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Starch digestion in pearl millet (*Pennisetum glaucum* (L.) R. Br.) flour from arid area of Algeria

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Summary

To assess the nutritive value of minor cereals cultivated in arid areas of Algeria, nine pearl millet landraces were sampled from two regions: Tidikelt and Hoggar. Some qualitative and quantitative characters of the panicle and grain were measured, as well as *in vitro* starch digestion of the grain flour. Considerable variation was recorded in seed color, endosperm texture and nutritional value of starch and protein content. *In vitro* starch digestion displayed a first-order kinetic model. For all pearl millet landraces, starch was digested to a different extent; the hydrolysis index (HI) ranged from 22.29 % to 35.52 % and the expected glycemic index (eGI) ranged from 27.41 to 38.82. The results show that there is diversity in the physical and chemical properties of pearl millet accessions from the arid areas of Algeria: Tidikelt and Hoggar. This study confirms that pearl millet has an acceptable nutritional value with a low glycemic index suitable for human health and nutrition.

Keywords: Pearl millet, Starch digestion, First-order kinetics, Glycemic index, Nutrition.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a small-seeded grass belonging to the Poaceae family and the Panicoideae subfamily. As it is a drought tolerant crop and has the ability to grow on low fertility soil and moisture (Fao and Icrisat, 1996), it is mostly cultivated in arid and semi-arid areas of the Sahel in Africa and in Asia, where it is a major food source. Millet is the 6th largest cereal crop in terms of world agriculture production. Furthermore, millets have resistance to pests and diseases, a short growing season, and are productive under drought conditions, compared to major cereals (Devi et al., 2014).
Millets are unique among the cereals because of their richness in calcium, dietary fiber, polyphenols and protein. Furthermore, as they do not contain gluten, they can be recommended for use by celiac patients (Devi et al., 2014). In addition to their nutritive value, several potential health benefits have been reported such as lowering the risk of cancer and cardiovascular disease, lowering blood pressure and risk of heart disease, lowering cholesterol and rate of fat absorption, and delaying gastric emptying by supplying gastrointestinal bulk (Saleh et al., 2013).
There are several plant characteristics that define grain quality, such as their structural and biochemical characteristics, digestibility, and bioavailability of nutrients. The structure of the grain kernels varies significantly because of environmental and genetic factors. Shape, size, proportion and nature of the endosperm, germ, and pericarp, the presence and absence of a subcoat, and color of the pericarp are all genetically determined (Rooney and Murty, 1982).

Grain digestibility also is important. Several works have been conducted to study the kinetics of starch digestion of different grains by alpha-amylase (Ezeogu et al., 2005; Frei et al., 2003; Goni et al., 1997). The glycemic index (GI) is an *in vitro* measurement based on glycemic response to carbohydrate-containing foods, and allows ranking of food on the basis of the rate of digestion and absorption of carbohydrates that they contain (Englyst et al., 1992; Jenkins et al., 1981). *In vitro* methods have also been used to classify foods based on their digestion characteristics similar to the *in vivo* situation, and to identify slow release of carbohydrate in foods (Schweizer et al., 1988; Jenkins et al., 1984). Food materials with GI values more than 70 %, between 56 and 69 % and lower than 55 % are classified as high, medium and low GI foods, respectively (Brand-Miller et al., 2003).
In the Sahara of Algeria, the Tidikelt and Hoggar regions are characterized by a typical desert climate; they are very hot and very dry. Moreover, rains have been rare during the last ten years. Aridity of the climate is extreme and the ambient temperature is very high in Tidikelt (in Salah), which is known to have temperatures ranging from 7.8 to 45.2 °C, a very low annual rainfall (16.9 mm), and irrigation is done with saline water. The Hoggar region is known to have temperatures ranging from 10 to 38 °C, with daily temperatures ranging over 23 °C, annual rainfall rate ranging from 7 to 160 mm, and irrigation is done with ground water. Despite these local hard climatic conditions, indigenous millet has maintained its original morphological diversity for centuries, and it has been able to accumulate significant genetic diversity between populations. However, these environmental factors have affected the starch properties in different *Sorghum* genotypes (Belhadi et al., 2012; Boudries et al., 2009; Mastsuki et al., 2003). For example, pearl millet; (*P. glaucum*), is a cereal that also is called mil, mil à chandelle (French), and Dokhen (Arabic); the local appellation is "bechnna" and "inélé", originally from West Africa, particularly, in the area north-east of the Senegal River. Millet was probably introduced during the eighth century into North Africa; its culture was intended to produce seed and fodder (Tostain, 1998). Actually, pearl millet production in these marginalized areas depends on traditional harvesting and processing. Most of the harvest is used as animal feed and rarely for human consumption. In the past, a wide range of traditional food products has been made from millet including kisra, porridge, and beverage.
One of the objectives of our laboratory research is to study the nutritional and quality traits of *Sorghum* and pearl millet grains as well as the isolation of starch and protein fractions and their beneficial characteristics for food and non-food uses (Souilah et al., 2014; Belhadi et al., 2012; Hadbaoui et al., 2010; Mokrane et al., 2010; Boudries et al., 2009; Mokrane et al., 2009). In a previous work, the protein nutritional quality of seven *Sorghum* cultivars cultivated in the Sahara of Algeria was assessed (Mokrane et al., 2010). High percentages of protein, up to 16 % db, were found in these cultivars with a favorable amino acid composition. The measure of *in vitro*

