS) with 10 min treatment and 0.639% (BS) without treatment for the ILLPA variety; in addition, 0.711% (BS) for 20 min treatment, 0.654% (BS) for 10 min treatment and 0.645 were available% (BS) without treatment for the CUPU variety, this pointed out that ultrasonic processes allow the decomposition of lipids, thus increasing the yield of oils in sprouted cereals, without affecting their content of essential fatty acids [23]. Wu et al. (2024) indicated that ultrasound allows the acceleration of metabolism, which leads to the use of fats, decreasing them through the process of respiration [31], as reported by Levent & Aktaş (2024) it does not coincide with the proposed study, since the control treatment had 3.40% (dry matter) and for lentils germinated with ultrasound of 35 kHz for 10 min it had 3.18% (dry matter). there being a decrease [28], in the same way it does not coincide with Chatchavanthatri et al. (2020) who studied sprouted brown rice with 35 kHz for 30 min; the fat content was 2.92% (dry weight) for brown rice and 2.85% (dry weight) for sprouted brown rice, where a reduction was seen [26]; likewise, the results do not coincide with what was reported by Jia et al. (2021) who point out that the fat content is related to energy, with respect to flaxseed with ultrasound for 22 minutes, this was reduced by an amount of 18.37% at 36 hours of germination, this was produced by energy consumption and nutritional requirement, metabolic activity during the germination process [32].

### **Carbohydrates**

The carbohydrate content for the ILLPA variety was higher in the control treatment with 76.3% (BS), followed by the sample with 10 min of ultrasound of 76.2% (BS) and finally the sample with 20 min of ultrasound of 74.5% (BS); on the other hand, the CUPU variety had a higher concentration in the control sample with 78.0% (BS). followed by the sample with 10 min of ultrasound with 76.7% (BS) and finally the sample with 20 min of ultrasound with 76.2% (BS). Regarding germination, enzymes activate changes significantly, where  $\alpha$  and  $\beta$ -amylases rapidly break down carbohydrates, increasing reducing sugars, ultrasound treatments lead to depressions, pores and cracks in starch granules from jets of rapid water [33]. Similarly, in the germination period of grain seeds, they supply energy from the degradation of both carbohydrates and lipids, which causes their decrease [30]. Likewise, the results do not coincide with Chatchavanthatri et al. (2020) who studied sprouted brown rice with 35 kHz for 30 minutes, in which the carbohydrate content was 85.58% expressed in dry weight, for brown rice; and 87.30% expressed in dry weight, for sprouted brown rice, where an increase was seen [26].

### **Antioxidant capacity**

Ultrasound at 40kHz for 10 min produced an antioxidant capacity of 35.7 mmol/mL TROLOX in the Cañihua ILLPA, and for the CUPU variety the same happened, the treatment with 10 min of ultrasound presented 31.2 mmol/mL TROLOX, these results compared to the values presented by the control. Exposure of food to ultrasound acts as an abiotic stressor, triggering protective responses in the plant, stimulating the manufacture of secondary metabolites thus improving their antioxidant activity during germination [11]. According to Callohuanca et al. (2021) in their analysis of the antioxidant capacity for Cañihua, it was obtained that the black variety presented 19.89 mmol TROLOX/ 100 g, the purple color 12.33 mmol TROLOX/ 100 g, the gold color 10.89 mmol TROLOX/ 100 g and the orange color 9.25 mmol TROLOX/ 100 g [34], resulting that the antioxidant capacity is greater in the varieties with an increase in color intensity, thus also concentrating flavonoids [34]. In addition, the activity that allows the elimination of DPPH free radicals obtained 80.51 mmol TROLOX/g (dry weight) in peanuts after executing germination for a time of 2 days [35], Deore et al. (2023) used 50 kHz with 10, 15 and 20 minutes in sorghum, with a gradual reduction of the values of antioxidant capacity with the increase of treatment time, the enzymatic synthesis that has a place during germination allows the improvement of polyphenolic compounds; and the antioxidant capacity that is directly associated with them also improves [6]. The antioxidant capacity of sprouts reveals the composition of the grain and its content with respect to bioactive compounds such as phenolic compounds, vitamins and GABA [23], in the same way, the antioxidant capacity is consistent with the content of polyphenols, it is attributed that the improvement of antioxidant activity is due to the content of antioxidants such as polyphenols, additionally due to the presence of vitamins E and C [1].

