LWT - Food Science and Technology 96 (2018) 527–534

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Quinoa germ and starch separation by wet milling, performance and characterization of the fractions

Jesica R. Mufari[a,b,*], Patricia P. Miranda-Villa[a,b], Edgardo L. Calandri[a,b]

[a] Instituto de Ciencias y Tecnología de los Alimentos - ICTA. Facultad de Ciencias Exactas Físicas y Naturales, Córdoba, Argentina
[b] Instituto de Ciencias y Tecnología de los Alimentos Córdoba - ICyTAC (CONICET), Córdoba, Argentina

ARTICLE INFO

Keywords:
Quinoa
Wet milling
Germ
Protein enrichment
Starch

ABSTRACT

Quinoa is a pseudocereal with remarkable nutritional characteristics due to high content of gluten-free biologically valuable protein. The grain is mostly consumed as a whole grain or flour; however, protein fraction is concentrated in the germ, which is external, represents one third of the whole seed and surrounding the perisperm, composed mainly of starch. The aim of this work was to purpose a method for germ and perisperm separation, through a wet milling stage in a roller mill. The method described enables to obtain an enriched fraction of the germ that represents a recovery of 96.5% of the proteins, 95.8% of lipids and 92.6% of ashes, with respect to the germ manually extracted. Additionally, the starch can be recovered in a further step. The extraction yields, nutritional losses and characteristics of the different fractions are discussed.

1. Introduction

At present there is a growing interest in vegetal as potential protein suppliers. Quinoa is a pseudocereal originated in the South American Andes, which recently won great relevance for its remarkable nutritional qualities. The most outstanding aspect lies in the quality of its proteins, which are gluten free and have a high nutritional value, with all the essential amino acids and in high proportion (Brakez, Daoud, Harrouni, Tachbibi, & Brakez, 2016; Koziol, 1992; Repo-Carrasco, Espinoza, & Jacobsen, 2003). Both whole flour, obtained from hammer or roller milling, and whole seed are incorporated to different food matrix (Föste, Elgeti, Brunner, Jekle, & Becker, 2015; Ramos Díaz et al., 2013). Additionally, numerous studies focused on obtaining new products from quinoa grains, such as protein isolates, starch, oil, malt, dyes, saponin derivatives with greater added value are detailed (Quiroga et al., 2014).

Quinoa germ or embryo is the reproductive part of the seed, composed by two cotyledons and a radicle. It is peripheral, constitutes approximately 30% of the grain and wraps the perisperm like a ring with a curvature of 320° (Prego, Maldonado, & Otegui, 1998). The germ is yellowish-white in color, 3.5–8.2 mm in length and approximately 0.35 mm width (Gallardo, Gonzáles, & Ponessa, 1997). On the contrary, cereals germ is found inside the grain and represents 1–2% and 5–14% of wheat and maize seeds, respectively (Siddiq, Nasir, Ravi, Dolan, & Butt, 2009). Most of quinoa proteins are located in the germ, representing 35–40% of the germ, while perisperm only contains 6.3–8.3%. Germ also contains lipids, fat-soluble vitamins and reserve minerals (Prego et al., 1998). Quinoa embryo can be separated from the rest of the seed and then be used in a wide range of products, due to its high nutritional value (Mufari, 2015). Cereal germ is generally obtained as a by-product of other industrial processes (Ma et al., 2014; Tsen, 1980). Although it concentrates most of proteins, it tends to be deficient in some essential amino acids (Serna-Saldivar, 2016, pp. 703–711) and, in the particular case of wheat, barley, oats and rye, are unsuitable for gluten-allergic people (Capriles & Arêas, 2014; Gallagher, Gormley, & Arendt, 2004).

Quinoa starch is an interesting by-product with excellent technological qualities due to small granule size, between 0.4 and 3 μm. It has been used to retain water and form stable gels (Li, Wang, & Zhu, 2016) to improve the behavior of gluten-free breads (Turkut, Cakmak, Kumcuoglu, & Tavman, 2016), to make biodegradable films (Araujo-Farro, Podadera, Sobral, & Menegalli, 2010) and in the fixation of aromas (Tari & Singhal, 2002; Tari, Annapure, Singhal, & Kulkarni, 2003). Most of the studies focused in the extraction of protein concentrates and quinoa starch from the wholemeal flours involves wet methods (Abugoch, Romero, Tapia, Silva, & Rivera, 2008; Elsohaimy, Refaay, & Zaytoun, 2015; Guerreo-Ochoa, Pedreschi, & Chirinos, 2015). Being starch granule size so small, the separation processes based on gravimetrics or particle size are difficult, giving rise to low purities and yields (Föste et al., 2015). There is a registered patent US 20140161950A1 (Pouvreau, Kanning, & Van de Velde, 2014) where milling of wet quinoa grains in a knife mill is proposed, and therefore,

* Corresponding author. Av. Vélez Sarsfield 1611, C.P. 5016, Córdoba, Argentina.
E-mail addresses: romi_mufari@hotmail.com, rominamufari@gmail.com (J.R. Mufari).

https://doi.org/10.1016/j.lwt.2018.06.010
Received 29 April 2018; Received in revised form 2 June 2018; Accepted 4 June 2018
Available online 06 June 2018
0023-6438/

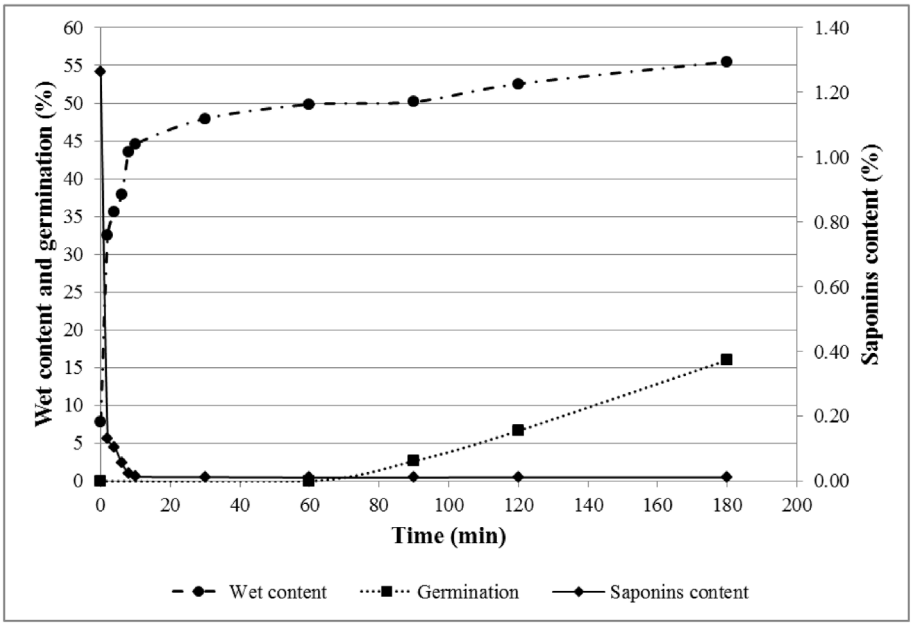

Fig. 1. Moisture content of quinoa, residual concentration of saponins and germination vs. soaking time.

