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EFFECTS OF EXTRACTABLE PROTEIN HYDROLYSATES, LIPIDS, AND POLYPHENOLIC COMPOUNDS FROM PEARL MILLET (*PENNISETUM GLAUCUM* (L.) R. BR.) WHOLE GRAIN FLOURS ON STARCH DIGESTIBILITY

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ABSTRACT

Pearl millet and other minor cereal production is marginalized in the Sahara of Algeria (Tidikelt and Hoggar regions). Their productions in these areas depend on traditional harvesting and processing. Pearl millet seeds are used as animal feed and rarely for human consumption. This work was to assess the starch digestion of pearl millet cultivated in the arid areas of Algeria. The seeds from this cereal could provide broad potential benefits to human health. However, their digestion properties have not been reported. Therefore, in this study, the *in-vitro* starch digestibility of pearl millet flour and the effect of processing on the expected glycemic index (eGI) were investigated. Grains from six pearl millet samples were chosen from two regions: Tidikelt and Hoggar. Five flours were prepared by dry milling (MF) and different treatments after dry milling such as extraction of phenolic compounds (MF-PP), lipid extraction (MF-L), protein hydrolysate extraction (MF-P) or lipid plus protein hydrolysate extraction (MF-L-P). The flours were then subjected to digestion, and the effects of grain treatments on the in vitro starch digestion were investigated. For all pearl millet samples, the kinetics of *in vitro* starch digestion displayed first-order model as substrates were digested to different extents; k (kinetic constant), C_∞ (percentage of starch hydrolyzed at infinite time), HI (hydrolysis index) and eGI (expected glycemic index) of the samples were also calculated. Significant increases in C_∞, HI and eGI ($P<0.05$) of the samples were observed after extraction of proteins or proteins plus lipids from flour. Four flours obtained after lipid extraction and five flours from extraction of phenolic compounds had low glycemic index (<55), with values ranging between 31.36 and 44.97. In contrast, flours obtained from protein hydrolysate extraction or lipids plus protein hydrolysates had the highest glycemic index (>69), with values ranging between 77.50 and 121.44. This study confirmed that some of the processed pearl millet seed flours have acceptable nutritional values suitable for human health and nutrition due to the low glycemic index values.

Key words: Pearl millet, Grain processing, Starch digestion, First-order model, Glycemic index

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INTRODUCTION

Millets are important crops in semi-arid and tropical regions of the world [1]. The most important species are pearl millet, finger millet, proso millet, and foxtail millet. Pearl millet accounts for almost half of global millet production in the world [2]. Millets are the most drought-tolerant cereal grain crops and require little input during growth, but as with other crops, yield better with good husbandry [1, 3, 4]. In Algeria, pearl millet [*Pennisetum glaucum (L.) R. Br.*] is an important crop. These millet grains are grown in Tidikelt and Hoggar regions, and their characteristics are defined and reported by Lemgharbi *et al*. [5, 6] who indicated that pearl millet was produced by small-scale farmers for household consumption. Pearl millet seeds are used as animal feed and rarely for human consumption in Algeria.

Millet grains have good nutritional value, in terms of proteins and amino acids, carbohydrates, fats, vitamins, minerals, and energy values. They are comparable to the popular cereals like rice, wheat, and barely [1, 3]. Millets are accepted as functional and nutraceutical food because they provide dietary fibre, protein, energy, minerals, vitamins, and antioxidants required for human health [7, 8].

Over several decades, the trend has been towards consumption of highly processed foods, which is linked to occurrence of a variety of chronic illnesses, for instance type 2 diabetes, hyperlipidemia, obesity, and cardiovascular problems [9, 10]. The main reason behind all this has been agreed upon to be due to overconsumption of digestible carbohydrates (starches), which are quickly absorbed from the gastrointestinal tract (GIT), and thus resulting in a “spike” of normal blood glucose levels [9]. Formulating foods which may regulate the levels of starch digestion, so as to prevent the high spike of blood glucose rates is consequently desirable.

The aim of this study was to evaluate the digestibility of starch in the Algerian arid areas pearl millet grain cultivars, by evaluating the effects of non-starch compounds (proteins, lipids and phenolic compounds) on the parameters of the in-vitro starch digestion kinetics and the expected glycemic index (eGI).

MATERIALS AND METHODS

Materials

Grains from five landraces and one introduced pearl millet (*P. glaucum (L) R. Br*) were sampled from the arid Sahara areas of south Algeria: Tidikelt and Hoggar. Table 1 lists sample codes, locality, region and status.