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pepsin digestibility shows that some cultivars exhibit high digestibility, whereas, other cultivars are characterized by their low digestibility (MOKRANE et al., 2010). The measurement of *in vitro* starch digestibility shows that the nine local *Sorghum* cultivars exhibit high digestibility of up to 90 % (SOUILAH et al., 2014).

The aim of the present study is to evaluate the digestibility of starch in Algerian arid area pearl millet grain cultivars by investigating starch *in vitro* digestion in pearl millet grain flour, assessing the *in vitro* digestion kinetic data, and evaluating the effect of landrace differences on kinetic parameters.

Materials and methods

Materials
Nine pearl millet [*Pennisetum glaucum* (L.) R. Br.] grains from local landraces were sampled from the arid Sahara areas of south Algeria: i.e., within Tidikelt and Hoggar. Landraces labeled as MLT.P, MLT.P.P, MLT.Saf, MDT.Smix, MLT.Ham, MDT.Sepl, MLH.Z, MLH.epc and MDH.Saf.T were harvested from different localities within Tidikelt and Hoggar; Djafou, Foggaret Ezzoua, El Malah, In Amghel, Tamanrasset and Abalessa. The samples were characterized by their kind of use (eg., cultivated millet, and domestic) and by different harvest years (2008, 2010 and 2011). Tab. 1 lists pearl millet landraces from arid areas of Algeria.

Millet grains were ground to flour in IKA Labotechik using an A10 sample mill. The flour was manually sieved using a 500 μm sieve. All reagents were analytical grade.

Methods
Pearl millet grain analysis
Some qualitative and quantitative characters of pearl millet grains (seed envelop, seed color, seed form, thousand seed weight and bulk density) were categorized (IBPGR and ICRISAT, 1993). Grain color was assessed by the Royal Horticultural Society (RHS) color codes (ICRISAT, 1993). Endosperm texture was defined as the proportion of corneous relative to floury endosperm in the grain, which was determined subjectively by viewing sectioned kernels using a stereomicroscope, and comparing them to *Sorghum* standards (TAYLOR and TAYLOR, 2008). The kernels were classified as corneous, intermediate or floury (ICC, 2008). Moisture content was determined according to AACC methods 44-15A. The crude protein content was determined according to Micro kjeldahl method using a nitrogen conversion factor of 5.83, based on an adaptation of the AACC 46-13A method (AACC, 2000). Total starch (TS) was determined by the enzymatic method of GONI et al. (1997).

In vitro starch digestion
In vitro starch digestion was determined according to the modified method of GONI et al. (1997). Around 600 mg of pearl millet flour was prepared in large tubes containing 25 ml of phosphate buffer (pH 6.9). To start starch hydrolysis, 5 ml of α-amylase (2×10^{-4} mg/ml), type VI.B from porcine pancreas (A3172, Sigma-Aldrich) was added. The prepared mixture was incubated at 37 °C for 2 h with constant shaking. Aliquots of 0.2 ml were withdrawn at 5, 10, 15, 20, 30, 60, 90 and 120 min. α-Amylase was inactivated immediately by placing the tubes in a boiling water bath for 5 min. Then, 0.6 ml of a 0.4 M sodium-acetate buffer solution (pH 4.75), and 0.2 ml of an enzyme solution containing 0.833 μl of amyloglucosidase from *Aspergillus niger* (300 U/ml, Sigma, A-7095) were added. In order to hydrolyze digested starch into glucose, the sample was incubated at 60 °C for 45 min. Finally, the volume was adjusted to 1-5 ml with distilled water and glucose concentration in the digesta was measured within the range (0.1-0.5 g/l) using an oxidase-peroxidase Kit (Biomaghreb, Tunisia).