### **CONCLUSIONS**

It was possible to apply 40 kHz ultrasound for 10 and 20 minutes before germinating the Cañihua of the ILLPA and CUPI variety where it was possible to know its positive effects on protein content, iron content, ashes, antioxidant capacity, total polyphenols and fats.

It was possible to determine the effects of ultrasound at 40 kHz on the properties of bioactive and nutritional compounds where the highest content was the ILLPA variety, with a 20-minute ultrasound treatment obtained 21.1% (BS) of proteins, 0.083 g/Kg (BS) of iron, 35.7 mmol/ml TROLOX in the antioxidant capacity and lower carbohydrate content with 74.5% (BS) both procedures such as germination and ultrasound allowed the compounds to be In addition, this variety has a higher content of the compounds indicated in relation to the CUPI variety, even so the CUPI variety presented higher values of some compounds, such as with a 20-minute ultrasound treatment of 3.97% (BS) of ash, 0.004 g/100g BS of polyphenols and 0.711% (BS) of fat, providing a health benefit to those who consume it.

### **Recommendations**

It is recommended to evaluate and compare the nutritional and bioactive compounds presented by Cañihua ILLPA during a germination process, as well as germination with ultrasound and germination by microwave.

It is recommended to evaluate the ultrasound treatment and germination of 40 kHz and times of 10 and 20 minutes in other varieties of Cañihua existing in our country.

It is recommended to determine and quantify the amino acid content present in the ILLPA and CUPI varieties after applying ultrasound and germination treatments at 40 kHz in the times of 10 and 20 minutes.

It is recommended to apply the Cañihua ILLPA with ultrasound and germination treatments in a product to facilitate its consumption and obtain the benefits presented.

### **Conflicts of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### **Funding**

The research was conducted independently by the researchers, and no funding was received.

### **Acknowledgement**

The authors would like to express their gratitude to all the participants and the authorities of the Universities, who allowed this investigation to take place.

### **Data availability**

The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

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## **REFERENCES**

1. Lidong W, Xiaoqiang L, Fei G, Ying L, Shuangjing L, Changyuan W, et al. Effect of ultrasound combined with exogenous GABA treatment on polyphenolic metabolites and antioxidant activity of mung bean during germination. *Ultrasonics Sonochemistry*. 2023; 94: p. 106311.
2. Castillo WE, Paucar LM. Rheological behavior of wheat flour bread doughs substituted with germinated quinoa and Cañihua flours. *INGnosis*. 2021; 7(1): p. 25–33.
3. Ponce-Quispe G, Siche R. Ultrasound-assisted grainhydration and its effect on starches:A review. *Agroind. sci*. 2022; 12(3): p. 365-374.

