ALGERIAN PEARL MILLET *Pennisetum glaucum* (L.) R. Br: A NEW POTENTIAL SOURCE OF AMINO ACIDS AND NON GLUTEN PROTEINS

MOKRANE Hind[1 *], BIBI Rafika[1], AMOURA Houria[1], BRIJS Kristof[2], BELHANECHE-BENSEMRA Naima[3] and NADJEMI Boubekeur[1,4]
[(1)]*Laboratoire des produits bioactifs et de la valorisation de la biomasse Ecole Normale Supérieure, Kouba, Algiers, Algeria*
[(2)]*Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), K.U. Leuven, Kasteelpark Arenberg 20, Leuven, Belgium*
[(3)]*Département Génie de l'environnement, Ecole Nationale Polytechnique, El Harrach, Algiers, Algeria*
[(4)]*Laboratoire d'études et de développement des techniques d'épuration, et de traitement des eaux et gestion environnementale, Ecole Normale Supérieure, Kouba, Algiers, Algeria*
E-mail: mokranehind@yahoo.fr

(Received December 2018 - Accepted 17 June 2019)

Abstract.- *In South Algeria, pearl millet is cultivated for feed and food. This cereal is well adapted to arid environment. The aim of this study was to identify the suitable cultivars to be used as gluten free source of protein and amino acids. For this purpose, eleven pearl millet cultivars of various colour and shape were sampled from the Algerian Sahara. Wide variability in protein contents (11.2 to 18.2 %.) was found in the studied cultivars. With the exception of one cultivar grown in Tamanrasset, all the local pearl millet samples were higher in protein content than the imported ones. Pearl millet samples were rich in essential amino acids (40% in average). The levels of lysine, threonine, phenylalanine, isoleucine and valine contents in all the sampled cultivars were higher than wheat, maize and sorghum. The extractability of pearl millet protein fractions (albumins, globulins, non reduced prolamins, reduced prolamins and glutelins) was investigated by a combination of size exclusion high performance liquid chromatography (SE-HPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The storage proteins (prolamins and glutelins) varied widely within cultivars and constituted the highest portion of the total extractable proteins in all pearl millet samples, the share of which ranged from 57% to 80%. Prolamins were the major protein fraction in the eleven cultivars (38 to 57% of all extracted proteins). The three pennisetin monomers with molecular masses (MM) of 27, 22 and 12 kDa were separated. This study shows that the sampled pearl millet cultivars from south Algeria, could constitute an alternative and good source of gluten free proteins and essential amino acids.*

Key words: Pennisetum glaucum, *non gluten protein, amino acid, protein extractability, SE-HPLC, SDS-PAGE.*

LE MIL PERLE ALGERIEN *Pennisetum glaucum* (L.) R. Br: UNE NOUVELLE SOURCE POTENTIELLE D'ACIDES AMINEES ET DE PROTEINES SANS GLUTEN

Résumé.- *Dans le sud Algérien, le mil perlé est cultivé pour l'alimentation animale et humaine. Cette céréale est très bien adaptée au climat aride. Le but de cette étude est d'identifier les cultivars les mieux appropriés à être utilisés comme sources de protéines sans gluten et d'acides aminés. Pour cela, onze cultivars de mil perlé de couleurs et de formes différentes ont été échantillonnées du Sahara Algérien. Une large variabilité dans la teneur en protéines (11,2 à 18,2 %) a été détectée dans les échantillons étudiés. A l'exception d'un échantillon de mil perlé cultivé à Tamanrasset, tous les cultivars locaux de mil perlé ont présenté de hauts pourcentages de protéines par rapport à ceux introduits. Les échantillons de mil perlé sont très riches en acides aminés essentiels (40% en moyenne). Les pourcentages de lysine, thréonine, phénylalanine, isoleucine et valine obtenus sont plus élevés que dans le blé, le mais et le sorgho. L'extractabilité des fractions protéiques*

(albumines, globulines, prolamines non réduites, prolamines réduites and glutélines) a été étudié par une combinaison de la chromatographie liquide à haute performance d'exclusion stérique (SE-HPLC) et de l'électrophorèse (SDS-PAGE). Les protéines de stockage (prolamines et glutélines) varient largement entre les cultivars (57 à 80%). Les prolamines appelées pennisetines sont la fraction protéique majoritaire dans les onze échantillons (38 à 57% des protéines extraites). Les trois formes monomériques de pennisetines de masses moléculaires (MM) 27, 22 and 12 kDa ont été séparées et clairement distinguées. Cette étude montre que les cultivars de mil perlé échantillonnés du sud de l'Algérie peuvent constituer une céréale alternative au blé et une bonne source de protéines sans gluten et d'acides aminés essentiels.

Mots-clés : *Pennisetum glaucum, protéines sans gluten, acides aminés, extractabilité des fractions protéiques, SE-HPLC, SDS-PAGE*

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is the sixth most important cereal in the word after wheat, rice, barley, maize and sorghum. Pearl millet grains could be produced in short growing season and under harsh conditions such scanty rain fall, infertile soil and intense heat [1-3]. Pearl millet is a gluten free cereal suitable for population suffering from celiac disease [4,5]. Carbohydrates usually make up about 70% of the dry grain, and consist almost exclusively of starch. The proteins constitute the second most abundant fraction, with a good balance of amino acids [2-4, 6].

In the past decade, several studies have aimed to improve cereal protein characterization using analytical methods such as SE-HPLC and SDS-PAGE. Thus, SCHALK *et al.* (2017) [7] combined these technics to extract and fully characterize gluten proteins from wheat, rye, barley and oats. The same analytical methods have been used to deeply characterize seed proteins of wheat [8], teff [9], cotton [10], rice [11], rye [12], oat [13], sorghum [14] and corn [15].

