

Characterization and comparison of tiger nuts (*Cyperus esculentus*) from different geographical origin. Physico-chemical characteristics and protein fractionation

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Abstract

In the present work, four types of marketable tiger nuts (*Cyperus esculentus*) from different geographical origins (Spain, Burkina Faso and Niger) were compared according to their physico-chemical characteristics and protein characterization. All batches presented a mixture of tubers of different shape, size and weight, of which the Spanish were the largest. Differences observed between their colours pointed to the variability in the skin color of tiger nuts in the market. The Spanish tubers were the richest in fat and protein (35.21 and 8.45%, respectively), while similar values were observed in the African tubers (from 25.35 - 28.19% in fat and 3.28 - 7.32% in protein). Fairly low percentages of tannins and phytates were also detected in all of them (0.08 - 0.18 g / 100 g and 9.31 - 22.55 mg / 100 g, respectively). Similar results were obtained in the protein fractionation and characterization in tubers. These data showed the albumins to be the main protein fraction (82.23 - 91.93%), while globulins, prolamins and glutelins were represented by a remaining ~ 3 - 7.5%. Albumins were characterized by a wide range of different molecular weight polypeptides (20, 25, 37, 55, 75 and ~

106 kDa), whereas in the other fractions, a lower number of bands was detected (at 18 - 78 kDa). These results suggested that physico-chemical characteristics and proximate composition of tiger nut batches in the current market are the most variable parameters, which are mainly dependable on their geographical origin.

Keywords: Tiger nuts, *Cyperus esculentus* L., physico-chemical characteristics, protein fractions, SDS-PAGE gel

1. Introduction

Tiger nuts (*Cyperus esculentus* L.) are little sweet tubers produced in the roots of an edible perennial grass-like plant, which is widely found around the Mediterranean countries (Coşkuner et al., 2002). These tubers are rich in energy (~ 400 - 450 kcal / 100 g), due to their content in starch and fat (26 - 30% and 21 - 25%, respectively). Major fatty acids of tiger nut oil are monounsaturated (> 60%), which is why its profile is comparable to olive or hazelnuts oils (Dubois et al., 2007). Tiger nuts also present a good percentage in protein (3 - 8%) and fiber (8 - 10%), as well as in vitamins (E and C) and minerals (phosphorous and potassium) (Adejuyitan, 2011; Arafat et al., 2009; Bosch et al., 2005; Chinma et al., 2009; Chukwuma et al., 2010; Tigernuts Traders, 2014). Owing to their composition, different studies have pointed the suitability of these tubers for diabetics and people with digestive disorders, and also in the prevention of heart diseases (Borges et al., 2008; Chukwuma et al., 2010; Tigernuts Traders, 2014). For this reason, these tubers are attracting industry interest, and the development of new tiger nut derived products has already been reinforced. These tubers are sold as snack food, and their flour has also been successfully used in bakery, in by-meat products and as a flavoring agent in ice cream production (Sánchez-Zapata et al., 2012). However,

tiger nuts are mainly used in the production of tiger nut drinks, non alcoholic beverages that are traditionally made in Spain (*horchata de chufa*) and Africa (*kunnun aya*). Current studies have evidenced the importance of tiger nut characteristics in the production of these quality derived products in terms of their physicochemical characteristics and nutritional profile, but despite most of these tubers being characterized and submitted to the sanitary official controls, the quality characteristics of some batches are still in doubt (Addy and Teshola, 1984; Bosch et al., 2005; Ejoh and Ndjouenkeu, 2007; Oladele and Aina, 2007; Sánchez-Zapata et al., 2010). Owing to all of this, the objective of the present work was to compare the quality characteristics of tiger nuts from different geographical origin which are commercialized in the European market for the production of derived products, in order to generate more information about the effect of site cultivation on their physicochemical characteristics. Furthermore, another goal was to obtain tiger nut protein fractions and determine partially their chemical characteristics, data about their chemical characterization that remains scarcely explored.

2. Materials and methods

2.1. Materials

Tiger nuts used in this study were obtained from different markets. Spanish tubers (VTN) were provided by Horchata Lider, S.L. (Polinyà, Spain) from the province of Valencia. At the same time, African tiger nuts were provided by Nectina, S.L. (Tarragona, Spain), from three different local markets of Burkina Faso (B1TN and B2TN) and Niger (NTN), which export several tons of tiger nuts every year to Europe. For each type of tiger nut, two carrier bags of 25 kg were collected from two different

containers of 1000 kg of tubers. Three representative subsamples (from three different parts of each carrier bag) were taken for the analyses carried out in this study.

2.2. Physical characterization

2.2.1. Shape

Shape of tiger nuts was determined measuring the length (L , in mm) and the thickness (T , in mm, considering the biggest diameter) of 300 tiger nuts of each carrier bag (100 tubers from three different parts of the bag), using an electronic digital caliper to an accuracy of 0.01 mm (Electronic Digital Vernier Caliper 0 - 150 mm, Barcelona, Spain). Tubers were divided in three groups according to the shape classification proposed by Morell and Barber (1983). In accordance to the ratio L/T , tubers were classified as round tubers ($L/T \leq 1.24$), oval tubers ($L/T < 1.25$) and elongated tubers ($L/T \geq 1.45$). In line with this classification, percentages of tiger nuts of every shape *per* carrier bag were also estimated.

