**Evaluation of the physicochemical and nutritional changes in two amaranth species
(*Amaranthus quitensis* and *Amaranthus caudatus*) after germination.**

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Abstract

Germination has been applied to different seeds to increase their nutritional values. Nevertheless, the potential impact of species has not been considered yet. This research evaluates the changes in the physicochemical and nutritional properties of *A. quitensis* (black specie) and *A. caudatus* (white specie) during germination. Hydration, thermal and rheological properties were evaluated. Also, starch and protein digestibility, phenolic compound, antioxidant activity and dietary fiber were determined. The reduction in pasting and viscoelastic properties as well as total and resistant starch content in *A. quitensis*, after being subjected to 24 h germination, were significantly higher ($P<0.05$), than the reduction observed in these respective properties in *A. caudatus*. These results revealed that during germination the enzymatic degradation of starch is greater in *A. quitensis* than *A. caudatus*. In addition, *A. quitensis* showed a significant lower glycemic index and higher protein digestibility than *A. caudatus* ($P<0.05$). **The data in this study demonstrates the potential impact of species in the application of germination to improve physicochemical or nutritional properties of amaranth.**

Keywords: Amaranth species; germination; physicochemical properties; nutritional properties; germinated flour; *Amaranthus quitensis*; *Amaranthus caudatus*.

1. Introduction

Amaranth is a pseudocereal with high nutritional value, particularly for its balanced amino acid content, dietary fiber content and antioxidant activity (Lamothe, Srichuwong, Reuhs & Hamaker, 2015). Amaranth contains higher amount of essential amino acids such a lysine, a limiting amino acid in most cereals. Also, amaranth is a gluten free pseudocereal, making it a good alternative

for gluten free diet. The nutritional properties of amaranth have attracted the attention of researches about the use of amaranth as functional ingredient. However, previous studies have demonstrated a considerable difference in nutrient properties between amaranth species such as aminoacid content, fatty acids content, mineral composition, among others (Venskutonis & Kraujalis, 2013; Kachiguma, Mwase, Maliro & Damaliphetsa, 2015). Certainly, the amaranth specie is a determinant factor not only for nutritional properties of the flour but also for its physiochemical properties. Kaur, Singh & Rana (2010) reported the variability of pasting, and textural properties among different amaranth cultivars. They suggested that cultivars with higher α -amylase activity showed a lower peak viscosity, breakdown, final viscosity and gel hardness.

Further, the nutritional properties of amaranth flour have been further improved by subjecting grains to germination, because many biochemical, nutritional and sensorial changes take place. During germination, hydrolytic enzymes, like α -amylase, β -amylase and proteases, are activated and they decompound starch and protein in sugars, soluble proteins and amino acids (Gamel et al., 2006; Ghumman, Kaur & Singh 2016; Xu, Chen, Ali, Yang, Chen, Wu, & Xu, 2017). But also, bioactive compounds such as GABA, γ -oryzanol and phenolic content can be improved with seeds germination, as has been reported in rice (Cáceres, Martínez-Villaluenga, Amigo & Frias, 2014). Particularly, in the case of amaranth, germination increases the amount of phenolic compounds, flavonoid content, protein digestibility and antioxidant activity, as well as decreases anti-nutritional compounds (Perales-Sánchez et al., 2014; Hejazi & Orsat, 2016, Colmenares & Bressani, 1990). Besides the improvement in nutritional properties, germination affects the physiochemical properties of the resulting flour (Gamel et al., 2006; Siwatch, Yadav & Yadav, 2017; Chauhan, Saxena & Singh, 2015). For instance, Chauhan et al. (2015) demonstrated that

germination affects significantly the pasting profiles of amaranth flour; decreasing the peak viscosity, breakdown, setback, final viscosity and pasting temperature. In addition, an increase of amaranth starch crystallinity has been observed after germination (Siwatch et al., 2017).

However, very few publications can be found in the literature that discuss the nutritional response of different species of amaranth to germination (Colmenares & Bressani, 1990). The aim of this study was to analyze the changes on the physicochemical and nutritional properties during germination of black (*Amaranthus quitensis*) and white (*Amaranthus caudatus*) amaranth species. The study will provide a useful information for the industrial application of germinated amaranth flour.

2. Materials and methods

2.1. Materials

Amaranthus quitensis (black specie) and *Amaranthus caudatus* (white specie) were harvested between March and April 2016 and provided by the National Institute of Agricultural Research from Ecuador (INIAP, Quito, Ecuador). All reagents were analytical grade.