A concentration of free glucose (0.459 g/l) was used to correct the starch digestion values. Starch digestion was expressed as percentage on the amount of starch present at the start of the reaction.

Modelling of starch digestograms
First-order exponential kinetics were used to estimate starch hydrolysis and glycemic indices in the food and feed studies (EZOGU et al., 2005; FREI et al., 2003; GONI et al., 1997). Starch amylolysis data was fitted to a first-order equation (Eq. (1)):

$$C_t = C_\infty (1-\exp[-kt]) \tag{1}$$

Where C_t corresponds to the percentage of starch hydrolysis at time t, C_∞ is the equilibrium percentage of starch hydrolyzed after 120 min, k is the kinetic constant and t is the time (min).

Glycemic and hydrolysis indices were determined from the area under the hydrolysis curve (AUC_{exp}), which was obtained by integrating Eq. (1) between times $t_0 = 0$ min and $t_f = 120$ min getting Eq. (2)

$$AUC_{exp} = C_\infty t_f - C_\infty / k (1-\exp[-k t_f]) \tag{2}$$

The hydrolysis index (HI) expressed the ratio of the AUC_{exp} of the sample from 0 to 120 min relative to the area under the hydrolysis curve of white bread (~ 7444 % min) (GONI et al., 1997). The expected glycemic index eGI was calculated by the equation eGI=8.198 + 0.862 HI as described by GRANFELDT et al. (1992).

Statistical analysis
All the parameters of pearl millet panicle and grain quality were measured in three replicates, and expressed as mean±SD. Data analyses were performed using SigmaPlot V.10.0 (Systat software Inc, Chicago, Illinois, USA) for windows.

Tab. 1: Pearl millet [*P. glaucum* (L.) R. Br.] landraces from the hyper area of Algeria: Tidikelt and Hoggar.

No.	Landraces codes	Locality	Region	Status	Harvest Date
01	MLT.P	Djafou	Tidikelt	Cultivated millet	2008
02	MLT.P.P	Foggarat Ezzoua	Tidikelt	Cultivated millet	2008
03	MLT.Saf	Foggarat Ezzoua	Tidikelt	Cultivated millet	2008
04	MDT.Smix	El malah	Tidikelt	Domestic[*]	2011
05	MLT.Ham	Djafou	Tidikelt	Cultivated millet	2010
06	MDT.Sepl	El Malah	Tidikelt	Domestic[*]	2011
07	MLH.Z	In Amgheul	Hoggar	Cultivated millet	2008
08	MLH.epc	Tamanrasset	Hoggar	Cultivated millet	2011
09	MDH.Saf.T	Abalessa	Hoggar	Domestic[*]	2011

Domestic[*]: introduced from neighboring countries; Mali, Niger, Sénégal… (Local appellation is Sudan)

Results and discussion

Pearl millet grain analysis

Some qualitative and quantitative characters of pearl millet grain were determined. As shown in Tab. 2, qualitative characters assessed were seed envelop (SE), seed color (SC) and seed form (SF). Seed envelop was assessed as exposed, intermediate or enclosed; seed color was grey, yellow, grey brown, brown, ivory or deep grey, and seed form was obevate, oblanceolate, hexagonal or globular. In general, pearl millet landraces showed variation in phenotypic characters, indicating that Algeria has a high diversity in millet traits.

The quantitative characters for all pearl millet landraces were thousand seed weight, bulk density, moisture, total starch and protein. Thousand seed weight varied from 6.20 ± 0.04 to 9.80 ± 0.12 g with a mean value of 8.75 g, which was in the range of the majority of millets varieties from the data collected by DENDY (1995), which varied between 2.5 and 14.7 g. The bulk density varied from 778.00 ± 3.66 to 782.60 ± 7.15 g/l with a mean value of 781.83 g/l; these mean values were lower than those reported for three pearl millets (850 g/l) looked at by JAIN (1997).