2.2.2. Weight

Tuber weight was determined by weighing 300 tiger nuts from each carrier bag (100 tubers from three different parts of the bag), using an electronic balance. The average weight of round, oval and elongated tubers were also determined by counting 100 tubers of every shape and weighing them.

2.2.3. Color

Surface color of tiger nuts was determined using a Hunter Lab colorimeter (MiniScan XETM, Hunter Associates Laboratory Inc., Reston, VA). Color coordinates were measured with an illuminant of D65 and a standard observer of 10°. The colorimeter

was calibrated against black and white tile standards. The Commission Internationale de l'Eclairage (CIE) L^* , a^* , and b^* values were measured in triplicate. The L^* value represents the lightness (with values from 0 (black) to 100 (white)), and the a^* (red-green) and b^* (blue-yellow) axes (with no specific numerical limits) represent chromatic components. Color differences (ΔE^*) were determined by the following equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, in which ΔL^* , Δa^* and Δb^* are the differences in the tristimulus coordinates between samples and a reference sample, in this case VTN.

All physical analyses were performed in triplicate.

2.3. Chemical characterization

2.3.1. Proximate composition

Before proximate analysis, tubers were ground in a laboratory grinder, passing through a 0.5 mm sieve (CISA, Barcelona). AOAC (2002) methods were used for the determination of moisture (method 925.10), fat (method 920.39), protein (method 955.04), crude fiber (method 962.09) and ash (method 923.03). The protein / nitrogen coefficient used was 6.25. Dietary fiber (soluble and insoluble) was determined according to the AOAC method 991.43 by using *KIT Megazyme K-TDFR 12/05* (Megazyme International, Wicklow, Ireland). Nitrogen free materials (NFM) were calculated by difference. Fat, crude fiber, ash, protein and NFM results were expressed in dry matter.

2.3.2. Antinutritional components: phytates and tannins

Phytate content of tiger nut flour was determined according to Haugh and Lantzsch (1983), following the variant *a* of the method. Results were expressed as μg of phytate /

mL of extract. Tannin content in samples was determined by ISO method 9648 (International Standards Organization, 1988). The tannin content was expressed as a percentage by mass of tannic acid in relation to the dry matter.

2.3.3. Protein fractionation

Tiger nuts were ground in a laboratory grinder and passed through a 0.5 mm sieve (CISA, Barcelona, Spain). Defatted tiger nut flour was obtained by the treatment with anhydrous ether in a Soxhlet apparatus (AOAC, 2002). The method proposed by Osborne (1924), with some modifications, was used for the extraction of the different protein fractions:

Albumins + Non Protein Nitrogen (NPN): Defatted tiger nut flour was first extracted with distilled water (0.1 g/mL), by three stirring steps of 1 h at 4 °C and then centrifuged at 10 000 *g* for 30 min at 4 °C. Supernatants were mixed and analyzed for total nitrogen and freeze-dried.

Globulins: The resulting residue from the previous extraction was extracted under magnetic stirring with 10% NaCl (0.1 g/mL) for 1 h at 4 °C and centrifuged at 10 000 *g* for 30 min at 4 °C. Supernatant was dialyzed against distilled water for 5 days at 4 °C, changing the water of dialysis daily. Dialyzed sample was analyzed for total nitrogen and freeze-dried.

Prolamins: The globulin residue was extracted under magnetic stirring for 1 h at 4 °C, with 70% 2-propanol (0.1 g/mL). Then, the aqueous sample was centrifuged at 10000 *g* for 30 min at 4 °C, and the supernatant was dialyzed against acetic acid (0.01%), during 5 days at 4 °C, changing the solution daily. Dialyzed sample was analyzed for total nitrogen and freeze-dried.

Glutelins: Glutelins were extracted from previous residue, under magnetic stirring for 1 h at 4 °C with 0.1 mol/L Na₂B₄O₇ + 1 g/L SDS (0.1 g/mL), according to Bernardino-Nicanor et al. (2006). Then, the samples were centrifuged at 10 000 *g* during 30 min at 4 °C, and supernatants were dialyzed against acetic acid (0.01%) during 5 days at 4 °C with daily changes of solution. Dialyzed sample was analyzed for total nitrogen and freeze-dried.

2.3.4. Nitrogen and total protein determination

The nitrogen content of each fraction (albumins + NPN, globulins,utelins, prolamins and insoluble residue) was determined by the Kjeldahl method, according to the AOAC (2002) method 955.04 by using a Kjeltec Auto Distillation 2200 apparatus (Barcelona, Spain).