4. Bender D, Schönlechner R. Recent developments and knowledge in pseudocereals including technological aspects. *Acta Aliment.* 2021; 50(4): p. 583-609.
5. Abderrahim F, Huanatico E, Repo-Carrasco-Valencia R, Arribas SM, Gonzalez MC, Condezo-Hoyos L. Effect of germination on total phenolic compounds, total antioxidant capacity, Maillard reaction products and oxidative stress markers in canihua (*Chenopodium pallidicaule*). *J. Cereal Sci.* 2012; 56(2): p. 410-417.
6. Deore A, Athmaselvi KA, Venkatachalapathy N. Effect of ultrasound and microwave pretreatment on sprouting, GABA, bioactive compounds, and other physicochemical properties of sorghum. *Grain & Oil Science and Technology.* 2023; 6(2): p. 91-99.
7. Paucar LM, Schmiele M, Lavado AA, Verona AL, Mollá C, Peñas E, et al. Andean Sprouted Pseudocereals to Produce Healthier Extrudates: Impact in Nutritional and Physicochemical Properties. *Foods.* 2022; 11(20): p. 3259.
8. Simpalo WD, Paucar LM. Effect of the partial incorporation of germinated kiwicha and Cañihua flour on the rheological characteristics of a bread dough. *INGnosis.* 2021; 7(1): p. 50-58.
9. Instituto Nacional de Calidad. NTP 209.262:2013 (revisada el 2023) INSTANT RECONSTITUTED COOKED FOODS. Determination of protein. Kjeldahl method. Norma Técnica Peruana. Instituto Nacional de Calidad.
10. Yüksel Y, Elgün A. Determination of the effect of high energy ultrasound application in tempering on flour quality of wheat. *Ultrasonics Sonochemistry.* 2022; 67: p. 105129.
11. Naumenko N, Potoroko I, Kalinina I. Stimulation of antioxidant activity and  $\gamma$ -aminobutyric acid synthesis in germinated wheat grain *Triticum aestivum* L. by ultrasound: Increasing the nutritional value of the product. *Ultrasonics Sonochemistry.* 2022; 86: p. 106000.
12. Instituto Nacional de Calidad. Norma Técnica Peruana - NTP 209.265:2013 (revised in 2018) - INSTANT RECONSTITUTION COOKED FOODS. Determination of ashes. Gravimetric method. 2nd Edition. Norma Técnica Peruana. Instituto Nacional de Calidad.
13. Elías JW, García CE, Pérez R, Yauris CR. Physicochemical Characterization of Bread with Partial Sustitución of wheat flour for Quinoa flour (*Chenopodium quinoa willd*) and Kiwicha (*Amaranthus caudatus* L.) Germinated. *SENDAS.* 2021; 2(2): p. 69-83.
14. AOAC. AOAC 945.40-1945, Iron in bread. Preparation of test sample. AOAC Official Method. AOAC International.
15. AOAC. AOAC 944.02-1944(1993), Iron in flour. Spectrophotometric method. AOAC Official Method. AOAC International.
16. González NE, Acosta JMM, Galeano MJ, Morínigo GM, Bonzi MC, Ovelar EC. Adequacy of micronutrient content in wheat flour with iron and vitamins in Paraguay during the period 2015-2019. *Perspectivas En Nutrición Humana.* 2023; 25(1): p. 31-43.
17. Multescu M, Marinas IC, Susman IE, Belc N. Byproducts (Flour, Meals, and Groats) from the Vegetable Oil Industry as a Potential Source of Antioxidants. *Foods.* 2022; 11(3): p. 253.
18. Tian W, Chen G, Zhang G, Wang D, Tilley M, Li Y. Rapid determination of total phenolic content of whole wheat flour using near-infrared spectroscopy and chemometrics. *Food Chem.* 2021; 344: p. 128633.
19. Huamaní F, Tapia M, Portales R, Doroteo V, Ruiz C, Rojas R. Proximate analysis, phenolics, betalains, and antioxidant activities of three ecotypes of Kañiwa (*Chenopodium Pallidicaule* Aellen). *PharmacologyOnLine.* 2020; 1: p. 229-236.
20. Mérida J, Rojas CC, Bergenstahl B, Purhagen J. Functional properties of starch cultivars of two Andean grains grown in Bolivia: *Amaranth* (*Amaranthus caudatus*) and canihua (*Chenopodium pallidicaule*). *Heliyon.* 2024; 10(15): p. e356140.
21. AOAC. AOAC 963.15-1973, Fat in Cacao Products - Soxhlet Extraction Met. AOAC Official Method. AOAC International.
22. Dávalos JZ, Tirado A, Romero V, Cisneros G, Gamarra F. Structural, thermal and energetic properties of Andean-pseudocereal flours with high nutritional values. *Journal of Thermal Analysis and Calorimetry.* 2023; 148: p. 7207-7215.
23. Liu H, Li Z, Zhang X, Liu Y, Hu J, Yang C, et al. The effects of ultrasound on the growth, nutritional quality and microbiological quality of sprouts. *Trends in Food Science & Technology.* 2021; 111: p. 292-300.