Moisture content in pearl millet grains ranged from 09.61% to 13.32%. Visual examination of endosperm texture varied in percentage of corneous (0 to 100%), intermediate (0 to 85%), and starchy (0 to 95%) fractions (Tab. 2). This variation in endosperm texture indicated that the grains should be classified as corneous, floury, mixed and intermediate endosperm type as described by the INTERNATIONAL ASSOCIATION FOR CEREAL SCIENCE AND TECHNOLOGY (2008). Total starch (TS) content of pearl millet flour ranged from

51.35 ± 5.35 to 69.07 ± 3.09% db with a mean value of 61.43% (Tab. 3). The grain chemical composition of pearl millet genotypes from the world collection at ICRISAT showed that starch composition was between 62.8% and 70.5% with a mean value of 66.7% (FAO, 1995). When compared to our results, the total starch content in the Algerian pearl millet samples was lower than the mean value. Moreover, the grain starch contents in the ten studied pearl millet landraces were lower than those in wheat (65%) and higher than those observed in rye (60%) and barley (55%) (CHOCT and HUGHES, 2000). However, our samples exhibited a lower total starch content than maize (75%) and rice (80%) (CHOCT and HUGHES, 2000).

The protein (P) content of pearl millet flour ranged from 09.62 ± 0.01 to 17.18 ± 0.58% db with a mean value of 14.01% (Tab. 3). The grain chemical composition of pearl millet genotypes from the world collection at ICRISAT showed that protein content was between 5.8% and 20.9% with a mean value of 10.6% (FAO, 1995). The grain protein contents in the ten pearl millet landraces studied were higher than the mean value.

A large variation for grain qualitative and quantitative traits was observed in Algerian pearl millet landraces. Based on this variation, probably due to environmental conditions, high genotype diversity is found among landraces (ROONEY and MILLER, 1982).

In vitro kinetic starch digestion and Modelling

Experimental data and computed digestibility curves were shown for all pearl millet flours in (Fig. 1). The kinetic curves show that the starches in pearl millet flours from Algeria landraces were hydro-

Tab. 2: Qualitative and quantitative characters of pearl millet grains.

N°	Landraces codes	SE	SC	SF
01	MLT.P	Exposed(3)	Grey (201)	Obevate
02	MLT.P.P	Intermediate(5)	Grey (201)	Oblanceolate
03	MLT.Saf	Exposed (3)	Yellow (8C)	Oblanceolate
04	MDT.Smix	Intermediate	Grey brown (199)	Hexagonal
05	MLT.Ham	Intermediate (5)	Brown (200)	Oblanceolate
06	MDT.Sepl	Enclosed (7)	Ivory (158A)	Obevate
07	MLH.Z	Exposed (3)	Deep grey (202B)	Oblanceolate
08	MLH.epc	Exposed(3)	Deep grey (202B)	Obevate
09	MDH.Saf.T	Exposed (3)	Yellow (8C)	Globular

SE: Seed envelop, **SC:** Seed color, **SF:** Seed form, **TSW:** Thousand Seed Weight, g, **BD:** Bulk density, g/L, **H:** Moisture, %, **TS:** Total starch, %, **P:** Protein, %.

N°	Landraces codes	TSW (g)	BD (g/L)	H (%)	TS (%)	P (%)	Endosperm texture (%)		
							Corneous	Intermediate	Starchy
01	MLT.P	9.50 ±0.15	782.60 ± 3.15	09.61	57.02 ± 1.92	17.18 ± 0.58	95	5	0
02	MLT.P.P	9.10 ±0.10	780.40 ± 6.32	11.35	58.82 ± 5.56	15.18 ± 0.71	5	85	10
03	MLT.Saf	9.20 ± 0.70	782.50 ± 4.56	10.95	65.29 ± 7.19	11.41 ± 0.20	0	5	95
04	MDT.Smix	6.86 ± 0.22	778.00 ± 3.66	10.42	58.44 ± 7.97	14.15± 0.12	90	10	0
05	MLT.Ham	9.50 ± 0.08	782.60 ± 7.15	11.75	65.81 ± 2.12	16.89 ± 0.76	90	10	0
06	MDT.Sepl	6.20 ± 0.04	782.60 ± 3.55	10.27	65.87 ± 2.48	13.27 ± 1.83	80	20	0
07	MLH.Z	9.40 ±0.14	782.60 ± 2.81	13.11	69.07 ± 3.09	14.87 ± 0.50	100	0	0
08	MLH.epc	9.20 ±0.05	782.60 ± 5.16	11.55	63.06 ± 4.19	13.30± 0.89	0	5	95
09	MDH.Saf.T	9.80±0.12	782.60 ± 4.46	10.00	59.53 ± 9.69	14.24± 1.55	0	5	95

MLT.P : Pearl Millet Local fromTidikelt, MLT.P.P : Pearl Poilue (Hairy), MLT.Saf: Safra(Yellow), MDT.Smix: Domesticated from Soudan mix, MLT.Ham: Hamra(Red), MDT.Sepl: Soudan epi log (Tall Panicle), MLH.Z: Millet Local from Hoggar. Zarga (Blue), MLH.epc : Local Hoggar epi court (Short panicle), MDH.Saf.T : Domesticated Hoggar Safra (Yellow) from Tidikelt.