Methods

Five flours were prepared from each sample. The first millet flour (MF) was prepared by dry milling. Other flours were prepared by dry milling combined with different treatments such as extraction of phenolic compounds (MF-PP), lipid extraction (MF-L), protein hydrolysate extraction (MF-P) or lipid plus protein hydrolysate extraction (MF-P-L). The processed flours were subsequently subjected to digestion assays. All the reagents used for analysis were of analytical grade.

***Dry milling*:** The millet grains were ground to flour in IKA Labotechik A10 sample mill. The obtained flours were manually sieved over a 500 µm sieve.

***Lipid Extraction*:** Flour samples (5 g) were treated with n-hexane using the Soxhlet apparatus by refluxing for 5 h. The defatted flour (MF-L) was dried directly in drying oven at 40 °C overnight.

***Protein Hydrolysates Extraction*:** Millet flour (MF) was treated with protease according to the method reported by Goni *et al*. [12] with modifications. One hundred and sixty milliliters (160 mL) of HCl-KCl buffer solution at pH 4.0 was added to 20 g of flour (MF) and the contained immersed in a water bath. To start protease treatments, 40 mL of pepsin (1 mg/mL) from porcine gastric mucosa (800-2,500 U/mg protein, Sigma-Aldrich (P7000)) was added. The prepared mixture was incubated at 40 °C for 1 h with constant shaking and then the suspension centrifuged (10 min, 28630 rpm). The supernatant was removed using a spatula and the residue (free of extractable protein hydrolysate) (MF-P) was dried directly in a drying oven at 40 °C overnight.

***Protein and Lipid Extraction*:** To prepare the flour (MF-L-P), sample flour obtained after lipid extraction was treated with pepsin and the protein hydrolysate removed as described above.

***Extraction of Phenolic Compounds*:** According to the method reported by Khadambi[13] with modification , the extracts of phenolic compounds were prepared by suspending a sample (3 g) of MF in 150 mL in aqueous acetone (75% v/v). The mixture was incubated at room temperature for 2 h with constant shaking. The suspension was centrifuged (5 min, 28630 rpm), the supernatant removed and the residue obtained (MF-PP) was dried directly in an oven at 40 °C overnight.

Sample Characterization

The moisture content of all the flours was determined according to AACC methods 44-15A. The crude protein content was determined according to micro-Kjeldahl method using nitrogen conversion factor of 5.83, an adaptation of the AACC 46-13A [14]. Total starch (TS) was determined by the enzymatic method [12]. Fat content was determined using the Soxhlet apparatus (n-hexane, 5 h) method [15].

In vitro Starch Digestion and Modelling of Starch Digestograms

The *invitro* starch digestion was determined according to method of Goni *et al*. [12] with modifications and a first-order exponential model in kinetics study was used to estimate starch hydrolysis or glycemic indices as previously described [6,12, 16].

Statistical Analysis

All the parameters of sample characterization were measured in three replicates, and expressed as Mean±SD. Data were analyzed by one-way analysis of variance (ANOVA) and mean differences were assessed by Fisher's least significant difference test at the level of $p < 0.05$ with the SPSS software, V.17. The data analyses were performed with the Sigma Plot V.10.0 (Systat software Inc, Chicago, Illinois, USA) for windows.

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RESULTS AND DISCUSSION

Sample Characterization

The pearl millet samples, selected for the study, differed in phenotypic characters, which is indicative of morphologic variation and biodiversity of millet cultivated in Tidikelt and Hoggar regions [6].

As shown in Table 2, the lipids content of pearl millet flours (MF) ranged from 8.27 to 11.18% with a mean value of 9.43%. Values obtained were higher than reported range of 4.1-6.1% for the Semi-Arid Tropics ICRISAT [16], as well as 1.5-6.8% [4] and 1.5-4.8% [18]. Moreover, the values obtained were higher than other cereals such as rice (2.7%), wheat (2%), maize (4.6%) and sorghum (3.1%) [18]. The percentage of extracted lipids after treatments ranged from 21.70 to 54.38%. This indicated that a high percentage of lipids remained in processed millet grains (MF-P-L).