2.2. Germination process and flours preparation

Germination was performance according to the methodology described in Cornejo & Rosell (2015). Briefly, grains were sterilized with hypochlorite solution for 30 min, then soaked for 7 h at 28 ± 1 °C and germination was carried out under darkness for 24 h at 32 ± 1 °C and 100% relative humidity. The germinated grains were dried at 50 ± 1 °C for 24 h.

Germinated and raw grains were milled and sieved (screen diameter 212 μm). Flours were stored at room temperature till further analysis.

2.3. Chemical composition

Standard methods were applied for moisture (AOAC 1996 method 930.15), ash (AOAC 1984 method 14006), protein (AOAC 1920 method 920.87) and fat (MO-LSAIA-01.03). Carbohydrate was calculated by difference.

Total amylose content was measured following Morrison & Laignelet (1983) method. Measurements were made in a UV-Visible Spectrophotometer (Thermo Fisher Scientific 201 Evolution, Madison, WI USA) at 620 nm. Amylose (Megazyme International Ltd, Wicklow, Ireland) was used for standard curve. The content of total sugars was analyzed by the Somogyi-Nelson method (Somogyi, 1952).

2.4. Determination of physicochemical properties

All physicochemical properties have been done in raw and germinated amaranth flours.

2.4.1. Flour and gel hydration properties.

Flour hydration properties were determined according to the procedure described by Cornejo & Rosell (2015). Water holding capacity (WHC), water binding capacity (WBC), water absorption index (WAI) and water solubility index (WSI) were calculated.

2.4.2. Thermal properties

Gelation properties were measured in a differential scanning calorimeter (model Q-200, TA instruments, Newcastle, USA) from 25 to 100 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C min}^{-1}$. The flour samples were mixed with deionized water at a ratio 1:3 (w/w) and sealed in a hermetic pan. The

samples were rest for 12 hours at room temperature prior to analysis. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, as well as the enthalpy of gelatinization (ΔH) were calculated.

2.4.3. X-Ray Diffraction

Crystallinity patterns were measured in a X-ray diffractometer (XRD) (X'Pert³ PRO PANalytical, Boulder, USA) in angular range from 5 to 40° at 0.03° step size and measuring time of 15 s. The XRD operated at 40 kV and 30 mA. The relative crystallinity was calculated as the percentage of the relation between crystalline area with total area. The software Origin®2017 (OriginLab Corporation, Northampton, USA) was used to calculate the areas.

2.5. Rheological properties

The rheological properties of amaranth gels were measured in Kinexus PRO rheometer (Malvern Instruments, Worcestershire, UK) at 25°C. The gels were prepared by mixing 0.5 g of flour with 4.5 ml of water and heated for 10 min at 90 °C. A cone-plate geometry with 40 mm diameter and 4° angle was used. Amplitude sweep was used to define the linear viscoelastic region at 1 Hz and shear strain from 0.1 to 100%. The viscoelastic properties (G' and G'') were measured at frequency from 0.01 to 10 Hz in the linear region. The apparent viscosity was measured by applying Ostwald's equation $\eta_{app} = K\dot{\gamma}^{n-1}$, at a share rate between $\sim 10^{-3}$ to 440 s⁻¹.

ICC standard method No 162 was applied to measure the pasting properties of raw and germinated amaranth flours, using a Rapid Visco Analyser model 4500 (Perten Instruments, Hägersten, Sweden). Peak viscosity, breakdown, final viscosity and setback were evaluated during analyses.

2.6. Nutritional properties

2.6.1. Total, Resistant and Non-Resistant Starch.

Megazyme assay kits (Megazyme International Ltd, Wicklow, Ireland) were used to measure total, resistant and non-resistant starch content. UV-Visible Spectrophotometer (Thermo Scientific 201 Evolution, Madison, USA) was used to measure the absorbance.

2.6.2. *In vitro* starch digestibility.

The kinetic of starch hydrolyses was determined following Goñi, Garcia Alonso, & Saura Calixto (1997) method, using a microplate reader (Biotek Instruments, Winooski, United States). The equation $C = C_{\infty} (1 - e^{-kt})$ was applied, where C is the percentage of hydrolyzed starch at time t , C_{∞} is the equilibrium hydrolyzed starch after 180 min and k is the kinetic constant. The expected glycemic index (eGI) was obtained using the equation $eGI = 8.198 + 0.862 HI$ ¹⁸. The hydrolysis index (HI) was calculated dividing the areas, under the hydrolysis curve, of each amaranth flours and white bread. The rapid digested starch (RDS), hydrolyzed at 20 min, and the slow digested starch (SDS), hydrolyzed between 20 and 120 min, were also considered.