the dispersions obtained enable to regain starch and proteins. Although it has lower losses because of the use of whole seeds instead of flour, the problems due to separation of the different fractions still remains. In recent works, processes of quinoa grains dry grinding were optimized, where sieving and classification by air current stages are incorporated. Therefore, high-starch and high-germ flours are obtained. From these last flours, proteins are removed by wet process, which improves the extraction procedure obtaining protein isolates of better final quality (Avila Ruiz, Arts, Minor, & Schutyser, 2016a; Föste et al., 2015).

The aim of this study is to propose innovative separation process through wet grain milling, allowing the complete separation of germ and starch, two products with numerous practical applications. Also, saponins might be regained from the waste streams of the process.

2. Materials and methods

2.1. Origin and conditioning of quinoa

The seeds of quinoa (*Chenopodium quinoa* Willd.) used were purchased from a producer in the province of Salta, Argentina. The grains were classified and cleaning by sieving, selecting the seeds retained in sieve ASTM No. 12 (> 1.68 mm).

Different washing and wetting conditions of the grains were tested in a ratio of 1:5 (seed g: mL water), with stirring, at room temperature, for 180 min. Samples were taken every 2 min for the first 10 min and then every 30 min until the end of time, the moisture content, the residual saponin content and the germination percentage were determined.

The determination of the moisture content was made by the standard method of analysis 44-01 of AACC (2002). To calculate the percentage of germinated grains, 100 grains were randomly selected and by visual inspection the amount of germinated grains was determined.

The extraction of residual saponins was assisted by microwaves (MAE). In this method, extractions of 1 g of seeds were carried out with 20 ml of methanol. The MAE extraction parameters were: equipment microwave (Litton 16650, BGH, Argentine) power: 900 W, extraction time: 20 min, and temperature controlled: 80 °C. For the spectrophotometric quantification, the saponins in the extracts were derivatized by the Libermann-Burchard reaction and the absorbances were measured at 528 nm. The calibration curves were made with oleanolic acid as a standard (Gianna, Montes, Calandri, & Guzmán, 2012). All the tests were performed in triplicate.

2.2. Wet milling of quinoa seeds and separation of fractions

The milling tests were carried out in a roller mill (5 cm diameter, 17 cm long) of stainless steel, built in the laboratory. The conditioned seeds are ground by different distances between rollers, starting with an opening of 0.5 mm, and then lowered to 0.3 mm and 0.15 mm.

The ground material is subjected to two successive washings with water in the ratio 1: 5 (ground material:water), with vigorous agitation for 15 min each. The sample is filtered through a No. 200 ASTM mesh, recovering two fractions a solid enriched in germ and a dispersion of starch. The germ-rich fraction was dried with air flow of 1 m^3/s, with a temperature between 45 and 50 °C for 30 min in fluidized bed (Armfield-Sherwood Scientific, United Kingdom), the starch dispersion was centrifuged at 3500 rpm (1000 G) for 15 min; and the solid residue so obtained was vacuum dried at 40 °C for 24 h. The determination of the particle size distribution in the germ-enriched fraction was made, sifting in a vibrating screen for 15 min, the dry sample through the standard ASTM mesh No. 20, 30, 40, 50 and collector (with mesh openings of 840, 590, 420, 297 and 0 μm, respectively).

In both stages, the yield was determined in each mesh, in triplicate of each milling condition tested, using equation (1):

$$Yield\ (\%) = \left({}^{g\ fraction}\Big/_{g\ starting\ material} \right) \times 100 \quad (1)$$

2.3. Determination of the proportion of germ in the grain and reference sample

The embryo proportion in the seed was established from 25 randomly selected quinoa grains, being weighed and wetted for 1 h. The whole germ of each grain was removed manually, with histological clamp. The germs thus obtained were washed with distilled water, dried in an oven at 60 °C and the dry weight was determined; five replications were made and the result expressed in dry base.

2.4. Physical-chemical characterization of fractions

For macronutrients measurements methods recommended by the

Table 1
Yields of germ and starch rich fractions related to the milling processes and particle size distribution of the germ-rich fraction.

	Rolls gap (mm)			R200 (g)						E200 yield %	Losses %
				>840 µm	>590 µm	>420 µm	>297 µm	>0 µm	yield %		
Milling steps	0.5			93.2 ± 0.3	3.0 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	2.21 ± 0.4	86.1 ± 0.2a	7.7 ± 2.2a	6.2
	0.3			91.1 ± 0.5	2.5 ± 0.1	1.6 ± 0.1	0.3 ± 0.1	4.5 ± 0.7	84.3 ± 0.5b	10.0 ± 0.8a	5.7
	0.15			27.9 ± 0.6	35.0 ± 1.4	29.6 ± 0.1	4.5 ± 0.3	3.0 ± 0.3	55.2 ± 1.0c	20.9 ± 0.6b	23.9
	0.3		0.15	28.1 ± 1.3	36.6 ± 0.8	30.7 ± 0.3	1.2 ± 0.2	3.4 ± 0.2	51.4 ± 0.6d	25.8 ± 0.9c	22.8
	0.5	0.3	0.15	24.3 ± 1.3	19.2 ± 0.9	54.5 ± 0.9	1.0 ± 0.6	1.0 ± 0.3	44.3 ± 0.8e	33.5 ± 1.0d	22.2

Mean of three independent milling trials with standard deviation, different letters in same columns, denote statistically significant differences ($p < 0.05$).

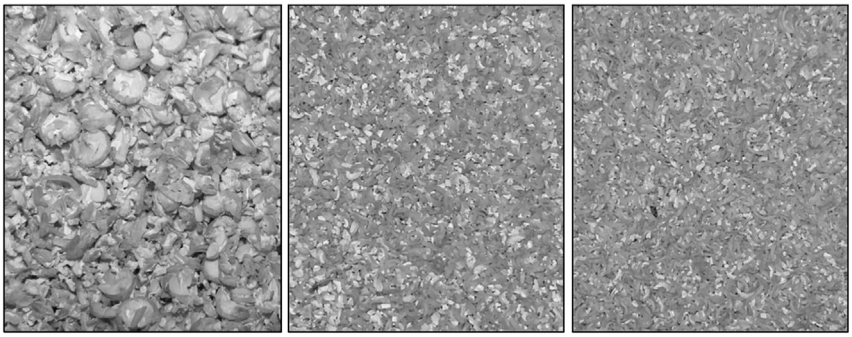

Fig. 2. Photograph of milling fractions.