The protein content of pearl millet flour (MF) ranged from 11.41% to 16.89% with a mean value of 14.31%. Values obtained are higher than reported mean value of 10.6 % [17], 11.21% reported for 10 Sudanese pearl millet varieties [19], and 11.8% reported [18], but are near the mean value of 14.5% reported by Taylor [3]. Moreover, the values obtained are higher than other cereals such as brown rice (7.9%), wheat (11.6%), maize (9.2%) and sorghum (10.4%) [18]. The percent of extracted proteins after the treatments ranged from 09.77 to 38.54%. The results indicate that a high percentage of protein remained in processed millet samples (MF-P-L). The Algerian pearl millet landraces, as shown, represent a satisfactory source of proteins and lipids for human nutrition.

In vitro Kinetics of Starch Digestion and Modeling

The starch digestion curves for all pearl millet flours in the present study are shown in Figures 1 to 6.

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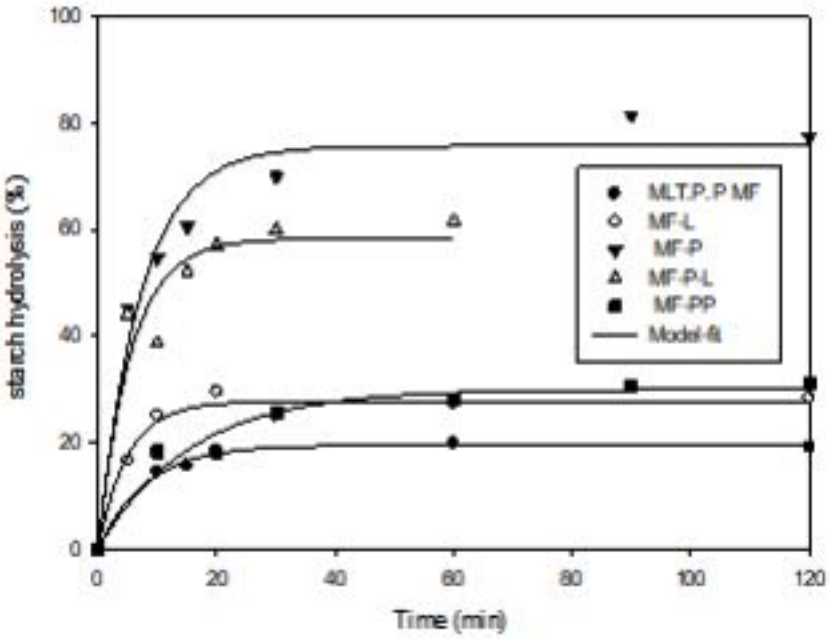

Figure 1: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MLT.P.P.
MF= Whole grain flour, MF-L = Flour after extraction of lipids, MF-P = Flour after extraction proteins hydrolysable, MF-P-L = Flour after extraction of lipids and hydrolysable proteins and MF-PP = Flour after extraction of phenolic compounds