In pearl millet, as in sorghum, maize, and coix, the major protein fraction consists of prolamins [16,17]. The pearl millet prolamins called pennisetins are extractable in alcoholic media. They on average make up approximately 50% of the total protein fraction [18]. Only few studies reported on the pearl millet protein composition and characterization, particularly its major fraction called pennisetin [19-22]. Pennisetin has been detected in three forms with respectively molecular masses of 27, 22 and 12 kDa. Only, the 22 and 27 kD-pennisetins were slightly similar in composition and sequence to α-prolamins from maize, sorghum and coix [20, 23-25].

In previous work, the protein composition of ten pearl millet genotypes cultivated in Sudan was investigated [26]. Later, MALIK *et al.* (2002) [27] studied the effect of cooking method on the protein and iron contents in pearl millet hybrids and varieties cultivated in India. A comparative study was carried out on nutritional and technological qualities of fourteen pearl millet cultivars sampled from Burkina Faso [28]. BAGDI *et al.* (2011) [29] investigated the nutritional potential of six Hungarian varieties of proso millet and two commercially available. To the best of our knowledge no similar study was performed on Algerian pearl millet cultivars.

The aim of this work was to gain insight in the amino acid composition, protein fractionation and characterization of Algerian pearl millet cultivars with an emphasis on

their prolamins. SE-HPLC and SDS-PAGE methods, originally developed for separating wheat proteins, were applied here with some minor modifications to pearl millet proteins.

1.- Material and methods

1.1.- Material

Eleven pearl millet cultivars varying in colour and shape were sampled in the arid Algerian Sahara areas (In Salah, Béchar, Timimoun, Aoulef and Tamanrasset). Table I shows the characteristics of the sampled pearl millet cultivars. The pearl millet samples were labelled PM1 to PM11. PM1, PM2, PM3 and PM6 were local cultivars from In Salah. PM4 was from Aoulef, PM5 from Timimoun, PM7 from Béchar, PM8 from Tamanrasset and PM9, PM10 and PM11 were introduced samples from Niger and Mali found in the local commerce.

The pearl millet grains were ground to flour in a Cyclotec 1093 sample mill (Tecator, Hogänäs, Sweden). The obtained flours were manually sieved over a 400µm sieve. All chemicals and reagents were from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade.

Table I.- Characteristics of the sampled pearl millet cultivars

Pearl millet cultivars	Origin	Cultivars	Color	Protein (%) ***
PM1	Ain Salah	El beldia etwilla	Yellow green	11.76 c
PM2	Ain Salah	El beldia elksira	Yellow	12.26 c
PM3	Ain Salah	El beldia elksira	Yellow green	18.20a
PM4	Aoulef	Elbechna lemekhelta	Yellow green	13.30 b,c
PM5	Timimoun	Bechna	Yellow	17.36 a
PM6	Ain Salah	Bechnet essoudan	Yellow green	14.48 b
PM7	Béchar	Bechnat ettouat	Yellow orange	17.38 a
PM8	Tamanrasset	Bechna	Grey	11.62 c
PM9	Commercial	Bechna	Yellow	11.19 c
PM10	Commercial	Bechna	Yellow	12.37 b,c
PM11	Commercial	Bechna	Yellow	12.96 c

1.2.- Protein and moisture analysis

Protein contents were determined using the Dumas combustion method, an adaptation of the AOAC Official Method [30] to an automated Dumas protein analysis system (EAS, VarioMax N/CN, Elt, Gouda, The Netherlands), using 6.25 as conversion factor [31]. Moisture analysis was carried out according to AACC method 44-15A [32].

1.3.- Amino acid composition

Amino acid composition was determined after acid hydrolysis of the pearl millet samples (containing 4.25 mg protein) in 1.0 ml of 6.0 M HCl during 24h at 110°C. The pearl millet cultivars samples (25 µl) was analysed on an analytical AminoPac PA 10 analytical column (2×250 mm) preceeded by an AminoPac PA 10 guard column (2×50 mm) (AAA-Diect Amino Acid Analyzer, Dionex Corporation, Sunnyvale, CA, USA) as previously described by LAMBERTS *et al.* (2008) [33]. The amino acid standard consists of 17 amino acids obtained from Sigma-Aldrich (Steinheim, Germany).

1.4.- Sequential extraction methods

Pearl millet flour was first defatted with n-hexane (1:10, w/v) overnight at room temperature with continuous shaking. Proteins were sequentially extracted by shaking defatted flour (500 mg) in 10.0 ml of different solvents, followed by centrifugation as described by MOKRANE *et al*. (2009) [14] for sorghum flour. All experiments were conducted three times. Five fractions were obtained. The albumins (A) were extracted with deionised water at room temperature. The globulins (G) were extracted from the residue with 5.0% (w/v) aqueous sodium chloride at room temperature. The non-reduced prolamins (PNR) were extracted under non-reducing conditions from the second residue with 70% (v/v) aqueous ethanol at 60°C. The residue after centrifugation was then further extracted with reducing agents, to render more cross-linked prolamins extractable. A first reduced prolamin fraction (PR1) was extracted with 1.0% (v/v) β-mercaptoethanol in 70% ethanol at 60°C. A second highly cross-linked protein fraction (PR2) was reduced and extracted at room temperature with 6.0 M urea containing 2.0% (w/v) SDS, and 1.0% (w/v) dithiothreitol (DTT). All extraction steps were repeated twice and were conducted during 30 min. Protein fractions were freshly analyzed to avoid protein aggregation during freezing and defreezing process.