Table 2
Proximal composition of the fractions obtained during wet milling, manually separated germ and quinoa grain. Comparison with wheat and corn germ.

Components	Three steps milling				Quinoa germ manual separation	Quinoa grain	Corn germ [1]	Wheat germ [2]
	E200	R200						
		R20	R30	R40				
Wet	5.27 ± 0.16	7.24 ± 0.02	7.79 ± 0.09	7.14 ± 0.13	7.10 ± 0.04	10.41 ± 0.01	11.26	8.40
Ash	0.45 ± 0.03a	2.61 ± 0.05b	3.65 ± 0.04c	4.26 ± 0.08d	4.60 ± 0.05	3.25 ± 0.03	4.10	4.26
Lipids	0.53 ± 0.21a	9.92 ± 0.10b	29.32 ± 0.40c	30.37 ± 0.29d	31.70 ± 0.27	8.89 ± 0.28	13.86	10.04
Proteins	7.23 ± 0.08a	18.26 ± 0.64b	33.11 ± 0.72c	35.18 ± 0.11d	36.47 ± 0.08	18.19 ± 0.27	14.24	35.59
Carbohydrates	91.79	69.21	33.92	30.19	27.23	69.67	67.80	50.11
Protein Yield (%, Eq. 2)	13.31	10.80	15.47	46.68				
Germ recovery (%)		31.29	92.49	95.80				

[1] Hernández, Guerra, & Rivero, 1999.
[2] Bejarano et al., 2002.
The results are expressed in g/100 g of sample, on a dry basis, mean with standard deviation are reported (n = 3). Different letters in same lines, denote statistically significant differences ($p < 0.05$).

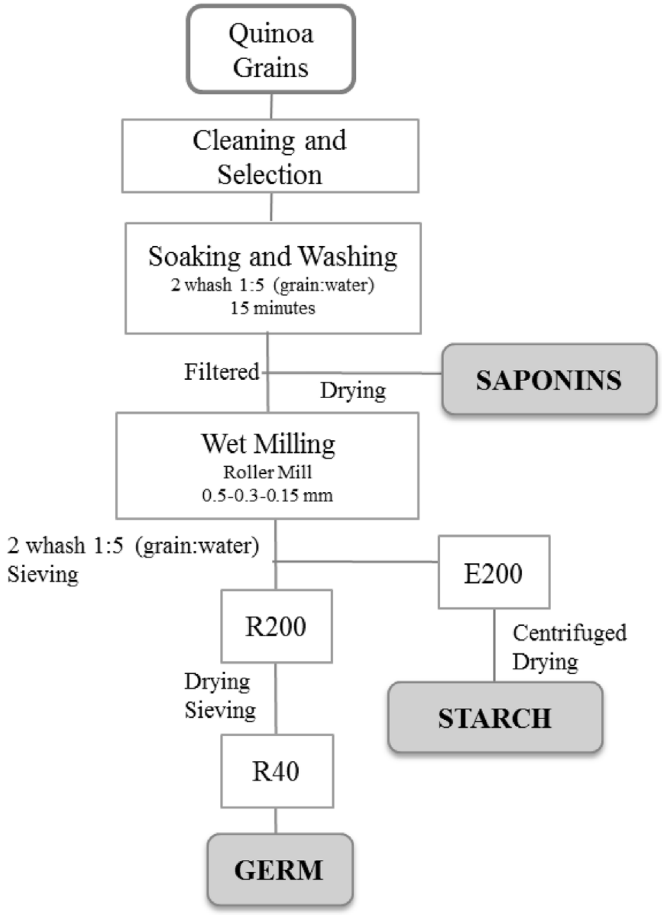

Fig. 3. Flow diagram of the general process.

AACC (2002) and AOAC (1999) were employed: moisture by drying at 100–105 °C for 24 h in a forced circulation oven (AACC 44-01). Crude protein by Kjeldahl (AACC 46-10), conversion factor of 6.25. Ash by calcination in muffle at 550 °C (AACC 08-12). Total lipids by Soxhlet, employing n-hexane as solvent (AOAC 920.39). The carbohydrates were calculated by difference. All determinations were made in triplicate and expressed on a dry basis.

The protein yield was determined using equation (2):

$$\textit{Protein Yield}\ (\%) = \left(\frac{\%\ protein\ of\ fraction\ \times g\ fraction}{\%\ protein\ of\ starting\ material\ \times g\ starting\ material}\right) \times 100 \quad (2)$$

Amino Acid Profile: the quinoa grains and the germ fraction were hydrolyzed in 6 mol/L HCl for 24 h (AOAC 994.12), the amino acids were derivatized with diethyl ethoxymethylenemalonate (Alaiz, Navarro, Girón, & Vioque, 1992) and quantified by HPLC on a Perkin Elmer 600 Chromatographer (United States), provided with a Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 5 µm) Agilent Technologies; solvents: sodium acetate buffer (25 mmol/L, pH = 6) and acetonitrile, UV detector (280 nm). External standards (AAS18, Fluka Analytical, Sigma Aldrich®) were used to identify and quantify amino acids. The samples were hydrolyzed in duplicate and each duplicate was injected 3 times. The mean value of the six determinations is reported, such as g of amino acids per 100 g of protein.

Fatty acid profile: for the determination, the cold extracted quinoa oils from the ground quinoa grains and the germ fraction, employing hexane as solvent and recovering the oil in a rotary evaporator at 40 °C. The fatty acids were transmethylated (Martínez, 2010), the identification and quantification of the fatty acids present in the oil was carried out by gas chromatography with mass spectrometry detector (GC-MS), Clarus 600 Perkin Elmer equipment, the data were acquired and processed with the TurboMass 5.4 program. The separation was carried out with a capillary column DB-5 (5% diphenyl and 95% dimethyl polysiloxane of low polarity, 60 m × 0.25 mm, 0.25 µm phase thickness) Perkin Elmer. The identification to the components of the sample was made by comparison with the mass spectra of the libraries of the NIST MS Search 2.0 program provided on the equipment. The fatty acid profile was performed in duplicate and the average relative percentages of the main esters of fatty acids present were reported with respect to the total of the same in the samples.