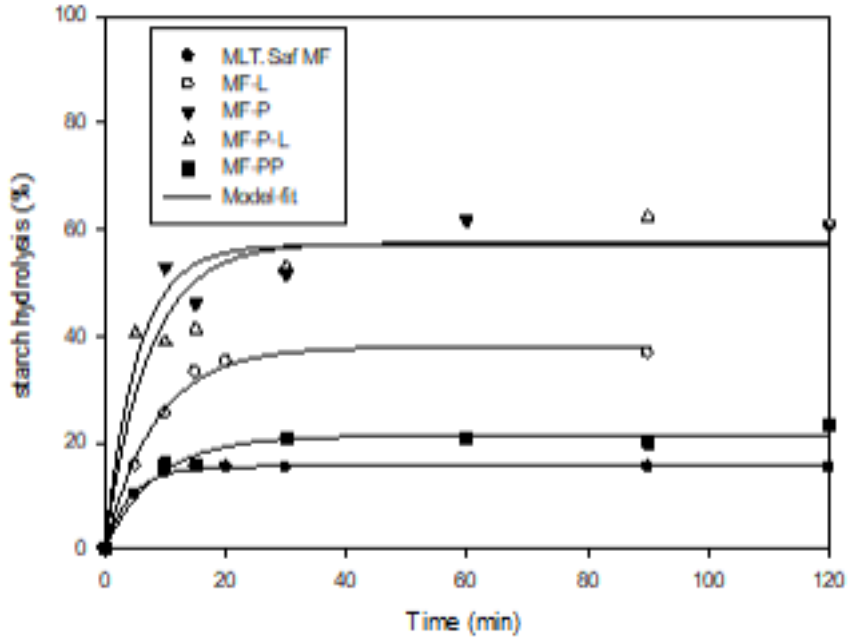

Figure 2: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MLT.Saf

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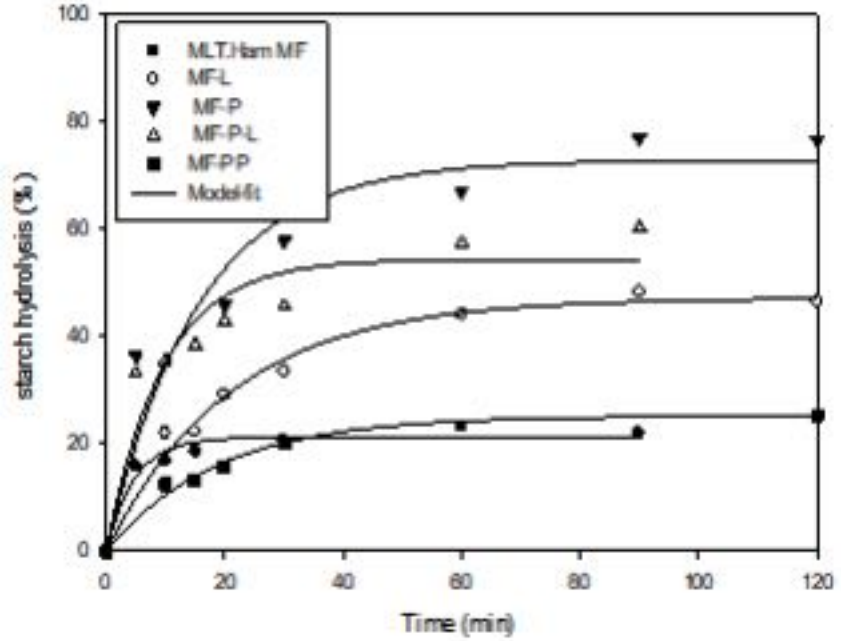

Figure 3: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MLT.Ham

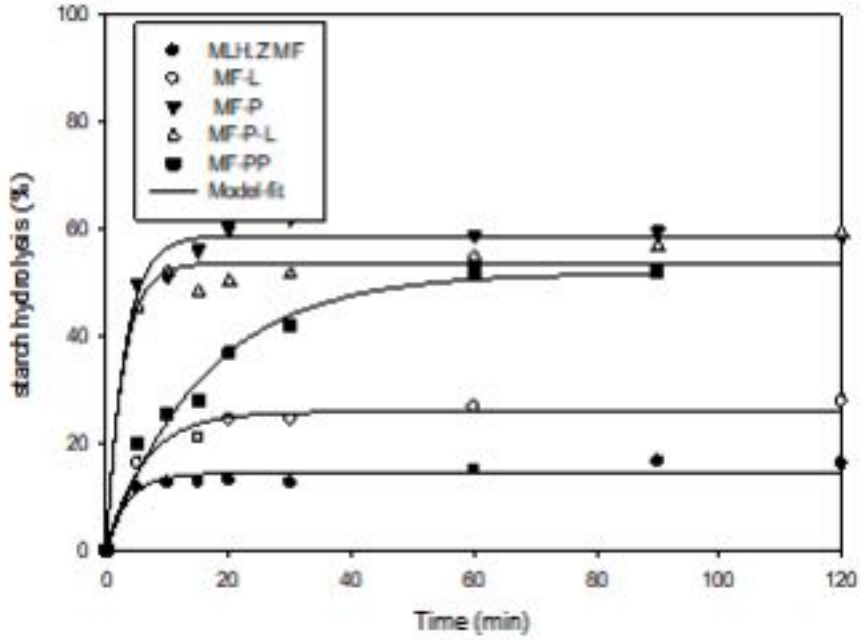

Figure 4: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MLH.Z.