2.6.3. *In vitro* protein digestibility.

The modified method of Hsu, Vavak, Satterlee & Miller (1977) was applied to calculate the *in vitro* protein digestibility of the flours. The method was described in Cornejo, Cáceres, Martinez, Rosell & Frias, 2015. The equation $Y = 210.464 - 18.1x$ was used to calculate the *in vitro* protein digestibility, where x was the change in pH after 10 min.

2.6.4. Antioxidant activity.

The oxygen radical absorbance capacity (ORAC) method was used to measure the antioxidant activity following the method described by Cáceres et al (2014). The results were expressed as μmols of Trolox equivalent/100 g.

2.6.5. Content of total phenols.

Total phenolic content was measured using the Singleton & Rossi (1965) method in a UV-Visible spectrophotometer (Beckman, Fullerton, USA). Results were defined as mg of gallic acid equivalents/100 g.

2.6.6. Total Dietary, soluble and insoluble fiber.

Insoluble and soluble dietary fiber were determined in the defatted samples by using total dietary fiber Megazyme Kit (Megazyme International Ltd, Wicklow, Ireland). The total dietary fiber (TDF) was the sum of insoluble (IDF) and soluble (SDF) dietary fiber.

2.7. Statistical Analysis

All results were statistically evaluated using Statgraphics Centurion XVI (Statistical Graphics Corporation, UK). Normal distribution analysis, multifactorial variance analysis (ANOVA) and multiple range test were applied to characterize and compare results. A correlation analyses was made using Pearson correlation coefficient (r) and P-value, where correlation coefficients equal or greater than 0.67 were considered meaningful. All analyses were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

As shown in Table 1, germination significantly affected the chemical composition of the amaranth flours ($P < 0.05$), with the exception of the carbohydrate content, likely due to the

shifting from large to shorter carbohydrates occurring during germination. It must be stressed that specie also induced a significant effect on the chemical composition of the resulting flours, with the exception of moisture content. After germination, a significant increase in protein content ($P<0.00$) was observed in both species. These results agree with previous findings in germinated amaranth flour (Chauhan et al., 2015; Hejazi & Orsat, 2016), although other authors reported no significant change in protein due to germination (Colmenares & Bressani, 1990; Kanensi, Ochola, Gikonyo & Makokha, 2011). Gamel et al. (2005) reported a significant reduction of amaranth protein content during germination, attributed to proteolytic enzyme activity. Indeed, there are many arguments that explain the behavior of the protein during germination. The increase of protein content has been ascribed to the release of packed protein in the seed structure when the α -amylase breaks down the starch granule during germination (Hejazi & Orsat, 2016), or to the protein synthesis (Chinma, Adewuyi & Abu, 2009; Chauhan et al., 2015). Xu et al. (2017) mentioned that protein content during germination results from the balance between synthesis and hydrolysis of proteins. Present results confirm that this balance also depends on the grain specie.

Fat content decreased significantly during germination ($P<0.00$) and the effect was dependent on the specie. According to Chinma et al. (2009) and Chauhan et al. (2015), the fat reduction could be due to the increased lipolytic activity during germination, which hydrolyses fat components and provides required energy for the seed growth (Xu et al., 2017). It is interested to notice that the percentage of reduction of fat content was higher in *A. caudatus* specie (32.10%) than *A. quintensis* specie (12.61%), showing that the rate of biochemical reaction during germination could be influenced by the specie. The ash content significantly increases with germination

($P<0.00$). Same trend has been observed in amaranth (Colmenares & Bressani, 1990; Chauhan et al., 2015), tigernut (Chinma et al., 2009) and rice (Chinma, Anuonye, Simon, Ohiare & Danbaba, 2015), and has been attributed to the reduction of total soluble solids (Chinma et al., 2015).

Germination did not significantly affect the carbohydrate content ($P>0.05$). Presumably, the rate of degradation of total carbohydrates after 24 hours of germination in the studied species, might be slower than in other reported studies (Chauhan et al., 2015). During germination, the apparent amylose content decreased and the total sugar increased. Although the reduction of amylose content during germination has been observed in other botanical sources, such as brown rice (Wu et al., 2013), lentil and horsegram (Ghumman et al., 2016); Gamel et al. (2005) found no significant change in amaranth. In brown rice, Wu et al. (2013) reported greater reduction in amylose than in amylopectin content. Taking into account those findings, present results suggested that degradation of starch by α -amylase might be initiated in amylose, releasing simple compounds as total sugars.