1.5.- SDS-PAGE

All extracted fractions (A, G, PNR, PR1 and PR2) of the pearl millet cultivars were dispersed in a Tris(hydroxymethyl)aminomethane-hydrochloric Tris-HCl sample buffer at pH 6.8 containing 125 mM Tris, 30% (w/v) glycerol, 4.0% (w/v) SDS, and 0.002% (w/v) bromophenol blue. The samples were boiled for 5 min, centrifuged at 11,000 g for 3 min and loaded. Electrophoresis was carried out on 20% (w/v) polyacrylamide gels in a PhastSystem unit (GE Healthcare, Uppsala, Sweden). All gels were silver stained (Development Technique file no. 210; GE Healthcare). The low MM markers (GE Healthcare) for gel electrophoresis were α-lactalbumin (14.4 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), ovalbumin (43.0 kDa), bovine serum albumin (67.0 kDa) and phosphorylase (94.0 kDa).

1.6.- Size Exclusion High Performance Liquid Chromatography

SE-HPLC of the protein fractions was conducted using an LC-2010 system (Shimadzu, Kyoto, Japan) equipped with automatic injection. The samples (30 µl), containing 0.1% (w/v) protein dissolved in the mobile phase, were loaded on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA, USA). The mobile phase was acetonitrile (ACN): deionized water (1:1, v/v) containing 0.05% (v/v) trifluoroacetic acid (TFA) [34]. Separation was at a flow rate of 1.0 ml/min and a temperature of 30°C. Protein elution was monitored by measuring UV absorption at 214 nm, and peak areas were determined by integration using the LC-2010 system software [14]. The relative level of any given extractable protein fraction was calculated as the ratio of the area of its chromatogram peak to the sum of the areas of the peaks of all extracted protein fractions. The column was calibrated with four MM markers, *i.e.* catalase (232 kDa), aldolase (158 kDa), ovalbumin (43 kDa) and ribonuclease (13.7 kDa) (Sigma-Aldrich).

1.8.- Data treatment

All statistical analyses were performed using the Statistical Analysis System software 8.1 (SAS Institute, Cary, NC, USA). The extraction procedure was carried out at least three times and the single extracts produced were analyzed in triplicate as well. The

analysis of variance of the protein contents and the amino acid composition was with a Tukey multiple comparison procedure on a 5% significance level [35].

2.- Results and discussion

2.1.- Protein contents

Measurements made on several hundred of pearl millet cultivars grown in different countries, have shown that the protein level ranged from 6 to 21% on dry matter basis (d.m.b) [36]. Among millets, pearl millet was the most widely used for human nutrition [1]. The variety, soil type, agricultural input and weather conditions during grain formation could influence the protein content of pearl millet [26, 28]. Table 1 shows the protein content of the sampled Algerian pearl millet cultivars. A wide range of protein contents were obtained, with high values ranging from 11.2% (PM9) to 18.2 % (PM3) on d.m.b. PM3 from Ain Salah, PM5 from Timimoun and PM7 from Béchar exhibited the highest protein content. Highly significant difference ($p \leq 0.001$) was observed in the total protein content (tab. 1)

In previous works, the pearl millet protein content was investigated in cultivars growing in different countries such as Sudan [26], India [37] and Burkina Faso [28]. ABDALLA *et al.* (1998) [26] investigated the protein content in ten pearl millet genotypes cultivated in Sudan, a wide variability was found with a range of (8.5-15.1 %). A comparative study was carried out on nutritional and technological qualities of fourteen pearl millet cultivars sampled from Burkina Faso, the proteins ranged from 8.7 to 17.1 % [28]. Similar study was performed on two pearl millet varieties cultivated in India their protein content were 9.3 and 11.6 % [37]. In the Algerian pearl millet cultivars analyzed in this study, the protein contents of three local cultivars were higher than 17 % (PM3, PM5 and PM7) and five exceeded 12 % (PM2, PM4, PM6, PM10 and PM11) with an average of 13.9 % (Table 1). A highly significant difference ($p \leq 0.001$) was observed. Thus, among all the sampled cultivars, PM3, PM5 and PM7 could be deeply used as rich and good protein source.

2.2.- Amino acid composition

Table II shows a comparison of the average amino acid compositions of the pearl millet samples with those of sorghum, wheat, maize, quinoa and a Food and Agriculture Organization/World Health Organization (FAO/WHO) reference protein with an amino acid composition that is optimally balanced for children of preschool age [38-41].

Based on the amino acid composition of the FAO/WHO reference protein, lysine and methionine were the most limiting essential amino acids in the analyzed pearl millet samples (tab. 2). In addition, the proportion of phenylalanine in pearl millet protein was lower than nutritionally required. The proportions of the remaining essential amino acids in pearl millet protein were all above the nutritional requirements.

As compared to sorghum [41], wheat [38] and maize [39] the analyzed pearl millet samples were on average richer in lysine, threonine, isoleucine, valine, serine and asparagine/aspartic acid, and poorer in proline (tab. II). In comparison to quinoa a pseudo cereal with a balanced amino acid composition, largely consumed in South American countries, pearl millet samples were richer in threonine, phenylalanine, leucine, alanine,

proline, serine, asparagine/aspartic acid and histidine [43].

Table II.- Comparison of the amino acid compositions (% of crude protein) in Algerian pearl millet to those in sorghum, wheat, maize and a FAO/WHO reference protein with an amino acid composition that is optimally balanced for children of preschool age.