Differential Scanning Calorimetry: was done in a DSC-821e Mettler Toledo equipment. For this purpose, we proceeded according to the technique of Steffolani, León, & Pérez, 2013 modified, 20 mg of each sample was weighed in an aluminum dish and 80 mg water (placed with microsyringe). The dish was hermetically sealed and allowed to stand at room temperature overnight. The thermograms were performed 30–120 °C at 10 °C/min and an empty dish as reference. Data was processed with STARe software version 9.0x (Mettler Toledo, Switzerland). Microphotographs: were performed in a laser confocal optical microscope, Olimpus OLS4000 (Japan), working with 5X, 20X and 100X magnifications.

2.5. Statistical analysis

Statistical analysis was performed with the software InfoStat version 2012 (InfoStat Group, FCA, National University of Córdoba, Argentina). The variance analysis (ANAVA) was used to estimate the differences between treatments and for those resulting statistically significant ($p \leq 0.05$), a multiple comparisons test was applied, DGC.

3. Results and discussion

3.1. Grain conditioning

The parameters involved in grain conditioning must be carefully selected, because it is a critical stage to achieve the correct separation of the different structural tissues of the grain (embryo and perisperm).

First, saponins present in the outer layers of the grain must be eliminated, before being consumed since they are considered an antinutritional factor, besides providing a bitter taste (Gee et al., 1996; Ruales & Nair, 1993; Vidueiros et al., 2015; Woldemichael & Wink, 2001). There are several methods to remove saponins, some consisting in dry abrasion while others use wet alternatives (Gianna et al., 2012). Dry methods produce surface damage to the grain and losses of germ and starch. In Fig. 1, it can be seen that removal of saponins was 99% in less than 10 min; the residual saponins were 0.012–0.014%, ten times lower than the maximum acceptable level of perception of bitter taste (0.12%) (Mujica et al., 2006).

On the other hand, the grain must reach a humidity between 40 and 50% to achieve the correct separation of the germ and the perisperm, considering these tissues have a differential behavior against compression when properly moistened; the germ becomes elastic and the perisperm breaks and is pulverized favoring the separation of these fractions (Belhadi, Djabali, Souilah, Yousfi, & Nadjemi, 2013; Calzetta Resio, Tolaba, & Suárez, 2009; Föste et al., 2015; Serna-Saldivar, 2010). The optimum humidity is achieved by macerating the grain in water at room temperature for more than 30 min (Fig. 1). By increasing the humidity of the grain, the germination process is activated, which causes the hydrolysis of the reserve nutrients, this process must be avoided in order to preserve the nutritional quality of the germ obtained (Othón Serna Saldívar, 1996); the germination begins after 60 min of moistening, so it must be worked with maceration times within this. Thus, the proper conditions for quinoa seeds soaking were fixed in 30 min at room temperature.

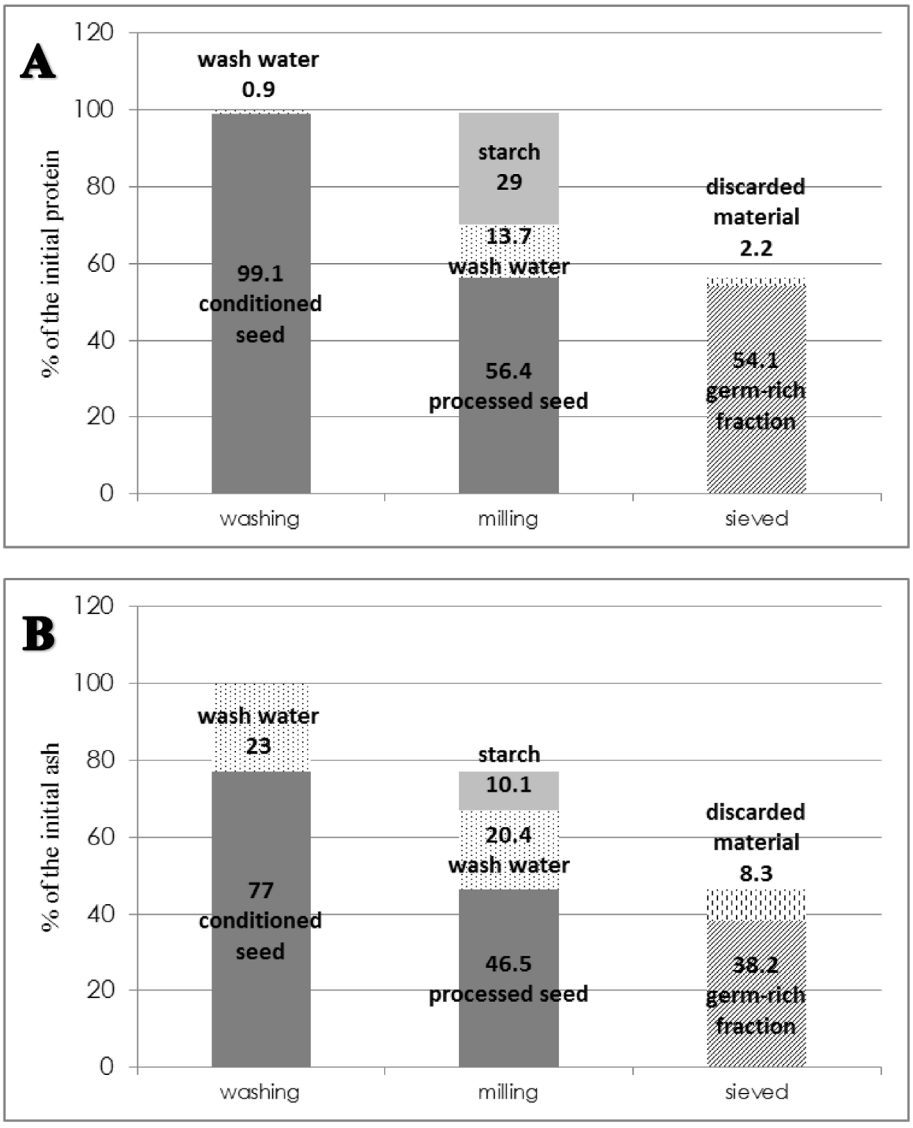

Fig. 4. Distribution of protein and ash content in the different stages of the process; (A) Protein, (B) Ash.

3.2. Wet milling of quinoa seeds and separation of fractions

As can be seen in Table 1, the smaller separation between rollers and the milling in consecutive stages with gradual reduction produced an increase in the fraction rich in starch (E200), with the consequent enrichment in germ of the other fraction (R200). The ideal conditions for the separation of the germ were three consecutive milling with gradual reduction of the distance between the rollers; as the quinoa seeds have an average thickness of 1 mm and the germ of 0.35 mm, a distance between rollers of 0.5 mm allows to compress the seed to half its original size and the successive reductions to 0.30 and 0.15 mm compressed the germ and disintegrated the perisperm, making possible their separation.