3.2. Physicochemical properties

The gel and hydration properties of the germinated and raw amaranth flour are presented in Table 2. After germination, hydration properties were significantly affected in both species ($P<0.00$), however they respond differently. In *A. caudatus*, WBC decreases significantly, otherwise in *A. quitensis*, it remains constant. Other diverse behavior associated to species was the wide increase of WSI in *A. quitensis* compared to *A. caudatus*. The increase of WSI was related to the release of water-soluble compounds during the enzymatic starch degradation (Chauhan et al., 2015). A strong negative correlation was found between WSI and total starch ($r = -0.86$, $P < 0.00$). The higher increment of WSI in *A. quitensis* could suggest a fragile starch

structure more susceptible to α -amylase attack during germination. A significant decrease of WAI was observed with germination ($P < 0.00$), which disagree with previous research in amaranth (Chauhan et al., 2015) and rice (Cornejo & Rosell, 2015). On the other hand, a significant increase in WHC was observed after 24 h of germination ($P < 0.00$). Same trend was noticed in brown rice (Cornejo & Rosell, 2015).

The correlation analysis carried out on the chemical composition indicates a positive correlation of fat content with WBC ($r = 0.71$, $P < 0.01$) and protein with WHC ($r = 0.89$, $P < 0.00$), as well as, a negative correlation of fat with WHC ($r = -0.78$, $P < 0.00$). Considering these results, a hydrophobic hydration might be present in amaranth, thus with lipid degradation a decrease of WBC is produced. In addition, the protein could entrap the water, increasing WHC. A negative strong correlation was found between protein and fat content ($r = -0.94$, $P < 0.00$). Overall, researches attribute the changes of hydration properties during germination to the increase of polar groups in proteins and polysaccharides (Gamel, et al., 2006; Chinma et al., 2009; Cornejo & Rosell, 2015; Ghumman et al., 2016).

The gelatinization properties of native and germinated amaranth flours can be found in Table 2. Germination did not affect the gelatinization behavior of flour, but specie induced significant changes ($P < 0.05$). The native and germinated *A. quitensis* flour presented higher gelatinization temperatures and gelatinization enthalpy than *A. caudatus*. Overall, no significant difference in gelatinization temperatures was observed within 24 hours of germination ($P > 0.05$). An increase in gelatinization temperatures was reported in amaranth after longer germination (48 h) (Gamel et al., 2005) and in brown rice flour (72 h germination) (Wu et al., 2013) and adlay seed (Xu et al., 2017). Wu et al. (2013) reported that gelatinization temperatures slightly decrease after

germinating 24 hours but increase with prolonged germinations. The effect on the gelatinization temperature has been associated to α -amylase activity, protease activity, sugar content, amylose content and protein content (Wu et al., 2013; Xu et al., 2017). Regarding species, *A. caudatus* exhibited an increment of ΔH due to germination, as reported by Gamel et al. (2005), but the opposite was observed in *A. quitensis*. Other botanical sources responded differently to germination, for instance, in brown rice flour ΔH decrease (Xu, Zhang, Guo & Qian, 2012) and in adlay seeds ΔH remains constant (Xu et al., 2017). The ΔH reflects the amount of energy needed to disrupt the starch granule during gelatinization, and some authors related ΔH to the crystallinity and molecular order of the starch. In fact, the molecular interaction of the starch with the flour components, particularly the water, influence the behavior of the starch during gelatinization (Tananuwong & Reid, 2004). As mentioned before, the results reveal that *A. quitensis* presents weaker starch structure that affects the thermal properties.

The X ray diffraction patterns of native and germinated amaranth (figure 1) showed A type crystallinity with three predominant peaks, supporting Siwatch et al. (2017) results. No significant difference ($P>0.05$) in crystallinity index was observed between species and germination process. Although, *A. caudatus* presented a slight increase, and *A. quitensis* a slight reduction, of crystallinity, suggesting different structure before germination ascribed to species. These results reveal that the hydrolysis of the starch after 24 hours of germination did not significantly affect starch at structural level.