2.3.5. Gel electrophoresis (SDS – PAGE) of protein fractions

The freeze-dried extracts obtained according to Osborne method were dissolved in a buffer solution at pH = 6.8 (Tris - HCl 0.5 mol/L, 10 mL/100 mL glycerol, 2 g/100 mL SDS and 0.05 g/100 mL bromophenol blue), in the proportion 5:1 (w:w). For reducing conditions, 5% β-mercaptoethanol was added to the samples before heating them at 95 °C for 5 min in a boiling water bath. In order to remove the residual fat, the samples were centrifuged at 2500 *g* for 30 min. 40 µL of supernatants were loaded onto SDS-polyacrylamide gels (4 and 12 % polyacrilamide for stacking and separating gel, respectively), according to Laemmli (1970). A molecular mass (MW) marker (wide range of 2-250 kDa, Precision Plus Protein™ Dual Xtra Standards, Bio-Rad Laboratories, España) was applied to each gel. Gels were run at 200 V using a Pharmacia Biothech power supply unit (model EPS 3500, Pharmacia Biotech, Uppsala,

Sweden). Proteins were fixed in the gel using 0.1% (w/v) Coomassie Brilliant Blue R250 in a 5:1:4 mixture of methanol, acetic acid and distilled water. Gels were destained with a solution of methanol (160 mL/L) and glacial acetic acid (10 mL/L). ImageMaster software (Amersham Pharmacia Biotech, Newcastle, UK) was used for the analysis of the scanned destained gels images. On the SDS-PAGE gel, the apparent molecular mass of the bands was estimated from the mobility of proteins in the gel when comparing with the mobility of the MW markers.

All chemical analyses were performed in triplicate.

2.4. Statistical Analysis

Data were processed by analysis of variance (ANOVA), using the general linear models procedure of Statgraphics (Statgraphics Inc., Chicago, IL, USA). Tukey test was used for comparison of sample data, and evaluations were based on a significance level of $P < 0.05$.

3. Results and discussion

Figure 1 displays a representative image of the different varieties of tiger nuts used in this study, in which physical impurities had already been eliminated. Note that in their reception, African tubers (B1TN, B2TN and NTN) showed a higher level of initial contamination (stones, sand, plant roots remains, damaged tubers or other grain shells) than Spanish, which nearly presented physical impurities.

3.1. Physical Characterization

3.1.1. Shape

Table 1 shows the values obtained in the shape classification of tiger nuts in accordance to their sizes. It was observed that batches of tiger nuts in the market present a mixture of tubers of different size. Length and thickness values ranged from 5.70 to 9.05 mm and from 7.10 to 14.37 mm, respectively. Spanish tiger nuts presented the largest sizes, whereas similar values were obtained among the African.

All carrier bags presented a mixture of elongated, oval and round tubers. In all of them, round tubers were the predominant (~ 50 - 72% of total), while elongated and oval tubers followed them in importance (~ 13 - 28% and ~ 16 - 27% of total, respectively). Similar results were described previously by Morell and Barber (1983) in their studies with marketable batches of tiger nuts from Spain. Linking the size of tubers with their shape classification, elongated tiger nuts were the biggest in all cases (Table 1). Djomdi et al. (2008) and Ejoh and Ndjouenkeu (2006) studied the influence of tiger nuts size on their soaking behavior. These authors observed that the rate of absorption was inversely proportional to the tuber size, whereas at ≥ 80 °C the water content equilibrium was reached in the same period of time, whatever the tuber dimension. Therefore, data about tubers size of marketable batches may be useful for the drink industry with respect to optimizing the soaking time of tubers in the tiger nut milk beverages performance. Shape properties of these tubers should also provide useful information for the design of equipment for processing and storing the tubers.

3.1.2. Weight

The average weight of analyzed tiger nuts is presented in Table 2. As shown, this parameter varied considerably, especially if comparing tiger nuts according to their origin. Note that weight depends on the grain size, and as observed, this parameter was widely variable. Spanish tubers were the heaviest (~ 0.40 g), whereas the African

reported similar specific weights to each other (0.27 - 0.30 g). If relating the shape of tubers with their weight, in general, elongated tiger nuts were the heaviest. Tuber weight depends on tuber thickness but, to the same thickness, the weight increase is in accordance to their length. Average specific weight ranged between 0.27 and 0.51 g in elongated, 0.24 - 0.39 in oval and 0.23 - 0.36 in round tiger nuts. In fact, the grain mass also increases according to the moisture content of grains, so that tubers with higher percentage of water are supposed to be the heaviest (Table 4).

3.1.3. Color measurement

Color coordinates of all analyzed tubers are shown in Table 3. Significant differences ($P < 0.05$) were observed in the lightness (L^*), and a^* (red - green) and b^* (blue - yellow) parameters between tubers (Table 3). Burkina tiger nuts B1TN and B2TN presented the highest values of L^* (more white), a^* (more red) and b^* (more yellow), and they did not show large differences between them. The lowest values of all chromaticity coordinates were obtained in VTN. NTN did not present significant differences in L^* and a^* parameters if comparing to VTN, although in Niger tubers b^* value was higher.

To the naked eye, tiger nuts were classified in two main groups: *lighter* or *yellowish* (B1TN ~ B2TN) and *darker* or *brownish* (NTN > VTN). In fact, other authors have previously classified these tubers in accordance to the skin colors *yellow* and *brown* (Chinma et al., 2009; Oladele and Aina, 2007). That subjective assessment was also evidenced by the results shown in Table 3. According to the ΔE^* parameter, which indicates the color differences between VTN and the others, NTN were close to VTN, while there were no general color differences between Burkina tubers, which were the most different related to VTN. This pointed to the variability in the skin color of tiger nuts in the market, although in some applications (as for example in the production of tiger nut drinks and snacks) tubers skin is finally removed.