The germ separated manually represented 27.4% of the grain, this value is near to that reported by Tapia and Fries (2007). Comparing this value with the percentages of recovery for R200 obtained in the best milling conditions (44.3%), it can be concluded that this fraction has part of the perisperm.

The sieving of R200 gave rise to three main fractions one with larger particles (>840 μm) which decreased as milling conditions improved, while the others, with particles between 420 and 840 μm, increased. This change in particle size distribution reflects the greater dissociation of the perisperm and consequent germ recovery. The images of Fig. 2, show broken and crushed grains for the fraction R20, while in R30 y R40 germs with white spots of adhering starch residues are observed. However, even in the best milling conditions the separation of the embryo and perisperm was not complete. For future studies, the design of a suitable mill for this type of grain could improve the separation of the embryo.

3.3. Physical-chemical characterization of fractions

In Table 2, the proximal composition of the different fractions R20, R30 and R40 are summarized, with an increase in lipid, protein and ash content as a result of the germ enrichment. The composition of R20 resembles that of the quinoa grain, while R30 and R40 approach that of extracted manually germ. To estimate the percentage of germ in each fraction, the relationship between the lipid content in each fraction and the hand-separated germ was calculated, due to the lipids are mainly located in quinoa germ and being insoluble in water so, its losses during washings are negligible. The fractions R40, R30 and R20 represent about 95.8%, 92.5% and 31.3% of germ recovery, respectively. The fraction R40 concentrates approximately one half of the total proteins (46.7%), the other fractions only have between 10.8 and 15.5%. The E200 fraction has 92% carbohydrates and 7% proteins (13.3% of the initial proteins), which suggests the need for greater purification to

Table 3
Aminoacid profiles (g/100 g of protein) from different sources.

Aminoacids	R40	Quinoa grain	Corn Germ [1]	Wheat germ [2]	Differences
Tryptophan	1	0.88	0.94	0.72	0.12
Threonine	3.58	1.72	3.56	3.35	1.86
Isoleucine	4.04	3.98	2.82	6.22	0.06
Leucine	6.60	5.67	6.58	3.99	0.93
Lysine	5.54	4.93	5.37	8.35	0.61
Methionine	8.58	6.91	1.81	0.9	1.67
Cysteine	2.47	11.93	2.08	–	−9.46
Phenylalanine	3.91	2.78	3.76	3.74	1.13
Tyrosine	2.75	1.82	2.62	2.81	0.93
Valine	4.73	2.72	4.9	5.04	2.01
Arginine	8.76	7.51	7.52	17.12	1.25
Histidine	3.44	2.24	2.82	3.02	1.2
Alanine	4.30	6.07	5.91	5.97	−1.77
Aspartic acid	8.14	5.04	7.72	5.86	3.1
Glutamic acid	14.53	11.02	12.62	18.31	3.51
Glycine	5.05	4.91	–	5.29	0.14
Proline	10.05	19.31	5.97	–	−9.26
Serine	3.54	1.49	3.83	5.14	2.05
% of Recovery	101.01	100.93	80.81	95.83	
Proteins Content (%)	36.47	18.19	14.9	27.8	

[1]Kim, Amezcua, Utterback, & Parsons, 2008.
[2]Arshad et al., 2007.

Table 4
Fatty acid profiles of oils from different sources.

Fattyacids		R40	Quinoa grain	Corn Germ [1]	Wheat germ [2]	Differences
Palmitic	**16:0**	9.93	9.16	11	35.6	0.77
Stearic	**18:0**	0.61	1.03	1.8	1.5	−0.42
Arachidonic	**20:0**	0.38	0.33	0.2	–	0.05
Oleic (ω9)	**18:1**	27.92	27.64	25.3	34.2	0.28
Linoleic (ω6)	**18:2**	54.48	54.98	60.1	27.1	−0.5
Linolenic (ω3)	**18:3**	5.4	5.67	1.1	0.6	−0.27
Gondolic	**20:1**	1.28	1.19	–	–	0.09
Saturated (S)		10.92	10.92	13	37.1	
Monounsaturated (MI)		29.2	29.2	25.3	34.2	
Poliunsaturated (PI)		59.88	59.88	61.2	27.7	
ω3/ω6		0.1	0.1	0.02	0.02	
PI/MI		2.05	2.05	2.42	0.81	

[1]Moreau, 2011.
[2]Yuldasheva, Ul'Chenko, & Glushenkova, 2010.

obtain good quality quinoa starch.

In Fig. 3, the complete process of obtaining the quinoa seed by wet milling is detailed. In the selected working conditions, nutritional losses were considered throughout the process (Fig. 4A and B).

During the obtaining of the fraction enriched in germ there is no loss of lipids, 96% remains in the fraction R200 and only 4% is retained in the starch. However, there is a 16.8% protein loss, mainly due to the washings after milling; because when the grains are crushed or split, the dissolution of the soluble proteins is favored. This dispersion of starch is centrifuged, before drying allowing the separation of the protein in the supernatant. In turn, the mineral losses reach 51.7%, due to the leaching during the washings. However, a continuous process could be considered, with recirculation of these effluents to concentrate proteins and minerals, reduce losses and recover the protein fraction by isoelectric precipitation.

The quinoa germ has more lipids and proteins than maize germ, and less carbohydrates, similar protein content and a third of the lipids than wheat germ. Amino acid profile in quinoa seeds is approximate to maize and wheat germ (Table 3), but the protein content is higher, therefore its nutritional contribution will be more important. Comparing this profile with grain of quinoa, marked losses of the cysteine, alanine and proline were observed, with a consequent increase in the other amino acids.

Table 4 shows the relative fatty acid composition of quinoa oil extracted from the germ fraction. The presence of saturated fats is either low, being the unsaturated oleic and linoleic acids its main components, a desirable property in oils. The profile is similar to maize and wheat germs, although the latter has a higher proportion of saturated fats. In comparison with the quinoa grain, there are losses of stearic, linoleic and linolenic acids.