3.3. Rheological properties

271 The pasting properties of the amaranth flour decreased after germination (Table 3), confirming
 272 previous findings in amaranth (Chauhan et al., 2015), brown rice (Wu et al., 2013; Cornejo &
 273 Rosell, 2015), lentil (Ghumman et al., 2016), tigernut (Chinma et al., 2009) and adlay seed (Xu
 274 et al., 2017). The starch degradation, amylase activity, hydrolysis and disruption of disulfide
 275 linkages of protein and fat content have been listed as responsible for those changes. The
 276 correlation analysis showed a strong correlation between pasting properties and lipid content,
 277 protein content, amylose content, ash and total starch, particularly non-resistant starch. The peak
 278 viscosity has strong positive correlation with lipid ($r=0.94$, $P < 0.00$), amylose ($r=0.82$, $P <$
 279 0.00), total starch ($r=0.87$, $P < 0.00$), non-resistant starch ($r=0.76$, $P < 0.00$); as well as strong
 280 negative correlation with ash ($r=-0.89$, $P < 0.00$). Final viscosity showed strong positive
 281 correlation with lipid ($r=0.95$, $P < 0.00$), total starch ($r=0.80$, $P < 0.00$), non-resistant starch (r
 282 $=0.81$, $P < 0.00$); as well as strong negative correlation with ash ($r=-0.89$, $P < 0.00$) and protein
 283 ($r=-0.98$, $P < 0.00$). Setback presents strong positive correlation with lipid ($r=0.72$, $P < 0.00$),
 284 total starch ($r=0.91$, $P < 0.00$), non-resistant starch ($r=0.91$, $P < 0.00$); as well as strong
 285 negative correlation with ash ($r=-0.94$, $P < 0.00$) and protein ($r=-0.87$, $P < 0.00$). Breakdown
 286 shows strong positive correlation with carbohydrate ($r=0.75$, $P < 0.01$), amylose ($r=0.89$, $P <$
 287 0.00), total starch ($r=0.68$, $P < 0.01$) and resistant starch ($r=0.94$, $P < 0.00$). These correlations
 288 confirmed that starch degradation by amylase, predominantly non-resistant starch degradation,
 289 affected the pasting properties. The results also revealed a significant intervention of lipids in
 290 peak viscosity, final viscosity and setback. Zhou, Zhang, Chen & Chen (2017) noted that the
 291 addition of saturated fatty acid to rice starch, decreased the peak viscosity, breakdown viscosity
 292 and setback. They mentioned that fatty acids restrain the starch granule from hydration, affecting
 293 pasting behavior. The positive correlation encountered in the present study, suggested that the

reduction of lipid content by germination negatively affected the paste properties. Thus, as mentioned earlier, a possible hydrophobic interaction of lipid with water might be involved. The weak intermolecular bonding allows the starch granule to swell freely and give higher paste viscosities. On the other hand, the extent of reduction of pasting properties differed between species, particularly in the breakdown (*A. quitensis* was 62.5% and *A. caudatus* remain equal) and setback viscosity (*A. quitensis* was 41.2 % and *A. caudatus* was 20.4%). It seems that *A. quitensis* underwent higher starch degradation after 24 h of germination.

The viscoelastic properties of native and germinated amaranth flour were also evaluated (table 3). The flow behavior indexes (n) of the native and germinated amaranth flours indicated a pseudoplastic behavior ($0 < n < 1$). No significant difference of n value was observed with germination process ($P > 0.05$). In addition, a significant reduction of K value was observed in *A. caudatus* ($P < 0.05$), while in *A. quitensis* did not change. A correlation between amylose content with n value ($r = 0.73$, $P < 0.01$) and K ($r = 0.92$, $P < 0.00$) was observed. Xie et al. (2009) had showed in corn starch that K increases with the increase of amylose content. Considering that K value is related to viscosity, it could explain the influence of amylose degradation during germination in the rheological behavior of the gel.

The elastic (G') and the viscous (G'') modulus decreased with germination (Table 3), corroborating previous findings in brown rice flour (Wu et al., 2013). Indeed, a significant difference of G' and G'' values was observed due to specie and germination process ($P < 0.00$). *A. quitensis* presented higher reduction of G' and G'' values than *A. caudatus*. In addition, the higher G' values compared to G'' values, indicated a weak gel structure, that were more elastic than viscous.