3.2. Chemical characterization

3.2.1. Proximate composition

Proximate composition of tubers is shown in Table 4. Although there were slight differences between samples in protein and fat composition, all tiger nuts presented a similar nutritional profile, whose principal components were NFM and fat (45.05 - 58.66 and 25.35 - 35.21%, respectively). Therefore, tiger nuts are considered a high energetic product (~ 400 - 450 kcal / 100 g), especially if compared with other tubers such as raw potato (*Solanum tuberosum*) or sweet potato (*Ipomoea batatas*), which are 71 and 100 kcal / 100 g, respectively (Chinma et al., 2009; CESNID, 2003; Tigernuts Traders, 2014).

Moisture content of analyzed tiger nuts was comprised in the range of 6.38 - 8.66%. Before tubers are stored, they are dried in order to reduce their percentage of water, down to almost ~ 10%, to ensure a longer shelf time inside the containers. This low moisture content emphasizes the importance of soaking tiger nuts before the production of milk beverages. The grain blending in this process is fundamental to facilitate their grinding, as well as to improve nutrient diffusion through the milky extract (Ejoh and Ndjouenkeu, 2006).

NFM were the principal component in all samples (Table 1). NFM percentages were $B2TN = NTN > B1TN > VTN$. Morell & Barber (1983) reported that ~ 65% of NFM corresponded to starch and fiber, which indeed are the main carbohydrates in all tubers. Between others, the variety and the ripening stage of tiger nuts will determine their content of carbohydrates (Coşkuner et al., 2002). Similar percentages of crude fiber were obtained in all analyzed tubers (8.42 - 9.31%). VTN and B1TN presented the highest percentages, similar to those described previously in tiger nuts from Turkey

(Coşkuner et al., 2002). Total dietary fiber in NTN, B1TN, B2TN and VTN was 18.52 ± 0.50 , 21.00 ± 0.42 , 25.84 ± 1.57 and $27.77 \pm 0.01\%$, respectively, and in all of them, ~90% of this fiber was represented by insoluble fiber. Total dietary fiber of tiger nuts has good profile for its application in different food products. In line with this, Sánchez-Zapata et al. (2010) noted the potential of the solid residue obtained in the production of tiger nut beverage as a possible ingredient for the elaboration of different meat products, such as dry cured pork sausages or pork burgers.

Fat content in all samples was comprised in the range of 25.35 - 35.21%. VTN were the fattiest (~ 35%), while Burkina tubers B1TN and B2TN, which did not present significant ($P > 0.05$) differences between them, showed the lowest fat percentages (25.35 and 25.77%). These values were within the usual range for those previously reported, which make evident the high fat content of tiger nuts in comparison to other tubers (0.13% in yacon, 0.16% in potato or 0.30% in cassava) (Adekanmi et al., 2009; CESNID, 2003; Lombardo et al., 2012; Maives et al., 2012; Scher et al., 2009).

Extracted tiger nut oil was characterized for a pale-yellow color and also for a smooth flavor. This product has recently been revalued for industry for different applications, for example as an accompaniment for cooking food, for nutritional purposes and cosmetic products or in the pharmaceutical industry, among others (Tigernuts Traders, 2014). Its profile is characterized for unsaturated fatty acids (~ 83%), showing a great similarity with the oils obtained from olive (~ 83%), maize (~83%) or soy (~ 82%) (CESNID, 2003). The major fatty acids in tiger nut oil are oleic, palmitic and linoleic (Coşkuner et al., 2002; Hunter, 2001; Kim et al., 2007; Linssen et al., 1988). Both site and variety have a significant influence on the different oil fractions, since it has been verified that the tiger nut fatty acid profile could be used as an alternative geographical authenticity marker (Ejoh and Ndjouenkeu, 2006; López-Cortés et al., 2013).

Furthermore, a moderate content of phytosterols (~ 0.5 - 0.6 g / g of oil) was also described in tiger nuts by Yeboah et al. (2012), which improves the value of tiger nut oil as a food source.

The protein was the most variable component among all analyzed samples (3 - 8.5%). Ejoh and Ndjouenkeu (2007) had already reported the variability among the protein content of tiger nuts from different geographical origin and variety (6.57 - 8.30%). In addition, Chinma et al. (2009) also noted that the protein percentage in tiger nuts increased with tubers germination (from 8.23 to 12.40%), due to the diminution of carbohydrates and fat contents. VTN were the richest in protein (8.45%), followed by Burkina (7.32 and 5.62% for B1TN and B2TN, respectively) and Niger tubers ($\leq 4\%$). Note that in comparison to other tubers, tiger nut protein content is high. In taro (*Colocasia esculenta*), cassava or yams (*Dioscorea spp.*), the percentage of protein is ~ 0,5 - 3% (Abara, 2011; Linssen et al., 1988; Maives et al., 2012)

The content of ash in all tiger nuts was similar, similar to that described in other tubers (Lombardo et al., 2012; Scher et al., 2009; Walter et al., 1984). This percentage in NTN was the lowest (1.60%), whereas the others were richer in minerals (1.90-1.97%) but not statistically different ($P > 0.05$) between them. These results were in accordance to those described by Valls *et al.* (1980) who characterized the tiger nut as a tuber rich in potassium (0.55%) and phosphorous (0.2%). For this reason, drinks made of tiger nuts have been reported to be healthy, since they are a good source of some useful minerals.