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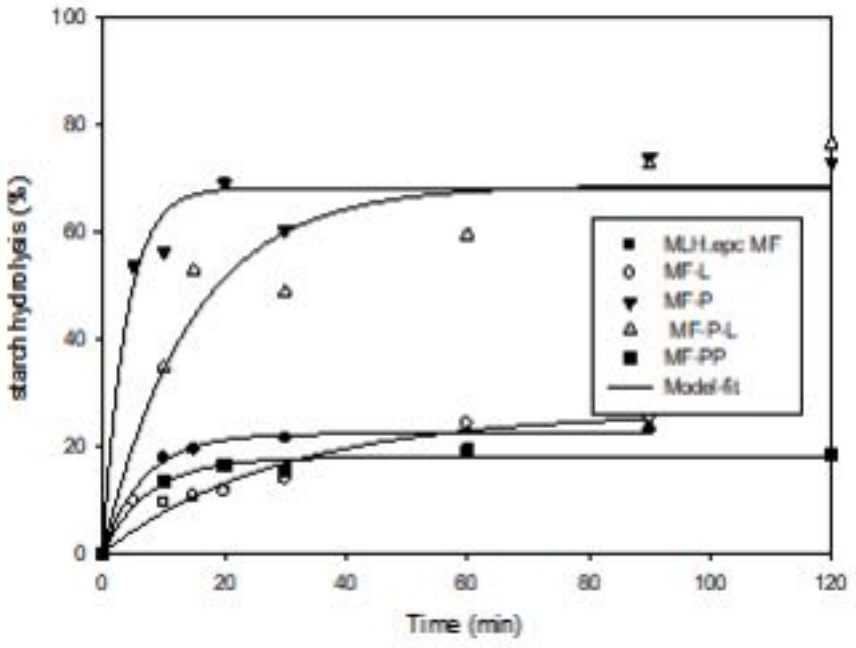

Figure 5: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MLH.epc.

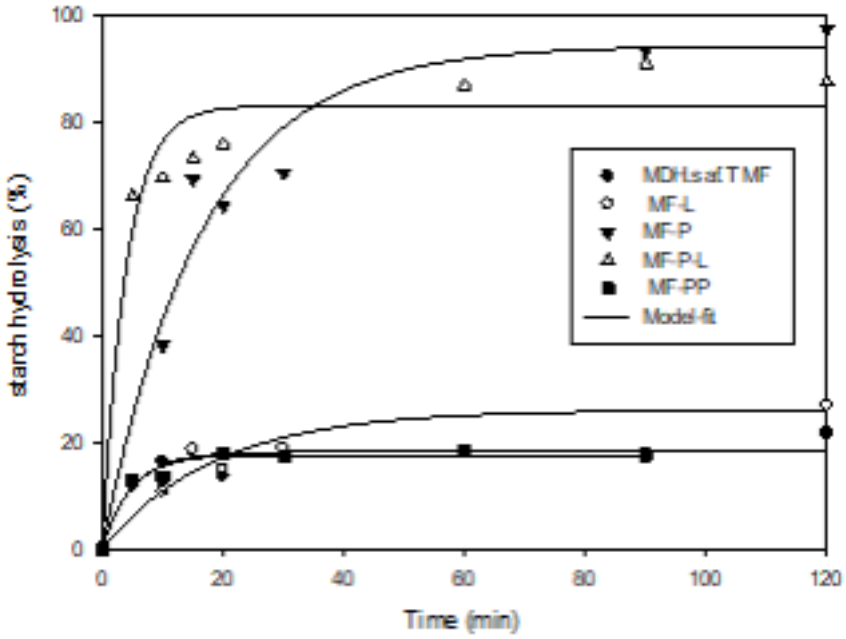

Figure 6: Starch digestibility curves for unprocessed and processed flours from pearl millet sample: MDH.saf.T.

The digestibility curves showed that the pearl millet flours from Algerian landraces were hydrolyzed by amylases in the chosen reaction conditions. The estimated parameters (k, C_∞, HI, eGI), by first-model, in starch hydrolysis were obtained by the fit to experimental data, where k was the rate constant (min^{-1}), C_∞ was the percentage of starch hydrolyzed at infinite time, recorded in 120 min, HI the hydrolysis index and eGI the expected