Cereal	**Pearl millet**		**Sorghum** [a]		**Wheat** [b]		**Maize** [c]		**Quinoa**[d]	**FAO /WHO**[e]
Essentials aminoacids (%)	**Average**	**Range**	**Average**	**Range**	**Average**	**Range**	**Average**	**Range**	**Average**	
Lysine	**2.8**	2-3.3	**1.9**	1.6-2.2	**2.3**	1.9-2.4	**2.7**	2.1-3.3	**4.6**	**5.8**
Threonine	**6.4**	6.0-6.6	**4.9**	4.7-5.1	**2.9**	2.6-3.0	**3.4**	2.5-4.2	**3.5**	**3.4**
Phenylalanine	**4.5**	4.1-5.0	**5.8**	4.3-6.2	**4.8**	4.4-5.1	**3.9**	2.5-5.3	**4.3**	**6.3**
Isoleucine	**4.1**	3.9-4.3	**4**	3.8-4.3	**2.6**	2.5-2.9	**3.4**	2.1-4.6	**7.4**	**2.8**
Leucine	**12.8**	12.2-13.7	**16.4**	15.8-17.3	**6.9**	6.6-7.1	**11.7**	7.1-16.2	**7.5**	**6.6**
Valine	**5.3**	5.1-5.5	**4.9**	4.6-5.1	**4.0**	3.7-4.4	**4.8**	3.0-6.5	**6.0**	**3.5**
Methionine	**1.4**	1.1-1.7	**1.3**	1.1-1.6	**1.6**	1.4-1.8	**1.9**	1.2-2.5	**2.3**	**2.5**
Tyrosine	**2.1**	1.9-2.2	**2.9**	2.7-3.2	**2.7**	2.5-2.9	**3.9**	2.6-5.2	**3.1**	
Non essentials										
Alanine	**7.3**	6.9-7.6	**8.5**	8.0-9.4	**3.1**	2.8-3.2	**7.0**	4.6-9.3	**5.7**	
Glycine	**3.2**	2.5-3.6	**2.5**	1.9-2.9	**3.6**	3.1-3.9	**3.7**	2.8-4.6	**6.1**	
Cystine	**0.3**	0.2-0.4	**0.4**	0.2-0.5	**2.2**	1.9-2.3		-	**2.2**	
Proline	**6.0**	5.7-6.4	**7.9**	7.6-8.3	**8.8**	8.6-8.9	**9.0**	6.2-11.7	**2.3**	
Serine	**9.4**	6.8-12.8	**3.6**	3.1-4.0	**4.4**	4.2-4.5	**4.7**	3.3-6.0	**3.8**	
Glutamic acid Glutamine	**16.4**	15.8-17.2	**16.7**	16.2-17.2	**35.1**	33.7-37.0	**17.8**	11.8-3.7	**16.7**	
Aspartic acid Asparagine	**7.2**	6.7-7.6	**5.9**	5.7-6.1	**4.5**	4.3-4.7	**6.3**	4.3-8.2	**6.7**	
Histidine	**2.2**	1.9-2.5	**1.9**	1.6-2.2	**2.3**	2.1-2.4	**2.7**	1.9-3.4	**1.9**	**1.9**

[a]Mokrane et al., [41], [b] Abdel-Aal and Hucl, [38]; [c] Harrigan et al., [39]; [d]Villa et al., [43], [e]FAO/WHO, [40].

Pearl millet samples were also richer in leucine than wheat, maize and quinoa proteins. According to DOI *et al.* (2005) [42], leucine is deeply involved in blood sugar regulation and muscle tissue growth and repair. In trauma or severe stress, leucine can assist to prevent the breakdown of muscle proteins. Hence, pearl millet could constitute a good source of leucine. Moreover, according to traditional use of pearl millet grain in south Algeria, large use in bone and trauma repair has been reported in traditional medicine. The phenylalanine levels in pearl millet protein were on average lower than in sorghum and similar to that in wheat [38, 41]. Although, pearl millet proteins were rich in essential amino acids, due to the lack of some specific essential amino acids, it should be

supplemented with other protein sources to make it nutritionally more suitable. Pearl millet could be a good alternative protein source in arid areas as quinoa and cañihua are in South American areas [43].

The above results could be a starting point for plant breeders to select the best pearl millet cultivars for nutrition. However, a genotype × environment interaction study is needed to assess the stability of high essential amino acid levels as a quality trait.

2.3.- SDS-PAGE

To further identify the pearl millet proteins, the SDS-PAGE profiles of all the extracted protein fractions, *i.e.* A, G, PNR, PR1 and PR2, were determined for pearl millet cultivar PM3 as example (Figure 1).

The A fraction in pearl millet (Figure 1, lane A) showed a wide range of protein bands with different MMs. However, in the G fraction (Figure 1, lane G) lower protein bands were observed.

Few studies have characterized the whole pearl millet protein fractions, most studies have aimed the separation by SDS-PAGE of only the most abundant fraction "the prolamins" [20,23,44]. The Algerian pearl millet prolamins showed SDS-PAGE profile comparable to those reported in previous works [18,20,45]. The silver-stained SDS-PAGE profiles of PNR and PR1 (Figure 1, lanes PNR and PR1) exhibited three major bands at 27, 22 and 12 kDa. In the PR1 fraction, the use of β-mercaptoethanol in the extraction process reduced the level of pennisetin dimers, trimers and oligomers (fig. 1, lane PR1). In the meantime, the protein bands appearing at 27 and 12 kDa become more intense in the PR1 fraction, which may confirm the decrease of crosslinking leading to the liberation of the 27 and 12 kDa pennisetin monomers. In the PR2 fraction, in addition to the remaining prolamins, a wide range of proteins could be detected probably corresponding to the highly cross linked glutelins witch need the use of urea and SDS to be extracted (fig. 1, lane PR2).