3.2.2. Antinutritional components

In this study, all tiger nuts showed low contents of tannins and phytates. These components are widely known to interfere with the bioavailability of some nutrients in the intestinal track, decreasing their absorption (xx). Table 5 shows the results obtained

in the tannin and phytate determination. The tannin content in tubers ranged from ~ 0.08 to 0.18%, the highest for B2TN, and the lowest for VTN. NTN and B1TN did not present significant differences ($P > 0.05$) between them. In Nigeria tiger nuts, Addy and Teshola (1984) reported a tannin content of 0.16%, similar results to those obtained in this study, but on the contrary, Chukwuma et al. (2010) reported a far lower concentration (~ 0.01%) in tiger nuts from the same origin. Tannins inhibit the activities of some enzymes (such as lipase or amylase) by binding proteins and consequently making them difficult to digest in the body. By contrast, these compounds have also been related with antimicrobial activities and also with the prevention of some bacterial infections (Sánchez-Zapata et al., 2012).

The phytate content of analyzed tiger nuts ranged between 9.31 and 22.55 mg / 100 g (Table 5). Phytate percentage in NTN was observed to be the highest, whereas no significant differences ($P > 0.05$) were observed between VTN and B1TN. The lowest content was shown in B2TN. These results were close to those reported by Adekanmi et al. (2009) in Nigeria tiger nuts (21.42 mg / 100 g), but higher than those described by Chukwuma et al. (2010) in tiger nuts from the same origin (1.97 mg / 100 g). Phytates have been found to form chelates with some divalent minerals (such as magnesium and calcium), and thereby the mineral absorption decreases in the intestine tract.

Other authors have also detected alkaloids, sterols, resins, cyanogenic glycosides, sponins and trypsin inhibitors in raw tiger nuts (Addy and Teshola, 1984; Chukwuma et al. (2010); Okafor et al., 2003). Despite this, these studies have demonstrated that soaking, boiling or toasting steps are supposed to reduce the percentage of these components, due to the heat sensibility of these antinutritional components and their diffusion into the soaking water.

3.2.3. Characterization of tiger nut protein fractions

Table 6 shows the percentages of the Osborne's protein fractions isolated in this study.

In accordance with the isolation protocol, the NPN was quantified together with albumins fraction. NPN is represented by non protein origin compounds that contain nitrogen, such as ammoniac, nitrites and nitrates, urea or uric acid, among others.

According to the literature, NPN in tiger nuts is ~ 0.58% (Morell and Barber, 1983).

As observed in all tiger nuts, the predominant protein fraction (~ 82 - 91%) corresponded to the water soluble fraction (albumins + NPN), whereas the other solubilised fractions (globulins, prolamins and glutelins) were in the range of ~ 0.63 - 3.96% of the total. The residual nitrogen differed from ~ 4.7% in VTN to ~ 12% in B1TN.

Tiger nuts presented a content in albumins significantly different ($P < 0.05$) to each other (82.23 - 91.93% of the total isolated protein). The percentage shown in VTN was the highest (~ 91%), followed by Nigerian (~ 84.07%), and both Burkina tubers (~ 82.23 - 82.75%). These results were in accordance to those reported by Morell and Barber (1983) in tiger nuts from Spain, whose albumins content was ~ 81%. Globulins, prolamins and glutelins fractions followed the albumins in importance. Globulins content also varied between samples, which accounted for between 2.10 and 3.96% of the total. Prolamins achieved 0.91 - 1.61% of total protein in VTN, B1TN and B2TN. In contrast, NTN presented the highest percentage in this fraction (~ 3.45%). VTN showed the lowest percentage of glutelins (0.63% of total), which was significantly ($P \leq 0.05$) different to the others (whose content was ~ 2%). Morell and Barber (1983), in their studies with Spanish tiger nuts, reported percentages of ~ 4.8, 2.3 and 0.9% for globulins, glutelins and prolamins, respectively.

Albumins and globulins are abundant fractions in tubers. In potato, Gorinstein et al. (1988) described that albumins and globulins represent 67 and 23% of the total protein content, whereas in taro (*Colocasia esculenta* L. Schott), Monte-Neshich et al. (1995) reported that ~ 11% of total protein corresponded to albumins and ~ 80% were globulins. The amount of these proteins changes over time, due to these proteins may enable the plant to survive in periods of adverse conditions (Shewry, 2003). Technologically, in tiger nuts, the existence of this soluble-in-water main fraction represents a benefit for optimizing the protein extraction in tiger nut milk beverage production. Sánchez-Zapata et al. (2012) demonstrated that liquid co-products of tiger nut milk production can be used as a food ingredient by the food industry, as a substitute for water addition to foods. In line with this, some tubers protein isolates, or the water extracts of tubers, have already been described as an ideal ingredient in different food applications, due to the functional properties of their proteins (emulsifying, foaming, gelling, antioxidative activity, etc.) (Hou et al., 2001; Nagai and Nagashima, 2006; Scher et al., 2009; Waglay et al., 2014).