Tab. 3: Starch digestibility and expected glycemic index parameters of the first-order model for the pearl millet flours [a].

N°	Landraces codes	k(min⁻¹)	R^2	SEE (%)	C_∞(%)	HI(%)	eGI
01	MLT.P	0.24	0.97	4.40	22.83	35.52	38.82
02	MLT.P.P	0.13	0.99	0.86	19.42	29.30	33.45
03	MLT.Saf	0.23	0.99	1.44	15.54	24.14	29.01
04	MDT.Smix	0.22	0.99	0.78	19.54	30.31	34.32
05	MLT.Ham	0.21	0.96	4.50	21.25	32.90	36.55
06	MDT.Sepl	0.17	0.98	2.18	22.23	34.08	37.57
07	MLH.Z	0.29	0.91	9.23	14.24	22.29	27.41
08	MLH.epc	0.15	0.99	0.94	22.22	33.83	37.36
09	MDH.Saf.T	0.20	0.90	6.64	18.15	28.04	32.37

[a] Values are estimated from fit to experimental data, with $R^2 > 0{,}9$ and standard Error of estimate (SEE) < 6% for most landraces.
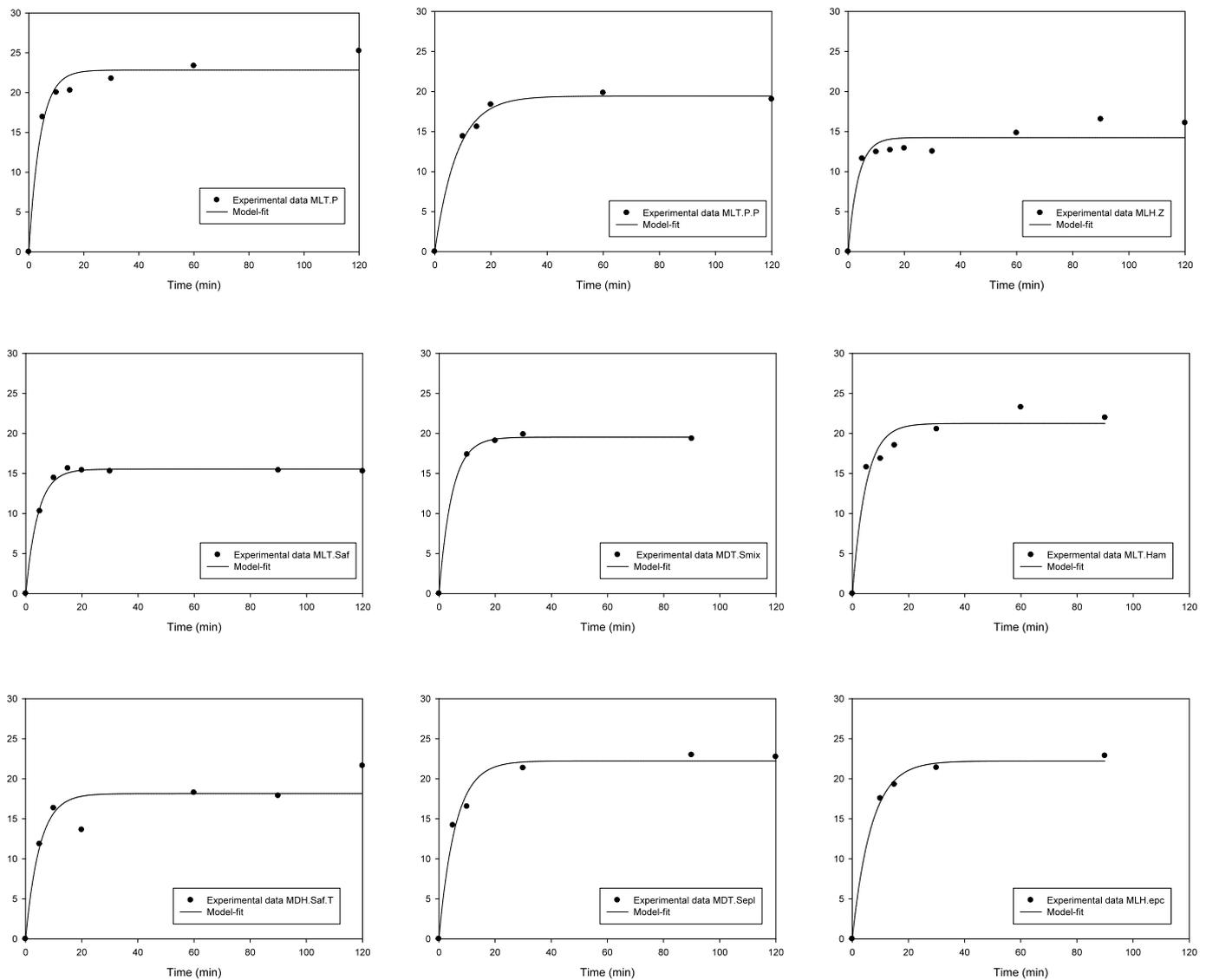
Fig. 1: Digestibility curves obtained for pearl millet flours.