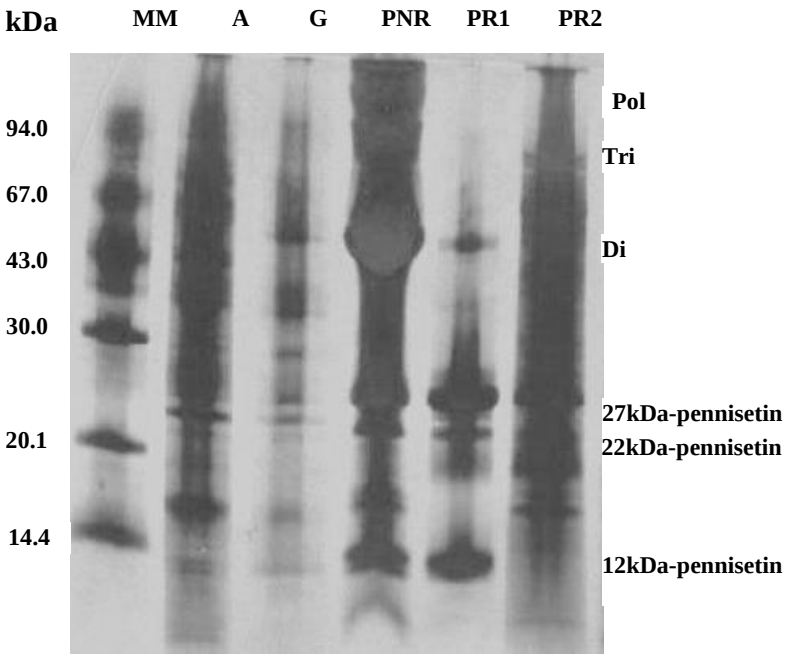

Figure 1.- SDS-PAGE pearl millet (PM3) protein fractions: lane A: Albumins; lanes G:

Globulins; lane PNR: non-reduced prolamin; lane PR1: first reduced prolamin; lane PR2: highly cross-linked prolamin and lane MM: Molecular masses markers. The sizes of the MM markers are indicated on the left side. The 27-, 22- and 12kDa-pennisetin monomers for pearl millet as well as the corresponding polymers (Pol), trimers (Tri) and dimers (Di) are indicated

2.4.- Size exclusion high performance liquid chromatography and yields of extracted protein fractions

Figure 2 shows the protein distribution over the extracted fractions for the eleven pearl millet samples, as calculated from their relative areas in SE-HPLC. The shares of A and G in total extractable protein ranged from 20% (PM7) to 43% (PM1) and were lower than that of the storage proteins (PNR+PR1+PR2) for all pearl millet samples.

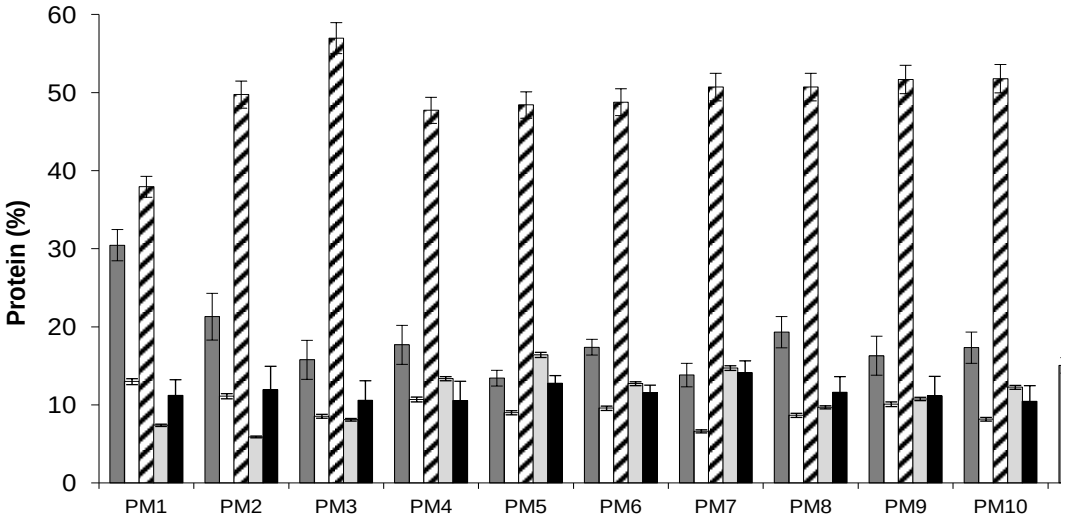

Figure 2.- Distribution of extractable protein in eleven pearl millet samples over albumin (A) ■, globulin (G) □, non-reduced prolamin (PNR) ▨, first reduced prolamin (PR1) ■ and highly cross-linked prolamin (PR2) ■ as calculated from their relative areas in SE-HPLC.

As shown in figure 2, the prolamin fraction (PNR+PR1) made up the larger part of the total extractable protein in all pearl millet samples, the share of which ranged from 45% (PM1) to 65% (PM7). The PNR fraction represented the most abundant prolamin fraction with 38 to 57% of all extracted proteins. The proportion of cross-linked prolamins, represented by the PR1 fraction, was very low in the pearl millet samples, *i.e.* ranging from 6% (PM2) to 16% (PM5). The PR2 levels were in the range of the PR1 levels or slightly higher. The pearl millet prolamin fraction was less cross-linked and more easily extractable than that of sorghum [14]. As shown in Figure 2, the share of the PNR fraction was the highest in all the pearl millet samples.

The extraction efficiency was evaluated by protein analysis using Dumas method. The assembled pearl millet fractions (A + G + PNR + PR1), ranged from 62% (PM4) to 77% (PM11) of total protein. The most efficient extraction was obtained for the commercial cultivars and those originating from Timimoun (PM5) and Tamanrasset (PM8). The extraction efficiencies were comparable to those obtained for sorghum [14].
MARCELLINO *et al.* (2002) [20] reported that, as in almost all the major cereals, the most

abundant seed storage protein fraction is composed of prolamin. In this study, the prolamin fraction called pennisetin accounted from 45 to 65% of the seed storage proteins.