3.2.4. Electrophoretic patterns

Fig. 2 shows the protein composition of the Osborne fractions analyzed by SDS-PAGE. Electrophoretic patterns corresponding to the different tiger nuts were similar to each other, and they were characterized by spanning the whole range of MW. Polypeptides with the same MW were isolated in the different fractions, which evidenced the existence of proteins with the same MW and different solubility.

Similar electrophoretic patterns were obtained in all albumins fractions. These fractions presented the highest number of bands (~ 18 - 20), which were characterized by a wide range of MW polypeptides (15 - 200 kDa). In all albumin patterns, bands with 20, 25, 37, 55, 75 and ~ 106 kDa were highlighted for their intensity, while four fair bands at 33, 41, 64 and 96 kDa were also observed. Apart from these, other diffuse bands were also isolated in all tiger nuts in the range of 15 - 200 kDa. These results are similar to those reported by Gorinstein et al. (1988), who isolated more than 10 different bands in the albumin fraction of potato, of which MW at 20, 25 and 45 kDa were the most intense. On the contrary, globulin fractions presented a pattern with fewer bands than albumins, whose MW mainly coincided with those observed for the water-soluble fraction. In all tubers, bands at 18 - 19, 25 and 31 kDa were detected, although in VTN and B2TN these bands were more intense. In taro, Monte-Neshich et al. (1995) isolated three major globulins at 12.5, 22 and 24 kDa; these proteins were believed to represent storage proteins, because their diminution was observed during tuber germination. Adding to this, it has been demonstrated in sweet potato that sporamin storage globulin (whose MW is at ~ 22 - 31 kDa) have trypsin inhibitory activity (Maeshima et al., 1985; Yao et al., 2001; Yeh et al., 1997). In fact, the presence of this protease inhibitor in tiger nuts has been previously reported by other authors in Nigerian tiger nuts, which would suggest the relationship between some of the isolated globulins with this inhibitory role (Addy and Teshola, 1984). In Spanish tiger nut fraction, two pale bands at 41 - 55 kDa were also observed. In reference to this Park et al. (1983), Racusen and Foote (1980) and Shewry (2003) isolated a glycoprotein in potato whose MW was ~ 40 - 45 kDa, the activity of which might be related to the biological defence of the plant against pests (Galliard, 1971; Shewry, 2003). This protein has been also shown to possess antioxidant

activity and has excellent foaming and emulsifying abilities as well (Van Koningsveld et al., 2006, 2002; Waglay et al., 2014).

Prolamins were the protein fraction with the lowest number of bands. In all tubers, the most intense band was at 18 - 19 kDa, whereas two diffuse bands at 25 and 31 kDa were also isolated. B2TN also presented an intense band at 20 kDa and a pale band at 50 kDa. In glutelin fractions, isolated bands were more intense than in prolamins. In VTN, NTN and B1TN, intense bands at 18 - 19, 20 and 25 kDa were observed, while in B2TN the same bands were isolated but slightly diminished. In VTN and NTN, three pale bands at 37, 50 and 78 kDa were also isolated, although in the range of 20 - 31 and 31 - 37 other diffuse bands were also appreciated. In potato, the major prolamin and glutelin bands were described at 12 and 25 KDa and at 12, 25 and 150 kDa, respectively (Goristein et al., 1988).

4. Conclusions

Results in this study showed that physico-chemical characteristics and proximate composition of tiger nut batches in the current market are quite variable and mainly dependable on their geographical origin. On the contrary, protein characteristics were more similar between tubers. In this respect, tiger nuts should be selected accordingly for derived products intended for manufacture by industry, in order to optimize the production processing and the physicochemical characteristics of tiger nut food products.

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Fig. 1. Comparative appearance of tiger nuts from Valencia - VTN (A), Niger - NTN (B) and Burkina Faso - B1TN (C) and B2TN (D). Note the appearance differences between tiger nuts from different origin.



660 **Fig. 2:** SDS-PAGE electrophoregrams of isolated fractions. Albumins (A), Globulins
661 (B), Prolamins (C) and Glutelins (D). VTN: Valencia tiger nuts; NTN: Niger tiger nuts;
662 B1TN and B2TN: Burkina Tiger nuts. MW: Molecular weight marker, corresponding to
663 15, 20, 25, 37, 50, 75, 100, 150 and 250 kDa.