3.4. Nutritional Properties

Table 4 presents the nutritional characteristics of native and germinated of amaranth flours. Both amaranth species presented high levels available starch, which represented more than 95% of total starch. A significant decrease in total, resistant and non-resistant starch content was noticed with germination ($P<0.05$) in both species, because the α -amylase action. It is interesting to notice that the reduction in total starch content and non-resistant starch was much more rapid in *A. quitensis* than in *A. caudatus*. In addition, *A. quitensis* exhibited a significant reduction of rapidly digested starch (RDS) and slowly digested starch (SDS) ($P<0.05$), while in *A. caudatus* an increment of RDS and no change in SDS was observed. Therefore, germination could improve the availability of starch in *A. caudatus*.

The parameters of the *in vitro* starch digestibility are also presented in Table 4. Each specie responded differently to germination. For example, in *A. caudatus* the rate of the hydrolysis (k), and rapid digested starch (RDS) increased significantly after germination, while in *A. quitensis* significantly decreased ($P<0.05$). Also, the estimated glycemic index (eGI) differed in both species after germination. *A. quitensis* showed significantly lower eGI ($P<0.05$) while in *A. caudatus* remained constant. Low glycemic index values are desirable to control diabetes, obesity and hyperlipidemia. Otherwise, no significant change was observed in the starch concentration at the equilibrium point (C_{∞}) ($P>0.05$).

Specie and germination of amaranth also affected protein digestibility significantly ($P<0.00$) (Table 4). *A. quitensis* showed higher protein digestibility after 24 hours of germination. According to Najdi Hejazi & Orsat (2017) the increment of protein digestibility was associated

to the protein released during starch breakdown in germination. It has been reported that germination improves the antioxidant activity in seeds like amaranth, quinoa, buckwheat and wheat, among others (Alvarez-Jubete, Wijngaard, Arendt & Gallagher, 2010; Perales-Sánchez et al., 2014; Chauhan et al., 2015). Nevertheless, the antioxidant activity remained constant in both species after 24 hours of germination. Therefore, it seems that the increment of antioxidant activity required longer germinations. Antioxidant capacity can be related to the presence of antioxidant compounds such as phenolic compounds and vitamins. Significant differences were observed between species ($P<0.05$), having higher phenolic content *A. caudatus*, and increased by three folds after being subjected to germination. No positive correlation was found between phenolic and antioxidant activity, which suggested that phenolic compounds released during 24 h germination might be free and esterified phenolics, since bound phenolic fraction presented higher antioxidant capacity than free and esterified phenolics (Liyana-Pathirana & Shahidi, 2006).

Insoluble dietary fiber content was significantly dependent on the amaranth specie ($P<0.00$), Overall, dietary fiber contents were significantly reduced after germination ($P<0.00$) (Table 4). Similar results were observed in *A. caudatus* and *A. cruentus* after 48 h of germination (Colmenares & Bressani, 1990; Gamel et al., 2005).

5. Conclusions

Short germination (24 hours) could be sufficient to produce significant changes in the physiochemical and nutritional properties of amaranth, revealing a physically efficient treatment to improve amaranth properties. Among the species studied, *A. quitensis* possessed weaker starch structure that significantly affected its response to germination, primarily due to the molecular interactions within the seed. Germination of *A. quitensis* led to flours with lower total starch

content and lower estimated glycemic index, but higher total dietary fiber than the germinated flour obtained from *A. caudatus*. With germination, *A. caudatus* present an increase of nutritional characteristics without extensively affect its nutritional properties of *A. quitensis*. According to our results, germinated *A. caudatus* flour could be used as a functional ingredient in bakery products and germinated *A. quitensis* flour could be used in beverages. Although the benefits of germination have been previous stated, this study points out the importance of prior selection of appropriate specie of amaranth for specific modification or improvement in physicochemical or nutritional properties for industrial application.

Acknowledgements

Authors acknowledge the financial support of the Ecuadorian Research Network of Universities and Polytechnic Schools (REDU) thought the project PI-REDU-2015-012, the Spanish Ministry of Economy and Competitiveness (Project AGL2014-52928-C2-1-R), Generalitat Valenciana (Prometeo 2017/189) and the European Regional Development Fund (FEDER).

Conflict of interest statement

We have no conflicts of interest to disclose.

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Figure 1. X-ray diffraction patterns of amaranth flour

A: *A. quitensis* germinated flour, B: *A. quitensis* native flour, C: *A. caudatus* germinated flour, D: *A. caudatus* native flour.

Table 1. Chemical composition from two species of native and germinated amaranth flour, expressed in g/100 g dry matter.