glycemic index. Overall, the 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 samples. The first-order kinetic model was suitable, including the estimated values k, C_∞, HI and eGI are summarized in Table 3. The analysis of the variance amongst the millet substrates, for six samples, revealed that the changes in C_∞, HI and eGI were significant ($P<0.05$) between free extractable proteins or proteins and lipids substrates, like untreated flour and free extractable phenolic compounds or free lipids substrates. However, the changes in k were non-significant ($P>0.05$) as shown in Table 4. Exponential model properties have been used to describe in vitro starch digestion of raw and processed food and feed [12, 16]. The starch digestion parameters have revealed the inherent susceptibility of starches to amylase hydrolysis [12, 20, 21]. The percentages of starch hydrolysis at infinite time (C_∞) ranged from 14.21 to 22.22% for all flour substrates (MF) and ranged from 53.50 to 83.10% after extraction of lipids and hydrolysable proteins (MF-P-L). The extent of reactions indicated that these substrates had low susceptibility to digestion. Two substrates after lipid extractions (MLT.Saf and MLT.Ham) and one substrate after phenolic compound extractions (MLH.Z) had medium susceptibility to digestion, C_∞ values ranged from 37.91 to 51.75%. High susceptibility to digestion was registered for all flour substrates after protein hydrolysate or lipids plus protein hydrolysate extractions with 53.50 to 94.38% C_∞ values. The results demonstrate that in studied pearl millet samples, extraction of protein hydrolysates from flours produced more effective starch substrates for hydrolysis than obtained from flour without treatments or flour after extraction of lipids or phenolic compounds. In contrast, the effect of lipids on the in-vitro starch digestibility of the kodo millet was found to be more significant than that of proteins [22]. When comparing the values of C_∞ in starch digestion, the differences between the substrates can be explained by the change in structure and architecture of grain endosperm and decrease in protein content. The protein hydrolysis processes led to the loss of a part of the endosperm crystallinity, the formation of smaller parts of the protein matrix and to the formation of starch voids in the endosperm. The previous change in the endosperm structure helped the external enzyme diffusion to the surface of the starch granule and the internal enzyme diffusion in the granule pores and channels. Thus, starch digestibility was high after extraction of protein hydrolysates. The flour substrates were composed of endosperm particle regions, which were extremely dense, hard, with high protein content and high resistance to enzymatic degradation [11]. The endosperm protein, associated with the type and location of protein, have been demonstrated to be responsible for many of the differences in starch digestion between slowly digested substrates and those that are rapidly digested [23].

The modeling of starch digestion kinetics is required to derive more quantitative information on digestibility properties. The HI and eGI are reported in Table 3. The eGI of millet flour substrates ranged from the lowest in MLH.Z (27.41) to the highest in MLH.epc (37.36). The values obtained were less than the values of wheat flour (37.53), rice flour (53.25) [22], Canadian barley (32), Indian wheat (43), Canadian wheat (60) made from whole kernels [24], and nine sorghum flours grown in Algeria (68.70-109.30) [25].

After lipid extraction, the eGI value of the millet flour (MF) increased from 29.01 to 57.22 for MLT.saf and from 36.55 to 62.21 for MLT.Ham, while for the remaining samples the range was between 38.78 and 44.97. In the previous work, the interaction between starch and lipid was known to have effects on the enzymatic-hydrolysis rates and physical properties of starches [26]. After extraction of phenolic compounds, there was not much difference of eGI with a range of 31.68-44.34, except for MLH.Z, which increased from 27.41 to 70.23. These results indicate that phenolic compounds had very little effect on starch digestion. The eGI range increased from 29.01-36.55 for the flours to 83.76-121.44 after extraction of protein hydrolysate and 77.50-119.82 after extraction of lipids plus protein hydrolysates. Previous results have confirmed that endogenous proteins and lipids can inhibit starch digestion in an important staple food with reduced glycemic-index [27, 28]. According to the classification of glycemic index content as previously suggested [29], the results indicate that starches in all the studied pearl millet flours, four lipids free and five free of extractable phenolic compounds can be classified as having a low GI (<55), with values ranging between 31.36 and 44.97. In contrast, all flours free of extractable proteins or lipids plus proteins had the highest glycemic index (>69), with values ranging between 77.50 and 121.44. These eGI values obtained for pearl millet processed by dry milling and extraction of hydrolysed proteins, are higher than different varieties of processed millet (cooked, porridge, steamed bread, pancake, muffin, extruded snack, couscous and roti) from 49.9% (Koko, porridge) to 69.4% (Foxtail, porridge) [27]. The eGI range values of some major cereals like rice, barley and oats in their cooked form were reported to be 60-102, 55-65 and 77-100, respectively [20, 30, 31, 32]. The eGI values of pearl millet substrates, prepared in this work, were in the following order: MF-P> MF-L-P >MF-L> MF-PP> MF. Compared with the results of Annor *et al*. [22], the eGI of kodo millet is in the following order: kodo millet starch (47.81) >MF-L-P (46.76) >MF-L (42.66) >MF-P (36.03) >MF (32.47). According to previous results [28], the eGI of cooked rice samples was in the following order: RF (rice flour) (88.9) >RF-L (91.5) >RF-P (92.3) >RF-L-P (94.5). These results showed that the treatment or processing applied on different grains or same grains but from different regions led to different effects on starch digestion.