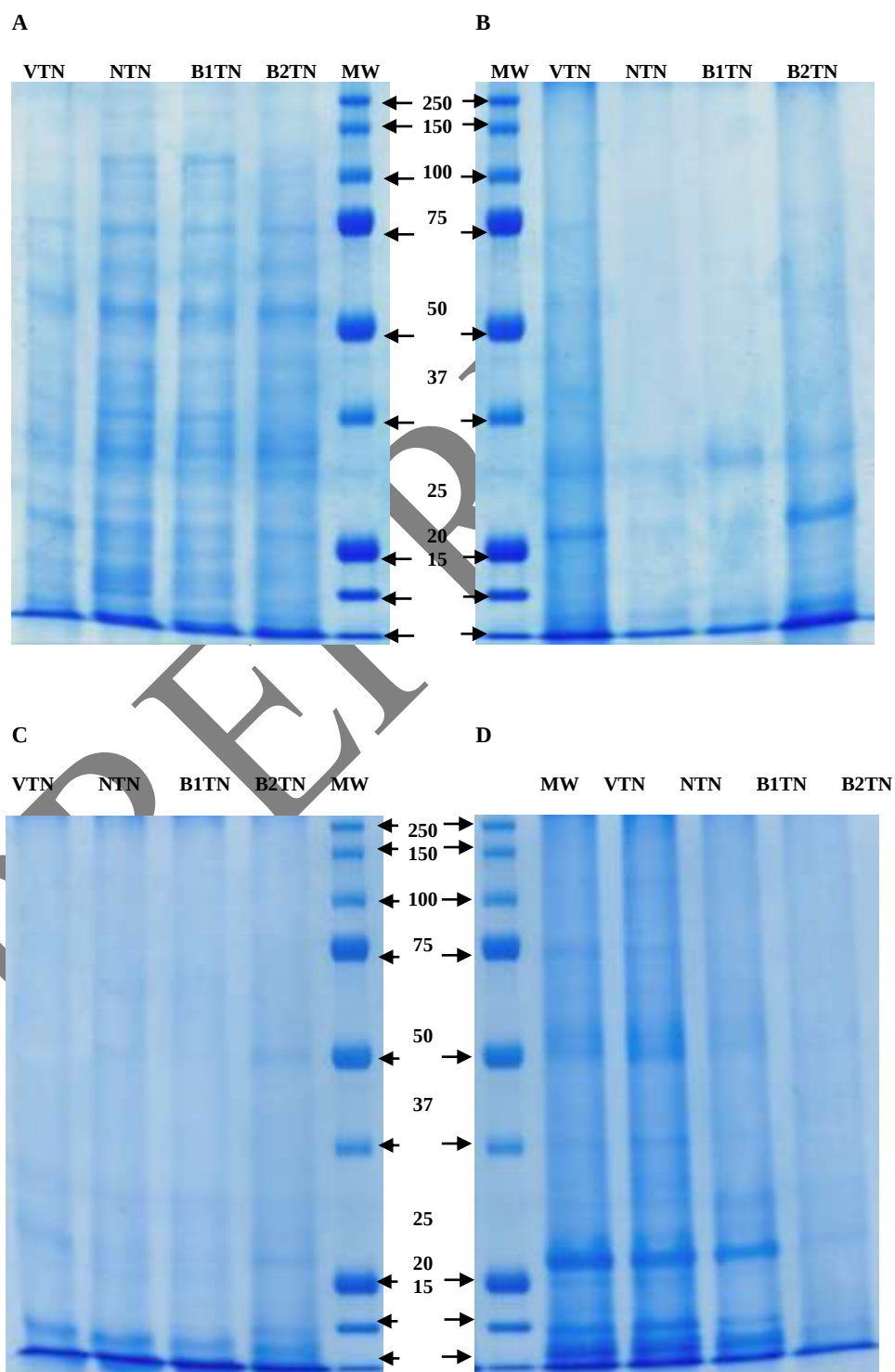


Table 1. Tiger nut shape and % of tiger nuts of every shape per carrier bag.

Type ¹	Shape ²	Length ³ (mm)	Thickness ³ (mm)	% per carrier bag
VTN	Elongated	14.37 ^a ± 1.59	8.21 ^a ± 1.95	25.67 ^y ± 2.08
	Oval	11.58 ^a ± 1.18	8.41 ^a ± 1.77	27.00 ^y ± 1.01
	Round	10.00 ^a ± 1.28	9.05 ^a ± 1.49	51.33 ^x ± 2.21
B1TN	Elongated	9.66 ^c ± 0.12	5.77 ^b ± 0.62	27.00 ^y ± 1.00
	Oval	9.29 ^b ± 0.88	6.49 ^c ± 0.65	22.01 ^y ± 2.00
	Round	7.62 ^c ± 0.11	7.48 ^c ± 0.88	49.79 ^x ± 2.16
B2TN	Elongated	12.03 ^b ± 0.94	7.61 ^a ± 1.22	13.33 ^y ± 1.53
	Oval	8.92 ^b ± 0.76	7.66 ^b ± 1.01	16.33 ^y ± 0.58
	Round	8.91 ^b ± 0.72	8.06 ^b ± 0.89	70.67 ^x ± 1.53
NTN	Elongated	9.05 ^c ± 0.11	5.70 ^b ± 0.65	28.00 ^y ± 2.02
	Oval	11.19 ^a ± 0.10	6.45 ^c ± 0.79	23.44 ^z ± 1.53
	Round	7.10 ^d ± 0.15	7.88 ^b ± 0.11	49.33 ^x ± 0.57

¹ VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

² According to the relationship proposed by Morell and Barber (1983).

³ Means ± standard deviations.

^{a-d} For every shape, values in the same column followed by different superscript differed significantly ($P < 0.05$).

^{x-z} For every type of tiger nut, values in the same column followed by different superscript differed significantly ($P < 0.05$).

Table 2. Weight of analyzed tiger nuts.