The differential scanning calorimetry (DSC) of germ showed two endothermic peaks (Fig. 5), the first at 66.62 °C corresponding to the starch gelatinization and the other at 97.32 °C for protein denaturation. The temperature of the starch peak significantly exceeds the value obtained for the purified starch from these seeds (Storani & Martini, 2010) and also exceeds those obtained from seven varieties of quinoa, with values between 50.5 and 61.7 °C (Lindeboom, Chang, Falk, & Tyler, 2005). Nevertheless, the peak of gelatinization of the starch in germ is within the values found for integral quinoa flours. This suggests positive interactions between starch and the other components of the germ (Li & Zhu, 2017). Conversely, the protein peak at 97.3 °C is coincident with the 97 °C obtained from protein isolates of quinoa (Avila Ruiz, Xiao, van Boekel, & Stieger, 2016b) and similar to 98 °C measured in isolates at pH 9 (Abugoch et al., 2008).

The first step to ensure the shelf life of quinoa germ would be to extract it whole, because the structural tissue has the function of protection against external agents, decreasing the possibility of oxidation and microbiological contamination, added to visual presentation as a product. In Fig. 6, it can be seen that this requirement is not met, because there are two types of characteristic break. In Figure A and B, the quinoa germ has lost one of the cotyledons and the rupture occurred at the meristematic apex of the sprout. During the maceration the cotyledons were separated and then in drying, handling and selection, the rupture occurred. On the contrary, in Figure C the rupture occurred on both sides of germ mainly in the insertion zone with perisperm of the grain, even the fraction thereof in the germ segment is observed. This rupture occurred during milling, probably because the grain was not sufficiently wet, as to soften the perisperm or since the initial compression of grain was not enough remove the germ. This broken embryos would reduce the shelf life of germ.

The addition of maize or wheat germ to enrich wheat flour, has been widely used to produce foods such as breads, cookies, muffins, pasta, among others; improving their nutritional value while modifying the textural characteristics (Arshad, Anjum, & Zahoor, 2007; Siddiq et al., 2009). It is expected that the quinoa seed has a more beneficial participation in products similar to those mentioned. On the other hand, it can be used as a starting material to obtain quinoa oil and protein isolates since there is a lower starch content which favors these extractive processes, as already mentioned in other similar research that start with flours enriched with the germ of quinoa (Avila Ruiz et al., 2016a; Föste et al., 2015).

4. Conclusions

The present work shows a possible and novel alternative to separate quinoa germ by wet milling, allowing saponins and starch recovering by applying simple purification, concentration and drying operations. The product has numerous practical applications due to its high nutritional value, and could be used as a dietary supplement in the formulation of foods for the celiac population, athletes, children or the elderly that require protein diets. It could also be used as raw material to obtain protein isolates and oil. Quinoa germ composition resembles to wheat and maize germs; and if it is compared to quinoa whole grain, it doubles the protein content and triples lipid content. Nevertheless, the profile of amino acids and fatty acids did not show great changes.

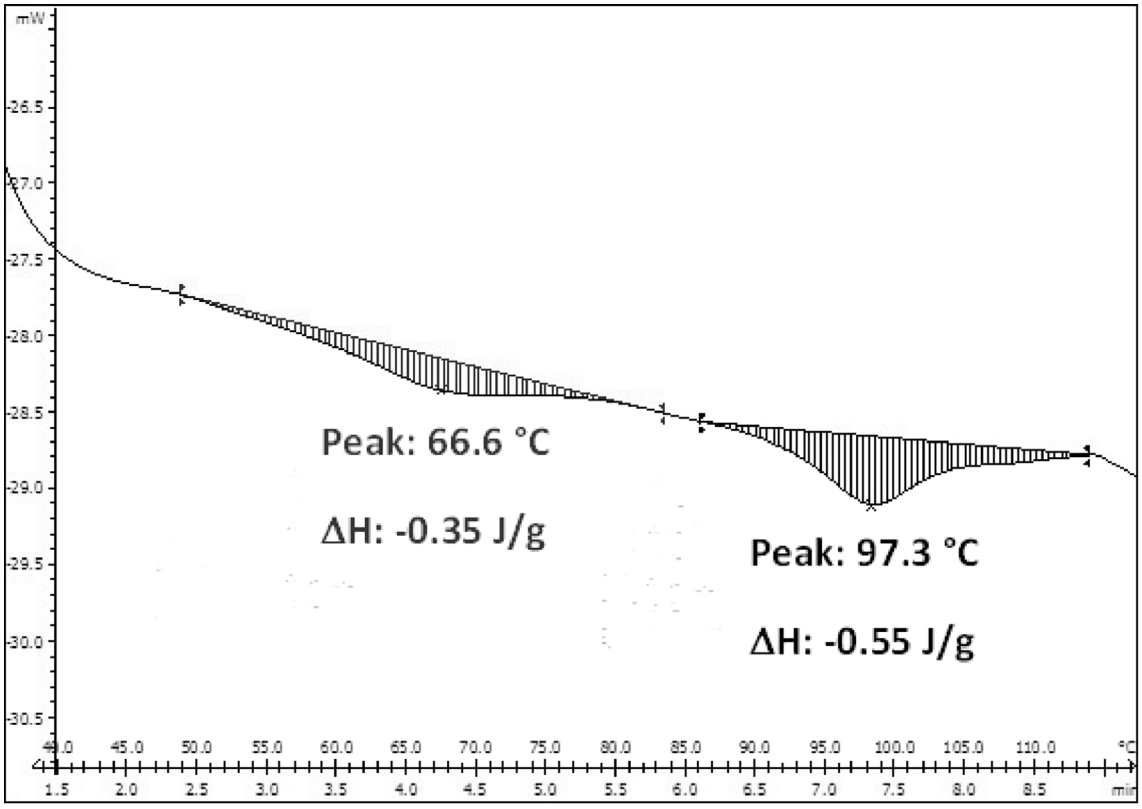

Fig. 5. Differential scanning calorimetry of germ.

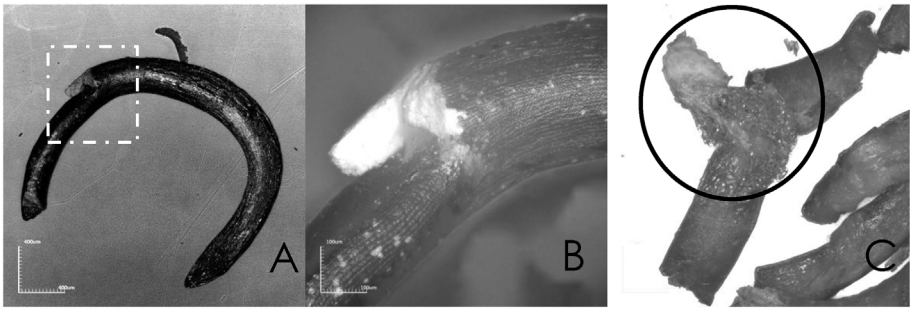

Fig. 6. Patterns of quinoa germ breaking during wet milling.