The SE-HPLC profiles of all the sampled pearl millet cultivars were comparable, slight differences were observed in the level of each fraction (A, G, PNR, PR1 and PR2) between cultivars as shown in figure 2.

Figure 3 shows the SE-HPLC profile of the storage proteins fraction (PNR, PR1, PR2 and the combined PNR+PR1 of pearl millet cultivar PM3 as example. The PNR prolamin fraction was the most abundant one and appeared in all pearl millet samples as three peaks, corresponding to 40, 27 and 12 kDa. These peaks probably represent trimers, dimers or monomers of the three pennisetin forms the 27kDa-, 22kDa- and 12kDa-pennisetin.

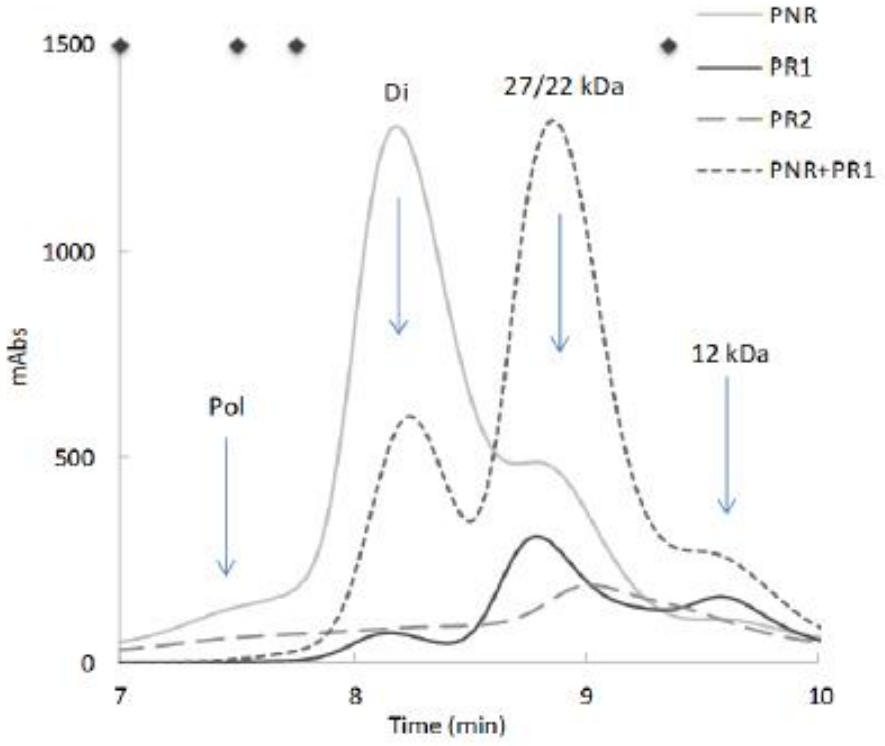

Figure 3.- SE-HPLC profiles of pearl millet proteins extracted from pearl millet cultivar PA1 with 70% ethanol at 60°C (PNR), with 70% ethanol in the presence of 1.0% β-mercaptoethanol at 60°C (PR1) and with 6.0M urea in the presence of 2.0% SDS and 1.0% DTT (PR2), with 70% ethanol in the presence of 1.0% β-mercaptoethanol at 60°C in one time (PNR+PR1 - - -). Elution times of markers with MM of 232 kDa, 158 kDa, 43 kDa and 13.7 kDa (from left to right) are indicated on top of the chromatogram. The mobile phase was (1:1, v/v) acetonitrile (ACN): deionized water containing 0.05% (v/v) TFA. The flow rate was 1.0 mL/min at a temperature of 30°C. Protein elution was monitored at 214 nm.

The SE-HPLC profile of the PR1 fraction (fig. 3) shows the presence of the most abundant monomer forms of 27 kDa- and 12 kDa-pennisetin. However, as shown in SDS-Page (fig. 1), the 22 kDa-pennisetin was less abundant than the 27 kDa- and 12 kDa-pennisetin monomers, which may explain that this form was not clearly distinguished in SE-HPLC.

The small peak at 8.1 min probably may correspond to remaining dimer forms of approx. 40 kDa; the latter is probably formed by the association of the two most abundant pennisetins 27kDa- and 12kDa-pennisetin. The PR2 fraction appeared as one peak which presumably represented highly cross-linked proteins extractable with SDS, urea and the reducing agent DTT.

Adding reducing agent to the PNR fraction allowed the complete disappearance of the polymer (Pol) form and the diminution of the dimer form (Di) and in the meantime the increase of the 27/22 kDa and 12 kDa peaks as shown in the figure 3 by blue arrows. The best extraction solvent would be the ethanol 70% containing 1.0% (v/v) β-mercaptoethanol at 60°C, from the residue of A and G extraction in the PNR+PR1 fraction.

Conclusion

In Algeria and, especially in arid Sahara areas of the South (In Salah, Béchar, Timimoun, Tamanrasset and Aoulef Tidikelt), several pearl millet cultivars varying in colour and shape are grown for animal feed or human food. As a gluten free cereal, pearl millet can be potentially used as alternative protein source in Algeria, thus it is necessary to explore within several cultivars the protein and amino acid composition to be exploited in the future.

The total protein content varied widely in the analyzed pearl millet cultivars *i.e.* from 11.2 to 18.2% of d.m.b (average: 13.8%) with highly significant difference ($p \leq 0.001$).

Pearl millet proteins exhibited high levels of essential amino acids, representing more than 40% of the total amino acid content. However, like many cereals, the sampled pearl millet cultivars were deficient in lysine but in spite of that the levels of lysine, threonine, isoleucine, valine, serine and asparagine/aspartic acid were richer than sorghum, wheat and maize.

