Algerian Journal of Environmental Science and Technology
March edition. Vol.7. Nº1. (2021)
ISSN : 2437-1114
www.aljest.org

Isolation of starch from seven Pearl Millet grain landraces by two processes; wet milling and Ultrasound application

S. Bouhallel[a,*], B. Belhadi[a,b], R. Souilah[a,c] , D. Djabali[a] , B. Nadjemi[a]

[a] Equipe valorisation de la biomasse. Laboratoire d'étude et de développement des techniques de traitement et d'épuration des eaux et de gestion environnementale, Ecole Normale Supérieure, BP92, Vieux Kouba, Algiers, Algeria.
[b] Département des Sciences et Techniques, Faculté de Technologie, Université Amar Telidji, Laghouat, Algeria.
[c] Département de Physique, Ecole Normale Supérieure, Laghouat, Algeria

*Corresponding author: bouhallelsarra@gmail.com ; Tel.: +213 5 60 28 39 63

ARTICLE INFO

Article History:

Received ***: 15/12/2019***
Accepted ***: 19/01/2020***

***Key Words*:**

Starch;
Isolation;
Ultrasound;
Pearl Millet.

ABSTRACT/RESUME

Abstract: *The objective of the present work was to add value to seven pearl millet landraces (Pennisetum glaucum (L.) R. Br) from south Algeria through isolation of the grain starch using a conventional process: the wet milling, adding sodium azide as a microbial growth inhibitor, and to enhance the yield by evaluation of a new process: Ultrasound-assisted isolation. The effects of pearl millet grain quality and starch isolation process on some properties of the isolated starches were evaluated in terms of yield, recovery, chemical composition, and some starch granules physical properties. The Ultrasound-assisted isolation efficiency was thus evaluated and gave better yields, higher recoveries, and purer starch, with ranges of 30.63-52.65 %, 46-75.70 %, and 91.32-94.58 % respectively, it showed a great potential for pearl millet starch isolation in a short period without generating any alkaline effluent.*

I. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is a cereal crop belonging to the Gramineae family; it has various health benefits due to a very interesting chemical composition (71.6 % starch, 8.6 – 19.4 % protein (higher than rice, maize and sorghum), 5.1 % lipids, 5 % fiber, and 1.6 – 3.6 % minerals), and a good productivity in a short growing cycle, with an excellent adaptability to high temperature and dry conditions. All these factors make this cereal having a major role in food security and could resurge as an easily available substitute, however, it remains understudied and underutilized [1-3]. Starch has extensive commercial and industrial utilization as a raw material in textile, paper, food, pharmaceutical, and as a thickener [4-6], the starch granules morphology and functionality vary between and within botanical species [4], their compact structure made them completely insoluble in cold water, amylose and amylopectin are organised in starch granules as dense, semi-crystalline entities [5, 7]. The starch isolation process is principally governed by the required operation scale, different processes have been established, and all were based on whether: grain steeping, wet milling, and starch recovery, or: dough making, dough washing, and starch recovery [8, 9]. For pearl millet, grains are steeped in water in the presence of a microbial growth inhibitor [9-11], wet grains are then washed and milled carefully to avoid granules heat damage, the slurry is finally sieved and the starch is separated from fibers and proteins by centrifugation [1, 9], a dark brownish layer is formed on the top of the centrifugation tubes and is removed by scraping with a spatula [9], the purification of the crude starch is performed by repeated washing and centrifugation because of the pericarp colour [8], the wet pure starch is either air dried, or oven-dried

at 40 °C as a maximum temperature to avoid annealing, the starch is then ground, sieved, and stored under dry conditions. A typical cereal starch sample should be bright white and contain less than 0.7 % proteins which affect the starch-derived products [4, 8, 9]. In the conventional isolation processes, the chemical products and the extended operation time can lead to microbial growth and starch degradation, thus, developing an environmentally friend isolation process is important [10, 12], the Ultrasound-assisted isolation (UAI) is a new technology for isolation of plants bioactive substances, with advantage in simplicity, reproducibility, yield, reduced time, and eliminating of post-treatment water, the required equipments are inexpensive, robust, and provide purer products [10, 12-14], the effects of Ultrasounds depend on amplitude, temperature, time, suspension concentration, and botanical origin. The application of Ultrasound at a laboratory scale for plant material is widely published, while it is still very challenging to attempt isolation on an industrial scale [13-15]. Many starch isolation processes have been evaluated in our laboratory on important cereals such as sorghum and pearl millet [16-18], in the current work; and looking for a procedure that would provide the best yield and purity in a short time with reducing chemicals use; the Ultrasound-assisted isolation process was investigated, we attempted to isolate starch from seven pearl millet landraces by both conventional and ultrasound application processes to evaluate the latter as an alternative.