Processing methods employed in this work improved the nutritional and starch digestion properties of millet grains. The whole grain flour (MF) had lower expected glycemic index (eGI) than flour substrates after treatments (MF-PP, MF-L, MF-L-P or MF-P) as well as flours or processed grains from major cereals [20, 27, 28, 30, 31, 32]. The presence of protein may be responsible for lower starch digestibility. The extraction of hydrolysed proteins from millet flour was shown to increase *in vitro* starch digestibility significantly.

Based on these results, processing had an effect on the starch digestion of the whole grain products from the pearl millet samples grown in arid regions of Algeria. Therefore, the whole grain flour and other flours obtained after lipid or phenolic compounds extraction with their hypoglycemic property can be a potential food source for controlling diabetes and obesity. The high glycemic index flour obtained after extraction of protein hydrolysates can be included in the diet. However, these foods should be consumed in association with foods of good nutritional quality that help to reduce post-prandial blood glucose level. However, the intake of flour that causes smaller increase in blood glucose

level is often preferred in some situations, for example, during hypoglycemia or for the maintenance of stored glycogen levels in athletes [33].

CONCLUSION

In this work, the kinetic studies showed that the first-order model could assess the modeling of starch digestion in uncooked pearl millet flours after pretreatments to extract lipids or phenolic compounds or proteins. There were different effects of some non-starch compounds in pearl millet grains on the *in vitro* starch digestion and glycemic index.

In general, the extraction of phenolic compounds caused no significant increases in the *in vitro* starch digestibility, while the extraction of lipids or protein hydrolysates had significant impacts. The effects of proteins on the *in vitro* starch digestibility of pearl millet were found to be more significant than those of lipids and phenolic compounds. Finally, the results suggest that there are good opportunities for the utilization of pearl millet grains grown in arid Sahara areas of south Algeria (Tidikelt and Hoggar), as nutritional agents with potential health benefits.

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Table 1: Pearl millet (*P. glaucum (L) R Br*) landraces from arid areas of Algeria: Tidikelt and Hoggar

No.	Samples codes	Locality	Region	Status
01	MLT.P.P	FoggaratEzzoua	Tidikelt	Landrace
02	MLT.Saf	FoggaratEzzoua	Tidikelt	Landrace
03	MLT.Ham	Djafou	Tidikelt	Landrace
04	MLH.Z	In Amghel	Hoggar	Landrace
05	MLH.epc	Tamanrasset	Hoggar	Landrace
06	MDH.Saf.T	Abalessa	Hoggar	Domesticate*

Domesticate*: introduced from neighboring country Niger

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Table 2: Protein, fat and total starch of pearl millet substrates

Landraces codes	**Samples**	Protein (%, dry weight)	Fat (%, dry weight)	Total starch (%, dry weight)[a]
MLT.P.P	MF	15.18 ± 0.71 [a]	10.18	58.82 ± 5.56
	MF-P-L	10.33 ± 0.94	07.97	-
MLT.Saf	MF	11.41 ± 0.20 [a]	08.27	65.29 ± 7.19
	MF-P-L	07.64 ± 0.12	05.91	-
MLT.Ham	MF	16.89 ± 0.76 [a]	08.43	65.81 ± 2.12
	MF-P-L	10.38 ± 0.36	11.18	-
MLH.Z	MF	14.87 ± 0.50 [a]	09.12	69.07 ± 3.09
	MF-P-L	12.64 ± 1.58	06.55	-
MLH.epc	MF	13.30± 0.89 [a]	11.18	63.06 ± 4.19
	MF-P-L	12.00 ± 0.62	05.10	-
MDH.Saf.T	MF	14.24± 1.55 [a]	09.44	59.53 ± 9.69
	MF-P-L	09.98 ± 0.60	07.29	-

MF=Whole grain flour**, MF-P-L**= Millet flour after extraction of lipids and hydrolysable proteins

[a]Published values from Lemgharbi *et al.* [5]

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Table 3: Kinetic parameters of first order reaction model, hydrolysis indices and expected glycemic indexes values for starch substrates prepared from pearl millet grains [a]