Prolamins made up the largest protein fraction in all pearl millet cultivars analyzed here. The combination of SDS-PAGE and SE-HPLC allowed the separation of the most abundant pennisetin forms. As gluten free cereal, pearl millet is a good alternative source of proteins to people suffering of celiac disease.

Further work is necessary to relate pennisetin compositions of pearl millet, and their nutritional quality to their growing conditions. The characterization of pearl millet proteins is essential to unleash pearl millet's capacity to be the cornerstone of food security in Africa as well as in many developing countries.

References

[1].- Amadou I., Gounga M. E. et Le G. W.; 2013.- Millets: Nutritional composition, some health benefits and processing-A review. Nutritional composition, some Emirates Journal of Food and Agriculture, 25: 501–508.

[2].- Saleh A. S. M., Zhang Q., Chen J. et Shen Q.; 2013.- Millet grains: nutritional quality, processing, and potential health benefits. Comprehensive Reviews in Food Science

and Food Safety, 12: 281–295.

[3].- Verma S., Srivastava S., et Tiwari N.; 2015.- Comparative study on nutritional and sensory quality of barnyard and foxtail millet food products with traditional rice products. Journal of Food Science and technology, 52: 5147–5155.

[4].- Ramashia S. E., Gwata E. T., Meddows-Taylor S., Anyasi T. A. et Jideani A. I. O.; 2018.- Some physical and functional properties of finger millet (Eleusine coracana) obtained in sub-Saharan Africa. *Food Research International*, 104: 101-118.

[5].- Taylor J. R. N. et Emmambux M. N.; 208.- Gluten-Free foods and beverages from millets. In Arendt EK, Bello FD (eds) Gluten-Free Cereal Products and Beverages. Academic Press, San Diego, U.S.A., Pp. 119-148.

[6].- Belton P. S. et Taylor J. R. N.; 2004.- Sorghum and millets: protein sources for Africa. Trends in Food Science and Technology, 15: 94–98.

[7].- Schalk K., Lexhaller B., Koehler P. et Scherf K. A.; 2017.- Isolation and characterization of gluten protein types from wheat, rye, barley and oats for use as reference materials. *PLoS ONE*, 12: 1–20.

[8].- De Brier N., Gomand S., Celus I., Courtin C. M., Brijs K. et Delcour J. A.; 2015.- Extractability and chromatographic characterization of wheat (Triticum aestivum L.) bran protein. Journal of food science, 80: 967–974.

[9].- Adebowale A. R. A., Emmambux Naushad M., Beukes M. et Taylor J. R. N.; 2011.- Fractionation and characterization of teff proteins. Journal of Cereal Science, 54: 380–386.

[10].- Gandhi K., Litoriya N., Shah A. et Talati J. G.; 2017.- Extraction, Fractionation and Characterization of Cotton (*Gossypium herbaceum* L.) Seed Proteins. Indian Journal of Agricultural Biochemistry, 30: 21–26.

[11].- Van Der Borght A., Vandeputte G. E., Derycke V., Brijs K., Daenen G. et Delcour J. A.; 2006.- Extractability and chromatographic separation of rice endosperm proteins. *Journal of Cereal Science*, 44: 68–74.

[12].- Redant L., Buggenhout J., Brijs K., et Delcour J. A.; 2017.- Extractability and chromatographic separation of rye (Secale cereale L.) flour proteins. Journal of Cereal Science, 73: 68–75).

[13].- Nałęcz D., Dziuba M. et Szerszunowicz I.; 2017.- Isolation of oat (Avena sativa L.) total proteins and their prolamin fractions for 2D electrophoresis. In Oat (Pp. 225-234). Humana Press, New York.

[14].- Mokrane H., Lagrain B., Gebruers K., Courtin C. M., Brijs K., Proost P. et Delcour J. A.; 2009.- Characterization of kafirins in Algerian sorghum cultivars. Cereal Chemistry, 86: 487–491.

[15].- Chaidez-Laguna Leslie D. Torres-Chávez Patricia I., Ramirez-Wong B., Medina-

Rodríguez Concepción L. et Carvajal-Millán E.; 2017.- Comparison of Solubility of Corn Proteins in Propanol, Ethanol, and tert-Butyl Alcohol Solutions on the Tortilla Process Samples. Cereal Chemistry, 94: 1052–1055.

[16].- Moroni A. V., Iametti S., Bonomi F., Arendt E. K. et Dal Bello F.; 2010.- Solubility of proteins from non-gluten cereals: A comparative study on combinations of solubilising agents. Food Chemistry, 121: 1225–1230.

[17].- Okoh P. N., Nwasike C. C. et Ikediobi C. O.; 1985.- Studies on seed protein of pearl millets. 1. Amino acid composition of protein fractions of early and late maturing varieties. Journal of Agricultural and Food Chemistry, 33: 55–57.

[18].- Chandna M. et Matta N. K.; 1990.- Characterization of pearl millet protein fractions.Phytochemistry, 29: 3395–3399.

[19].- Hadimani N. A., Muralikrishna G., Tharanathan R. N. et Malleshi N. G.; 2001.- Nature of carbohydrates and proteins in three pearl millet varieties varying in processing characteristics and kernel texture. Journal of Cereal Science, 33: 17–25.

[20].- Marcellino L. H., Junior B. C. et Gander E. S.; 2002.- Characterization of pearl millet prolamins. Protein and peptide letters, 9: 237–244.

[21].- Sainani M. N., Lachke A. H., Sahasrabudhe N. A. et Ranjekar P. K.; 1989.- Viscometric characterization of pennisetin from pearl millet. Biochemical and Biophysical Research Communications, 165: 334–341.