Shape	Weight (g) ^{1,2}			
	VTN	B1TN	B2TN	NTN
Mix ³	0.40 ^a ± 0.16	0.27 ^{bc} ± 0.07	0.25 ^c ± 0.02	0.30 ^b ± 0.74
Elongated	0.51 ^{ax} ± 0.17	0.29 ^{bx} ± 0.07	0.27 ^{bx} ± 0.09	0.30 ^{bx} ± 0.08
Oval	0.39 ^{ay} ± 0.15	0.25 ^{by} ± 0.05	0.26 ^{bx} ± 0.06	0.24 ^{by} ± 0.09
Round	0.36 ^{ay} ± 0.15	0.25 ^{cy} ± 0.07	0.23 ^{cx} ± 0.07	0.30 ^{bx} ± 0.06

¹ Means ± standard deviations.

² VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

³ Mix of tiger nuts of different shape.

^{a-c} Values in the same row with different superscripts differed significantly ($P < 0.05$).

^{x-y} For every type of tiger nut (VTN, B1TN, B2TN and NTN), values in the same column followed by different superscript differed significantly ($P < 0.05$).

Table 3. Skin color parameters of tiger nuts.

Type ¹	Color parameter ²			ΔE^*
	L*	a*	b*	
VTN	37.58 ^b ± 0.79	9.80 ^c ± 0.48	10.03 ^c ± 1.35	-
B1TN	43.31 ^a ± 0.61	11.33 ^a ± 0.08	22.38 ^a ± 0.46	6.23 ^a ± 0.75
B2TN	44.18 ^a ± 0.79	10.75 ^b ± 0.13	21.18 ^a ± 0.30	7.02 ^a ± 0.79
NTN	37.81 ^b ± 0.22	10.37 ^{bc} ± 0.27	17.15 ^b ± 0.29	2.00 ^b ± 0.25

¹VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

² Means ± standard deviations.

^{a-d} Values in the same column followed by different superscript differed significantly ($P < 0.05$).

Table 4. Proximate composition (as % dry matter) of tiger nuts.

Major nutrients (%)	Tiger nuts ^{1,2}			
	VTN	B1TN	B2TN	NTN
Moisture	8.66 ^a ± 0.04	7.75 ^b ± 0.27	6.38 ^c ± 0.45	7.45 ^b ± 0.31
Fat	35.21 ^a ± 3.07	25.77 ^c ± 0.77	25.35 ^c ± 1.85	28.19 ^b ± 0.37
Protein	8.45 ^a ± 0.20	7.32 ^b ± 0.20	5.62 ^c ± 0.09	3.28 ^d ± 0.10
NFM	45.05 ^c ± 3.13	55.94 ^b ± 0.91	58.66 ^a ± 1.82	58.19 ^a ± 0.76
Fiber	9.31 ^a ± 0.09	9.07 ^{ab} ± 0.21	8.42 ^c ± 0.15	8.75 ^{bc} ± 0.59
Ash	1.97 ^a ± 0.01	1.90 ^a ± 0.04	1.95 ^a ± 0.16	1.60 ^b ± 0.02

¹ Means ± standard deviations.

² VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

^{a-c} Values in the same row with different superscripts differed significantly ($P < 0.05$).

Table 5. Tannin and Phytate contents (as dry matter) of tiger nuts.

Antinutritional Components	Type ^{1,2}			
	VTN	B1TN	B2TN	NTN
Tannins (g / 100 g)	0.08 ^c ± 0.00	0.12 ^b ± 0.03	0.18 ^a ± 0.01	0.11 ^{bc} ± 0.02
Phytates (mg / 100 g)	16.27 ^b ± 0.88	17.11 ^b ± 1.06	9.31 ^c ± 1.44	22.55 ^a ± 1.23

¹ Means ± standard deviations.

² VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

^{a-d} Values in the same row with different superscripts differed significantly ($P < 0.05$).

Table 6. Osborne protein fractions of tiger nuts.

Protein fractions	Tiger nuts (%) ^{1,2}			
	VTN	B1TN	B2TN	NTN
Albumins (+ NPN)	91.93 ^{aw} ± 0.39	82.23 ^{ay} ± 0.50	82.75 ^{ay} ± 0.19	84.07 ^{ax} ± 0.65
Globulins	1.11 ^{cdz} ± 0.04	2.76 ^{cx} ± 0.10	3.96 ^{cw} ± 0.03	2.10 ^{dy} ± 0.12
Prolamins	1.61 ^{cx} ± 0.36	1.13 ^{dx} ± 0.20	0.91 ^{ex} ± 0.17	3.45 ^{cw} ± 0.41
Glutelins	0.63 ^{dx} ± 0.34	1.96 ^{cw} ± 0.04	1.92 ^{dw} ± 0.13	1.98 ^{dw} ± 0.01
Insoluble residue	4.72 ^{bz} ± 0.33	11.93 ^{bw} ± 0.44	10.42 ^{bx} ± 0.52	7.90 ^{by} ± 0.62

¹ Means ± standard deviations.

² VTN: Valencia tiger nuts; B1TN and B2TN: Burkina Faso tiger nuts; NTN: Niger tiger nuts.

^{a-e} Values in the same column followed by different superscript differed significantly ($P < 0.05$).

^{w-z} Values in the same row with different superscripts differed significantly ($P < 0.05$).

Highlights

1. Physico-chemical characteristics of tiger nuts are mainly dependable on their geographical origin.
2. Low percentages of antinutritional components were detected in all tubers.
3. The albumins were the main protein fraction.
4. Isolated polypeptides were characterized by a wide range of molecular weights (15-200 KDa).