lyzed by amylases. The extent of the reaction indicates that these flours have a low susceptibility to digestion.

Predicted values for variables affecting starch amylolysis (C_∞ and k), that were obtained from the first-order model, fit to the experimental data. Overall, computed digestibility curves provided a very good fit to all experimental data, with $R^2 > 0.9$ and standard Error of estimate (SEE) < 6% for most landraces.

Predicted values (C_∞, and k) were obtained from the first-model fit

to the experimental data, reported in Tab. 3. The constant k ranged between 0.13 and 0.29 min^{-1}; starch hydrolysis at infinite time C_∞ varied from 14.24 to 26.38 %, and model-fit analysis of digestibility data was particularly well-suited to these studies.

First-order model properties have been demonstrated in *in vitro* starch digestion of raw and processed food and feed (Ezogu et al., 2005; Frei et al., 2003; Goni et al., 1997). The kinetic constant k of amylolysis has been proposed as a reliable index of the inherent susceptibility of flour starches to amylase hydrolysis (Frei et al., 2003; Goni et al., 1997).

Modeling of starch digestion kinetics is required to derive more quantitative information on digestibility properties. The hydrolysis index HI and expected glycemic index eGI are reported in Tab. 3. The hydrolysis index (HI) obtained by use of a first-order model fit to the experimental data ranged from the lowest in MLH.Z (22.29 %) to the highest MLT.P (35.52 %). This variation in starch digestibility of pearl millet flours was due to grain quality differences in the sampled pearl millet landraces (Rooney and Pflugfelder, 1986). The expected glycemic index eGI varied from 27.41 to 38.82. Relative to the glycemic index suggested by Brand-Miller et al. (2003), for Algerian pearl millet landraces (eGI < 55), the results of the GI for the eight local cultivars of *Sorghum* were high and ranged from 74.02 to 94.14 (Souilah et al., 2014). In the 2002 edition of the international table of Glycemic Index and Glycemic Load reported by Foster-Powell et al. (2002), the glycemic index of boiled millet (Canada) was found to be 101, while that of millet flour porridge (Kenya) was 153 ± 14. Mani et al. (1993) also found that GI ranged from 55 ± 13 to 104 ± 13 after testing six commonly consumed *Sorghum* foods of India. The results indicate that starches from pearl millet samples in our work can be classified as having a low GI, although the GI values of *Sorghum* flours grown in Algeria are high with a GI value of up to 70 (Souilah et al., 2014).

The HI and eGI values of pearl millet flours grown in Algeria were much lower, possibly due to differences in genetic source, growing conditions, and the employed methods used to determine HI and eGI.

Conclusions

The present study points out that differences in some morphological characteristics and biochemical components of the panicle and grains demonstrate diversity in the phenotype of pearl millet landraces in Algeria. Moreover, measure of *in vitro* starch digestibility shows that the nine local landraces examined exhibited low digestibility (< 40 %) and a low glycemic index (< 55). First-order kinetic analysis was used to model starch digestion of uncooked pearl millet flours. The digestibility properties of starches in pearl millet landraces showed high variability. This result suggests that there are good opportunities for utilization of pearl millet grain, grown in the Sahara of Algeria, for nutritional purposes and for potential health benefits especially for dealing with diabetes.

Conflict of Interest

All authors declare that they have no conflict of interest.

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