[22].- Shewry P. et Halford N.; 2003.- The prolamin storage proteins of sorghum and millets. AFRIPRO Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa. Pretoria, South Africa, 63p.

[23].- Ricks C. B., 2007.- The prolamins of pearl millet, Master thesis, Brigham Young University, 91p.

[24].- Sivakumar S., Franco O. L., Thayumanavan B., Murad A M., Manickam A, Mohan M. et Mridula M.; 2006.- Cloning and structural analysis of an Indian little millet (Panicum sumatrense) zein-like storage protein: Implications for molecular assembly. Biochemistry. (Moscow), 71: 1183–1191.

[25].- Tatham A. S., Fido R. J., Moore C. M., Kasarda D. D., Kuzmicky D. D., Keen J. N. et Shewry P. R.; 1995.- Characterisation of the major prolamins of tef (Eragrostis tef) and finger millet (Eleusine coracana). Journal of Cereal Science, 24: 65–71.

[26].- Abdalla A. A., El Tinay A. H., Mohamed B. E. et Abdalla A. H.; 1998.- Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chemistry, 63: 243–246.

[27].- Malik M., Singh U. et Dahiya S.; 2002.- Nutrient composition of pearl millet as influenced by genotypes and cooking methods. Journal of food science and technology, 39: 463-468.

[28].- Cheik A. T. O., Aly S., Yaya B. et Alfred T. S.; 2006.- A comparative study on

nutritional and technological quality of fourteen (14) cultivars of pearl millets [Pennisetum glaucum (L) Leeke] in Burkina Faso. Pakistan Journal of Nutrition, 5: 512–521.

[29].- Bagdi A., Balázs G., Schmidt J., Szatmári M., Schoenlechner R., Berghofer E. et Tömösközi S.; 2011.- Protein characterization and nutrient composition of Hungarian proso millet varieties and the effect of decortication. Acta Alimentaria, 40: 128–141.

[30].- AOAC, 1995.- Association of Official Analytical Chemists. Official methods of the Association of Official Analytical Chemists. 16th Edition, AOAC, Washington D.C., U.S.A, 231p.

[31].- Johns C. O. et Brewster J. F.; 1916.- Kafirin, an alcohol-soluble protein from kafir, andropogon sorghum. Journal of biological chemistry, 28: 59–65.

[32].- AACC, 2000.- American Association of Cereal Chemists: Approved Methods of the American Association of Cereal Chemists. 10th Edition, AACC International, Inc., St. Paul, MN, U.S.A., 452p.

[33].- Lamberts L., Rombouts I., Brijs K., Gebruers K. et Delcour J. A.; 2008.- Impact of parboiling conditions on Maillard precursors and indicators in long-grain rice cultivars. Food Chemistry, 110: 916-922.

[34].- Batey I.L., Gupta R. B. et Macritche F.; 1991.- Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. Cereal Chemistry, 68: 207–209.

[35].- SAS. Statistical Analysis System, Software Version 8.2, SAS Institute Inc., Cary, 334 NC, USA.

[36].- Serna-Saldivar S. D., McDonough C. M. et Rooney L. W.; 1991.- Handbook of cereal science and technology, Lorenz K.J. et Kulp K., eds., New York, Marcel Dekker.

[37].- Florence Suma P.; 2012.- Studies on the nutritional composition, functionality and utilization of pearl millet *(Pennisetum typhoideum).* Doctoral dissertation, University of Mysore, Mysore, India, 354p.

[38].- Abdel-Aal E.-S. M. et Hucl P.; 2002.- Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. Journal of Food Composition and Analysis, 15: 737-747.

[39].- Harrigan G. G., Stork L. G., Riordan S. G., Reynolds T. L., Taylor J. P., Masucci J. D., Cao Y., LeDeaux J. R., Pandravada A. et Glenn K. C.; 2009.- Impact of environmental and genetic factors on expression of maize gene classes: Relevance to grain composition. Journal of Food Composition and Analysis, 22: 158-164.

[40].- FAO/WHO, 1991.- Food and Agriculture Organization/World Health Organization. FAO/WHO Nutrition Meetings, Report Series 51, Viale delle Terme di Caracalla,

FAO, Rome, Italy, 91p.

[41].- Mokrane H., Amoura H., Belhaneche-Bensemra N., Courtin C. M., Delcour J. A. et Nadjemi B.; 2010.- Assessment of Algerian sorghum protein quality [Sorghum bicolor (L.) Moench] using amino acid analysis and in vitro pepsin digestibility. Food Chemistry, 121: 719–723.

[42].- Doi M., Yamaoka I., Nakayama M., Mochizuki S., Sugahara K. et Yoshizawa F.; 2005.- Isoleucine, a blood glucose-lowering amino acid, increases glucose uptake in rat skeletal muscle in the absence of increases in AMP-activated protein kinase activity. Journal of Nutrition. 135: 2103-2108.

[43].- Villa D. Y. G., Russo L., Kerbab K., Landi M. et Rastrelli L. ; 2014.- Chemical and nutritional characterization of Chenopodium pallidicaule (cañ ihua) and Chenopodium quinoa (quinoa) seeds. Emirates Journal of Food and Agriculture, 26: 609–615.

[44].- Abdelrahman A., Hoseney R. C. et Varriano-Marston E.; 1984.- The proportions and chemical compositions of hand-dissected anatomical parts of pearl millet. Journal of Cereal Science, 2: 127–133.

[45].- Bugs M. R., Forato L. A. , Bortoleto-Bugs R. K., Fischer H., Mascarenhas P. Y., Ward R. J. et Colnago L. A.; 2004.- Spectroscopic characterization and structural modeling of prolamin from maize and pearl millet. European Biophysics Journal, 33: 335–343.