24. Quispe AJ, Moreno MC, Leon AM, Bouchon P, Medina WT. Design of Cañihua-rice: Development and characterization of an analogue of rice by warm-extrusion of Cañihua (*Chenopodium pallidicaule* Aellen) and rice (*Oryza sativa*) flours. *Food and Humanity*. 2024; 2: p. 100193.
25. Chen J, Shao F, Igbokwe CJ, Duan Y, Cai M, Ma H, et al. Ultrasound treatments improve germinability of soybean seeds: The key role of working frequency. *Ultrasonics Sonochemistry*. 2023; 96: p. 106434.
26. Chatchavanthatri N, Junyusen T, Moolkaew P, Arjharn W, Junyusen P. Effect of soaking and sprouting treatment on germination rate of paddy. *E3S Web of Conferences*. 2020; 187: p. 04016.
27. Estivi L, Brandolini A, Condezo L, Hidalgo A. Impact of low-frequency ultrasound technology on physical, chemical and technological properties of cereals and pseudocereals. *Ultrasonics Sonochemistry*. 2022; 86: p. 106044.
28. Levent H, Aktaş K. The effect of germinated black lentils on cookie quality by applying ultraviolet radiation and ultrasound technology. *Journal of Food Science*. 2024; 89(5): p. 2557-2566.
29. Xia Q, Tao H, Li Y, Pan D, Jinxuan C, Liu L, et al. Characterizing physicochemical, nutritional and quality attributes of wholegrain *Oryza sativa* L. subjected to high intensity ultrasound-stimulated pre-germination. *Food Control*. 2020; 108: p. 106827.
30. Altıkardeş E, Güzel N. Impact of germination pre-treatments on buckwheat and Quinoa: Mitigation of anti-nutrient content and enhancement of antioxidant properties. *Food Chemistry: X*. 2024; 21: p. 101182.
31. Wu M, Zhou Q, Zhou L, Wang J, Ren T, Zheng Y, et al. Enhancement of  $\gamma$ -Aminobutyric Acid and the Characteristics of Nutrition and Function in White Quinoa through Ultrasound Stress at the Pre-Germination Stage. *Foods*. 2024; 13(1): p. 57.
32. Jia C, Tang L, Huang F, Deng Q, Huang Q, Zheng M, et al. Effect of Ultrasound or Microwave-Assisted Germination on Nutritional Properties in Flaxseed (*Linum usitatissimum* L.) with Enhanced Antioxidant Activity. *ACS Food Science & Technology*. 2021; 1(8).
33. Estivi L, Brandolini A, Condezo L, Hidalgo A. Impact of low-frequency ultrasound technology on physical, chemical and technological properties of cereals and pseudocereals. *Ultrasonics Sonochemistry*. 2022; 86: p. 106044.
34. Callohuanca MA, Mamani E, Mamani J, Canaza AW. Perigonium color and the antioxidant capacity of Cañihua (*Chenopodium pallidicaule* Aellen). *Revista de Ciencias Agrícolas*. 2021; 38(2): p. 99-110.
35. Zhou Z, Fan Z, Meenu M, Xu B. Impact of Germination Time on Resveratrol, Phenolic Acids, and Antioxidant Capacities of Different Varieties of Peanut (*Arachis hypoEAGa* Linn.) from China. *Antioxidants*. 2021; 10(11): p. 1714.