Acknowledgments

The authors thank the National Council for Scientific and Technical Research (CONICET) and the Secretariat of Science and Technology (SECyT) (30720130101068CB) for funding. To the Institute of Food Science and Technology (ICTA) - the National University of Córdoba (UNC) and the Institute of Food Science and Technology Córdoba (ICyTAC) - CONICET - UNC, for providing the workplace. Finally, to the center of electronic microscopy LAMARX - UNC.

References

AACC (2002). Approved methods. *AACC international method*(10th ed.). St. Paul, MN: American Association of Cereal Chemists.

Abugoch, L. E., Romero, N., Tapia, C. A., Silva, J., & Rivera, M. (2008). Study of some physicochemical and functional properties of quinoa (*Chenopodium quinoa* Willd.) protein isolates. *Journal of Agricultural and Food Chemistry, 56*(12), 4745–4750.

Alaiz, M., Navarro, J. L., Girón, J., & Vioque, E. (1992). Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. *Journal of Chromatography A, 591*(1–2), 181–186. http://doi.org/10.1016/0021-9673(92)80236-N.

AOAC (1999). *Official methods of analysis of AOAC international.* Association of Official Analysis Chemists International.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. A., & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willdenow) starch. *Carbohydrate Polymers, 81*(4), 839–848. http://doi.org/10.1016/j.carbpol.2010.03.051.

Arshad, M. U., Anjum, F. M., & Zahoor, T. (2007). Nutritional assessment of cookies supplemented with defatted wheat germ. *Food Chemistry, 102*(1), 123–128.

Avila Ruiz, G., Arts, A., Minor, M., & Schutyser, M. (2016a). A hybrid dry and aqueous fractionation method to obtain protein-rich fractions from quinoa (*Chenopodium quinoa* Willd). *Food and Bioprocess Technology, 9*(9), 1502–1510.

Avila Ruiz, G., Xiao, W., van Boekel, M., Minor, M., & Stieger, M. (2016b). Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (*Chenopodium quinoa* Willd). *Food Chemistry, 209*, 203–210. http://doi.org/10.1016/j.foodchem.2016.04.052.

Bejarano, E., Bravo, M., Huamán, M., Huapaya, C., Roca, A., & Rojas, E. (2002). *Tabla de composición de alimentos industrializados.* Lima - Perú: Ministerio de Salud de Perú.

Belhadi, B., Djabali, D., Souilah, R., Yousfi, M., & Nadjemi, B. (2013). Three small-scale laboratory steeping and wet-milling procedures for isolation of starch from sorghum grains cultivated in Sahara of Algeria. *Food and Bioproducts Processing, 91*(3), 225–232. http://doi.org/10.1016/j.fbp.2012.09.008.

Brakez, M., Daoud, S., Harrouni, M. C., Tachbibi, N., & Brakez, Z. (2016). Nutritional value of Chenopodium quinoa seeds obtained from an open field culture under saline conditions. *Halophytes for Food Security in Dry Lands* (pp. 37–47). . http://doi.org/10.1016/B978-0-12-801854-5.00003-0.

Calzetta Resio, A. N., Tolaba, M. P., & Suárez, C. (2009). Correlations between wet-milling characteristics of amaranth grain. *Journal of Food Engineering, 92*(3), 275–279.