Species	Treatment	Moisture	Ash	Protein (N x 6.25)	Lipid	Carbohydrate	AAC	Total sugars
<i>A. caudatus</i>	Native	11.28 ± 0.20 ^a	3.25 ± 0.26 ^c	16.04 ± 0.07 ^d	8.20 ± 0.07 ^a	61.23 ± 0.33 ^a	12.79 ± 0.96 ^a	2.55 ± 0.02 ^b
	Germinated	8.57 ± 1.37 ^b	4.40 ± 0.26 ^b	20.70 ± 0.04 ^b	5.57 ± 0.23 ^c	60.77 ± 1.33 ^{ab}	10.06 ± 0.46 ^b	2.69 ± 0.02 ^a
<i>A. quitensis</i>	Native	11.48 ± 0.73 ^a	3.67 ± 0.28 ^c	18.95 ± 0.06 ^c	6.50 ± 0.34 ^b	59.40 ± 1.10 ^{bc}	7.17 ± 0.17 ^c	1.13 ± 0.01 ^d
	Germinated	8.44 ± 0.38 ^b	5.47 ± 0.13 ^a	22.17 ± 0.02 ^a	5.68 ± 0.26 ^c	58.25 ± 0.53 ^c	5.79 ± 0.15 ^d	2.13 ± 0.03 ^c
P-value	Treatment	0.000	0.000	0.000	0.001	0.151	0.000	0.032
	Species	0.930	0.002	0.000	0.040	0.002	0.000	0.004

AAC: Apparent Amylose Content. Values followed by different letters within a column denote significant differences (P<0.05). Mean ± standard deviation (n=3).

Table 2. Physical properties from two species of native and germinated amaranth flour.

	<i>A. caudatus</i>		<i>A. quitensis</i>		P-value	
	Native	Germinated	Native	Germinated	Treatment	Species
Hydration Properties ^A						
WHC (g/g)	3.17 ± 0.00 ^c	4.61 ± 0.10 ^{ab}	3.80 ± 0.16 ^{bc}	5.30 ± 0.91 ^a	0.000	0.029
WBC (g/g)	1.87 ± 0.07 ^a	1.52 ± 0.12 ^b	1.79 ± 0.08 ^a	1.73 ± 0.09 ^a	0.014	0.433
WAI (g/g)	7.46 ± 0.51 ^a	5.51 ± 0.57 ^b	7.71 ± 0.19 ^a	4.93 ± 0.62 ^b	0.599	0.000
WSI (g/g)x100	10.39 ± 1.12 ^c	34.91 ± 1.77 ^b	5.07 ± 0.57 ^d	53.66 ± 3.50 ^a	0.000	0.141
Thermal Properties ^B						
T _o (°C)	54.57 ± 0.60 ^c	61.82 ± 0.75 ^b	78.60 ± 1.11 ^a	77.88 ± 1.24 ^a	0.048	0.000
T _p (°C)	56.64 ± 0.85 ^c	65.63 ± 1.05 ^b	82.58 ± 1.97 ^a	81.29 ± 0.60 ^a	0.066	0.000
T _c (°C)	59.59 ± 0.90 ^c	69.13 ± 0.65 ^b	85.40 ± 3.83 ^a	83.99 ± 0.60 ^a	0.089	0.000
ΔH _{gel} (J/g)	7.94 ± 1.68 ^c	11.58 ± 0.51 ^b	16.73 ± 0.98 ^a	11.56 ± 0.77 ^b	0.640	0.022
Crystallinity (%)	31.47 ± 3.54 ^a	33.06 ± 0.45 ^a	29.90 ± 1.93 ^a	27.74 ± 4.30 ^a	0.872	0.077

^AWHC: Water Holding Capacity, WBC: Water Binding Capacity, WAI: Water Absorption Index, WSI: Water Solubility Index. ^B T_o= onset temperature, T_p= peak temperature, T_c= conclusion temperature, ΔH_{gel}= enthalpy change. Values followed by different letters within a row denote significant differences (P<0.05). Mean ± standard deviation (n=3).

Table 3. Rheological properties of amaranth flour.