Landraces codes	Substrates[b]	k (min^{-1})	Std Error	C_∞ (%)	HI (%)	eGI	R^2
MLT.P.P	MF [c]	0.130	**0.86**	19.42	29.30	33.45	0.994
	MF-L	0.200	**3.06**	27.61	42.65	44.97	0.977
	MF-P	0.140	**2.13**	75.93	114.66	107.04	0.968
	MF-L-P	0.180	**5.26**	58.26	89.57	85.41	0.924
	MF-PP	0.064	**1.02**	29.90	41.93	44.34	0.954
MLT.Saf	MF [c]	0.230	**1.45**	15.54	24.14	29.01	0.996
	MF-L	0.120	**0.93**	37.91	56.87	57.22	0.992
	MF-P	0.180	**4.54**	57.02	87.66	83.76	0.947
	MF-L-P	0.140	**3.92**	57.36	86.96	83.16	0.893
	MF-PP	0.110	**1.61**	21.25	31.66	35.50	0.972
MLT.Ham	MF [c]	0.210	**4.50**	21.25	32.90	36.55	0.958
	MF-L	0.047	**0.42**	47.21	62.66	62.21	0.984
	MF-P	0.064	**1.42**	72.90	102.22	96.31	0.910
	MF-L-P	0.100	**2.63**	54.41	80.44	77.50	0.895
	MF-PP	0.054	**0.46**	25.22	34.39	37.84	0.985
MLH.Z	MF [c]	0.290	**9.22**	14.21	22.29	27.41	0.910
	MF-L	0.160	**2.60**	25.74	39.33	42.10	0.970
	MF-P	0.330	**5.20**	58.64	92.14	87.63	0.981
	MF-L-P	0.360	**8.54**	53.50	84.25	80.82	0.966
	MF-PP	0.062	**0.75**	51.75	71.91	70.23	0.974
MLH.epc	MF [c]	0.150	**0.94**	22.22	33.83	37.36	0.996

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	MF-L	0,034	**0.96**	26.19	32.05	35.82	0.902
	MF-P	0.260	**6.44**	68.24	106.50	99.98	0.954
	MF-L-P	0.069	**1.54**	68.60	97.23	92.01	0.914
	MF-PP	0.130	**1.91**	17.81	26.87	31.36	0.974
MDH.Saf.T	MF [c]	0.200	**6.64**	18.15	28.04	32.37	0.898
	MF-L	0.054	**1.00**	26.02	35.48	38.78	0.935
	MF-P	0.061	**0.89**	94.38	131.37	121.44	0.959
	MF-L-P	0.250	**5.83**	83.10	129.49	119.82	0.950
	MF-PP	0.210	**3.58**	17.60	27.25	31.68	0.973

[a] Values are estimated from fit to experimental data, with $R^2 > 0.9$ and standard Error of estimate (SEE) < 6% for most landraces. k is the rate constant (min^{-1}), C_∞ is the percentage of starch hydrolyzed at infinite time (recorded after 120 min of digestion), HI is the hydrolysis index and eGI is expected glycemic index

[b]Substrates: **MF=** Whole grain flour; **MF-L=** Flour after extraction of lipids; **MF-P=** Flour after extraction hydrolysable proteins; **MF-P-L=** Flour after extraction of lipids and hydrolysable proteins; and **MF-PP**=Flour after extraction of phenolic compounds.

[c] **Published values from Lemgharbi *et al.* [6]**

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Table 4: Effects of non-starch compounds on digestibility and expected glycemic index (eGI) parameters for pearl millet starch obtaibed from different grain processing treatments

Treatments	k (min^{-1})	C_∞ (%)	HI (%)	eGI
MF	0.20 ± 0.06b	18.46 ± 3.15a	18.47 ± 3.14*	32.69 ±3.97ab
MF-L	0.10± 0.07a	31.78 ± 8.88b	44.84 ± 12.24a	46.85 ± 10.55b
MF-P	0.17 ± 0.11ab	71.18 ± 13.64c	105.76 ± 15.88b	99.36 ± 13.69c
MF-L-P	0.17 ± 0.11ab	62.54 ± 11.42c	94.66 ± 17.97b	89.79 ± 15.50c
MF-PP	0.10 ± 0.06a	27.25 ± 12.88ab	39.00 ± 17.04a	41.82 ± 14.71ab

Data presented as mean±SD (n=6). ANOVA followed by Fisher's least significant difference (LSD) test was performed to search for parameters differences in the samples. In a row, means followed by the same letters (a,b and c) are not significantly different ($p>0.05$) between treatments. * The mean difference is significant at the 0.05 level. k the rate constant (min^{-1}), C_∞ is the percentage of starch hydrolyzed at infinite time (recorded after 120 min of digestion), and HI is the hydrolysis index

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