http://doi.org/10.1016/j.jfoodeng.2008.11.005.
Capriles, V. D., & Arêas, J. A. G. (2014). Novel approaches in gluten-free breadmaking: Interface between food science, nutrition, and health. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 871–890. http://doi.org/10.1111/1541-4337.12091.
Elsohaimy, S. A., Refaay, T. M., & Zaytoun, M. A. M. (2015). Physicochemical and functional properties of quinoa protein isolate. *Annals of Agricultural Science, 60*(2), 297–305.
Föste, M., Elgeti, D., Brunner, A. K., Jekle, M., & Becker, T. (2015). Isolation of quinoa protein by milling fractionation and solvent extraction. *Food and Bioproducts Processing, 96*, 20–26. http://doi.org/10.1016/j.fbp.2015.06.003.
Gallagher, E., Gormley, R., & Arendt, E. K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science & Technology, 15*(3–4), 143–152. http://doi.org/10.1016/j.tifs.2003.09.012.
Gallardo, M., Gonzáles, J., & Ponessa, G. (1997). Morfología del fruto y semilla de *Chenopodium quinoa* Willd. (quinoa). Chenopodiaceae. *Lilloa,* 71–80.
Gee, J. M., Wortley, G. M., Johnson, I. T., Price, K. R., Rutten, A. A., Houben, G. F., et al. (1996). Effects of saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations in vitro. *Toxicology in Vitro, 10*(2), 117–128. http://doi.org/10.1016/0887-2333(95)00113-1.
Gianna, V., Montes, J. M., Calandri, E. L., & Guzmán, C. A. (2012). Impact of several variables on the microwave extraction of *Chenopodium quinoa* Willd. saponins. *International Journal of Food Science and Technology, 47*(8), 1593–1597. http://doi.org/10.1111/j.1365-2621.2012.03008.x.
Guerreo-Ochoa, M. R., Pedreschi, R., & Chirinos, R. (2015). Optimised methodology for the extraction of protein from quinoa (*Chenopodium quinoa* Willd.). *International Journal of Food Science and Technology, 50*, 1815–1822.
Hernández, B. D., Guerra, M. J., & Rivero, F. (1999). Efecto del fraccionamiento sobre las características del germen de maíz desgrasado. *Food Science and Technology (campinas), 19*(1), 107–112.
Kim, E. J., Amezcua, C. M., Utterback, P. L., & Parsons, C. M. (2008). Phosphorus bioavailability, true metabolizable energy, and amino acid digestibilities of high protein corn distillers dried grains and dehydrated corn germ. *Poultry Science, 87*, 700–705.
Koziol, M. (1992). Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd.). *Journal of Food Composition and Analysis, 5*(1), 35–68. http://doi.org/10.1016/0889-1575(92)90006-6.
Lindeboom, N., Chang, P. R., Falk, K. C., & Tyler, R. T. (2005). Characteristics of starch from eight quinoa lines. *Cereal Chemistry, 82*(2), 216–222. http://doi.org/10.1094/CC-82-0216.
Li, G., Wang, S., & Zhu, F. (2016). Physicochemical properties of quinoa starch. *Carbohydrate Polymers, 137*, 328–338. http://doi.org/10.1016/j.carbpol.2015.10.064.
Li, G., & Zhu, F. (2017). Physicochemical properties of quinoa flour as affected by starch interactions. *Food Chemistry*. http://doi.org/10.1016/j.foodchem.2016.10.137.
Martínez, M. L. (2010). *Extracción y caracterización de aceite de nuez (Juglans regia L.): Influencia del cultivar y de factores tecnológicos sobre su composición y estabilidad oxidativa.* Doctorado en Ciencias de la IngenieríaFacultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba.
Ma, S., Wang, X., Zheng, X., Tian, S., Liu, C., Li, L., et al. (2014). Improvement of the quality of steamed bread by supplementation of wheat germ from milling process. *Journal of Cereal Science, 60*(3), 589–594. http://doi.org/10.1016/j.jcs.2014.07.010.
Moreau, R. (2011). Corn oil. In F. Gunstone (Ed.). *Vegetable oils in food Technology* (pp. 1–352). (1st ed.). Oxford, Reino Unido: Blackwell Publishing.
Mufari, J. R. (2015). Germen. In F. Grasso (Ed.). *Aprovechamiento integral del grano de quinoa* (pp. 101–106). Córdoba, Argentina: Repositorio digital de la Universidad Nacional de Córdoba. http://rdu.unc.edu.ar, Accessed date: 20 January 2018.
Mujica, A., Ortiz, R., Bonifacio, A., Saravia, R., Corredor, G., & Romero, A. (2006). *Informe final.* Proyecto Quinua: Cultivo multipropósito para los países andinos.
Othón Serna Saldívar, S. R. (1996). In Primera (Ed.). *Química, almacenamiento e industrialización de los cereales*. D.F. México: A.G.T. Editor.
Pouvreau, L., Kanning, M., & Van de Velde, F. (2014). *Processing quinoa for improved protein-to-carbohydrate formulations.* US Patent application 13/566,391. Patent application number 20140161950A1, 2014-06-12.
Prego, I., Maldonado, S., & Otegui, M. (1998). Seed structure and localization of reserves in Chenopodium quinoa. *Annals of Botany, 82*, 481–488.
Quiroga, C., Escalera, R., Aroni, G., Bonifacio, A., González, J., Villca, M., et al. (2014). Procesos tradicionales e innovaciones tecnológicas en la cosecha, beneficiado e industrialización de la quinua. In D. Bazile, D. Bertero, & C. Nieto (Eds.). *Estado del arte de la quinoa en el mundo en 2013* (pp. 728). (1ra ed.). Santiago de Chile, Chile y Montpellier, Francia: FAO y CIRAD.
Ramos Díaz, J. M., Kirjoranta, S., Tenitz, S., Penttilä, P. A., Serimaa, R., Lampi, A. M., et al. (2013). Use of amaranth, quinoa and kañiwa in extruded corn-based snacks. *Journal of Cereal Science, 58*(1), 59–67. http://doi.org/10.1016/j.jcs.2013.04.003.
Repo-Carrasco, R., Espinoza, C., & Jacobsen, S. E. (2003). Nutritional value and use of the andean crops quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*). *Food Reviews International, 19*(1–2), 179–189. http://doi.org/10.1081/FRI-120018884.
Ruales, J., & Nair, B. M. (1993). Saponins, phytic acid, tannins and protease inhibitors in quinoa (*Chenopodium quinoa*, Willd) seeds. *Food Chemistry, 48*(2), 137–143. http://doi.org/10.1016/0308-8146(93)90048-K.
Serna-Saldivar, S. O. (2010). Cereal grains: Properties, processing, and nutritional attributes. *Cereal Grains: Properties, processing, and nutritional attributes* (pp. 57–61). . http://doi.org/10.2135/cropsci2010.12.0005br.
Serna-Saldivar, S. O. (2016). *Cereals: Dietary importance. Encyclopedia of food and health.* http://doi.org/10.1016/B978-0-12-384947-2.00130-6.
Siddiq, M., Nasir, M., Ravi, R., Dolan, K. D., & Butt, M. S. (2009). Effect of defatted maize germ addition on the functional and textural properties of wheat flour. *International Journal of Food Properties, 12*(4), 860–870. http://doi.org/10.1080/10942910802103028.
Steffolani, M. E., León, A. E., & Pérez, G. T. (2013). Study of the physicochemical and functional characterization of quinoa and kañiwa starches. *Starch/Stärke, 65*(11–12), 976–983.
Storani, F., & Martini, J. (2010). *Desarrollo del proceso de obtención de almidón a partir de granos de Chenopodium quinoa Willd. mediante la utilización de métodos químicos, físicos y/o enzimáticos.* Facultad de Ciencias Exactas, Físicas y Naturales - Universidad Nacional de Córdoba.
Tapia, M. E., & Fries, A. M. (2007). *Guía de campo de los cultivos andinos.* Anpe-Perú: Fao.
Tari, T. A., Annapure, U. S., Singhal, R. S., & Kulkarni, P. R. (2003). Starch-based spherical aggregates: Screening of small granule sized starches for entrapment of a model flavouring compound, vanillin. *Carbohydrate Polymers, 53*(1), 45–51. http://doi.org/10.1016/S0144-8617(02)00293-X.
Tari, T. A., & Singhal, S. R. (2002). Starch-based spherical aggregates: Stability of a model flavouring compound, vanillin entrapped therein. *Carbohydrate Polymers, 50*(4), 417–421. http://doi.org/10.1016/S0144-8617(02)00055-3.
Tsen, C. C. (1980). Cereal germs used in bakery Products: Chemistry and nutrition. *Cereals for food and beverages*Academic press, Inchttp://doi.org/10.1016/B978-0-12-370960-8.50019-9.
Turkut, G. M., Cakmak, H., Kumcuoglu, S., & Tavman, S. (2016). Effect of quinoa flour on gluten-free bread batter rheology and bread quality. *Journal of Cereal Science, 69*, 174–181. http://doi.org/10.1016/j.jcs.2016.03.005.
Vidueiros, S. M. M., Curti, R. N. N., Dyner, L. M. M., Binaghi, M. J. J., Peterson, G., Bertero, H. D. D., et al. (2015). Diversity and interrelationships in nutritional traits in cultivated quinoa (*Chenopodium quinoa* Willd.) from Northwest Argentina. *Journal of Cereal Science, 62*, 87–93. http://doi.org/10.1016/j.jcs.2015.01.001.
Woldemichael, G. M., & Wink, M. (2001). Identification and biological activities of triterpenoid saponins from Chenopodium quinoa. *Journal of Agricultural and Food Chemistry, 49*(5), 2327–2332. http://doi.org/10.1021/jf0013499.
Yuldasheva, N. K., Ul'Chenko, N. T., & Glushenkova, A. I. (2010). Wheat germ oil. *Chemistry of Natural Compounds, 46*(1), 97–98.