Species	Treatment	Viscoelastic properties		Power-law equation parameters		Pasting properties			
		G' (Pa)	G'' (Pa)	K (Pa s ⁿ)	n	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
<i>A. caudatus</i>	Native	25.81 ± 3.91 ^a	8.40 ± 0.40 ^a	12.63 ± 1.94 ^a	0.41 ± 0.04 ^a	1086 ± 13 ^a	133 ± 3 ^a	1099 ± 13 ^a	147 ± 2 ^a
	Germinated	9.29 ± 0.04 ^c	1.81 ± 0.42 ^c	6.98 ± 1.27 ^b	0.38 ± 0.05 ^a	583 ± 27 ^c	144 ± 11 ^a	555 ± 19 ^c	117 ± 3 ^b
<i>A. quitensis</i>	Native	16.46 ± 0.09 ^b	5.42 ± 0.44 ^b	2.29 ± 0.72 ^c	0.36 ± 0.02 ^a	724 ± 5 ^b	24 ± 5 ^b	848 ± 9 ^b	148 ± 6 ^a
	Germinated	1.32 ± 0.56 ^d	0.44 ± 0.11 ^d	1.42 ± 0.31 ^c	0.38 ± 0.04 ^a	413 ± 1 ^d	9 ± 1 ^c	491 ± 4 ^d	87 ± 3 ^c
P-value	Treatment	0.000	0.000	0.014	0.344	0.001	0.964	0.000	0.000
	Species	0.000	0.000	0.000	0.106	0.073	0.000	0.306	0.375

Values followed by different letters within a column denote significant differences ($P < 0.05$). Mean ± standard deviation (n=3).

G': storage modulus; G'': loss modulus. K: consistency index; n: flow behavior index.

Table 4. Nutritional properties of amaranth flour

	<i>A. caudatus</i>		<i>A. quitensis</i>		P-value	
	Native	Germinated	Native	Germinated	Treatment	Species
Total starch (g/100g)*	66.92 ± 0.92 ^a	54.69 ± 1.24 ^b	56.62 ± 1.86 ^b	27.07 ± 1.75 ^c	0.000	0.000
Resistant starch (g/100g)*	0.76 ± 0.02 ^a	0.64 ± 0.03 ^b	0.37 ± 0.01 ^c	0.23 ± 0.02 ^d	0.000	0.000
Non-resistant starch (g/100g)*	66.16 ± 0.91 ^a	54.05 ± 1.22 ^b	56.25 ± 1.86 ^b	26.84 ± 1.77 ^c	0.000	0.000
In vitro starch digestibility						
C _∞ (g/100 g)	71.25 ± 2.37 ^a	61.27 ± 2.10 ^b	68.80 ± 3.82 ^{ab}	68.55 ± 8.97 ^{ab}	0.103	0.558
k (min ⁻¹)	0.026 ± 0.002 ^b	0.048 ± 0.016 ^a	0.024 ± 0.003 ^b	0.009 ± 0.001 ^c	0.500	0.019
RDS	28.24 ± 1.39 ^b	35.85 ± 5.64 ^a	28.06 ± 0.41 ^b	12.96 ± 1.39 ^c	0.335	0.010
SDS	35.24 ± 1.61 ^{ab}	32.11 ± 6.68 ^{ab}	43.61 ± 13.61 ^a	25.44 ± 8.96 ^b	0.043	0.779
eGI	55.00 ± 0.84 ^a	54.45 ± 1.58 ^{ab}	52.70 ± 1.30 ^b	36.50 ± 1.26 ^c	0.002	0.002
Protein digestibility (%)	75.04 ± 0.07 ^d	77.18 ± 0.16 ^c	79.42 ± 0.09 ^b	80.12 ± 0.12 ^a	0.000	0.000
Antioxidant capacity ORAC ^{A*}	32.19 ± 6.66 ^a	35.74 ± 5.27 ^a	29.79 ± 1.29 ^a	33.08 ± 3.73 ^a	0.229	0.364
Total phenolic content ^{B*}	17.61 ± 0.16 ^c	58.55 ± 0.11 ^a	11.71 ± 0.11 ^d	36.65 ± 3.99 ^b	0.000	0.015
Soluble dietary fiber (g/100 g)*	2.11 ± 0.05 ^a	0.60 ± 0.36 ^b	2.01 ± 0.26 ^a	0.59 ± 0.25 ^b	0.000	0.686
Insoluble dietary fiber (g/100 g)*	7.13 ± 0.21 ^c	3.90 ± 0.65 ^d	19.03 ± 0.12 ^a	12.45 ± 0.22 ^b	0.000	0.000

*Dry basis. C_∞: equilibrium concentration of starch hydrolyzed after 180 min, k: kinetic constant, RDS: rapidly digesting starch, SDS: slowly digesting starch, eGI: glycemic index. ^A μM Trolox equivalent (ET)/g sample, ^B mg gallic acid equivalents (GAE)/ 100 g sample. Values followed by different letters within a row denote significant differences (P<0.05). Mean ± standard deviation (n=3).