II. Materials and methods

Pearl millet (*Pennisetum glaucum* (L.) R. Br) samples were introduced to the laboratory collection [19], they were originally collected from two areas in the Algerian Sahara; Tidikelt and Hoggar, Tidikelt is characterized by low annual rainfall and high temperature reaching a monthly mean of 45.2°C, temperatures in Hoggar vary from 10 to 38°C and the annual rainfall there vary from 7 to 160 mm. Experimental data were collected on seven pearl millet landraces after a cleaning and sampling operation, experiments were conducted on grains kept in the laboratory, then stored at 6°C for a week to allow uniformity of moisture distribution, before each test, the required quantities were allowed to warm up to room temperature.

II.1. Pearl Millet grain quality and chemical composition

Thousand grains weight (TGW) was determined according to [19], the grains color was determined using the Munsell Color System, the starchy endosperm percentage was determined according to [20] who reported that endosperm texture was defined as the proportion of corneous relative to starchy endosperm in the grain, the bulk density of grains was determined according to [21], standard AACC 44-19 and AACC 46-10 methods were used respectively to determine the moisture (H%) and the protein content (Prot%) in pearl millet flour [22], total starch (TS%) was determined according to [17,18,23,24]. All the experiments of this work have been repeated three times.

II.2. Starch isolation methods

II.2.1. The Wet Milling method (WMM)

Pearl millet starch was isolated according to [2,7], grains were steeped in distilled water containing 0.01% sodium azide (to inhibit microbial growth) during 24 hours at 4°C, the steeping solution was discarded and the grains were washed and wet-milled, the slurry was sieved through 160 and 100 µm opening screen sieves, and the material remaining on the sieves was subjected to repeated blending and sieving process, the final residues of the sieves were dried, weighed, and kept for starch content analysis. The recovered starch was separated from fiber and protein by centrifugation at 2000 rpm for 20 min; and the dark brownish layer formed on the top of the centrifugation tubes was separated by scraping with a spatula, then dried, weighed, and kept for protein content analysis. Starch purification was performed by repeated centrifugation and the cleaned starch obtained was air-dried for 18 hours then redispersed in water and wet sieved through 100 µm sieve, the sieved slurry was centrifuged at 2000 rpm for 20 min, and the starch was air dried and stored in dry conditions.

II.2.2. The Ultrasound-assisted isolation

Pearl millet starch was isolated according to [25], a sufficient volume of distilled water was warmed up to 35°C before adding pearl millet flour, the slurry was stirred for 5 min before the onset of the sonication, amplitude was set at 75 % during 60 min; the sample temperature was controlled at 40 °C. After the ultrasound treatment, the same procedure as described above was followed for wet-milling, sieving, centrifugation, and protein removal, the final sieving residues and the protein layer were dried, weighed, and kept for starch and protein contents analysis respectively, the obtained starch was dried at 45 °C for 48h, and stored in dry conditions.

II.3. The isolated starch characterization

II.3.1. Chemical composition

The isolated starch chemical composition was determined using methods cited in subsection II.1.

II.3.2. Water solubility index, swelling power, and water holding capacity

To study the nature of the associative bonding forces within granules, the swelling power (SP) and the solubility behaviour in an aqueous system (Water Solubility Index; WSI) have been determined according to [26] with slight modification, 0.1 g of starch (W_0) and 6 mL of distilled water were mixed and incubated in a thermostatically controlled water bath for 30 min in the temperature range of 55 – 95 °C at 10°C intervals with occasional shaking. The suspension was rapidly cooled to room temperature and centrifuged for 30 min at 8000 xg, the supernatant was carefully separated and dried to constant weight at 130 °C (W_1), and the swollen sediment adheringed to the wall of the centrifuge tube was weighed (W_s). WSI was determined from the amount of dried solids recovered by evaporating the supernatant, whereas SP is reported as the ratio of swelling starch granules sediment to dry starch. WSI and SP were calculated with equations (1) and (2).

$$WSI = (W_1/W_0)x100\ (\%) \quad (1)$$

$$SP = W_s/\big(W_0(100\% - WSI)\big)\ (g/g) \quad (2)$$

Water holding capacity (WHC) was realized according to [27], a suspension of 0.1 g starch in 10 mL distilled water was kept for 1 h in a previously weighed centrifuge tube at ambient temperature then centrifuged at 10,000 rpm for 15 min, the supernatant was decanted and the tube was weighed after removal of the adhering drops of water, the weight of water (g) retained in the sample was reported as WHC and calculated with equation (3).

$$WHC(g(H_2O)/g(Starch)) = (Wet\ starch\ mass - Dry\ starch\ mass)/Dry\ starch\ mass \quad (3)$$

II.3.3. Starch iodine absorbance spectrum blue value and light transmittance

Iodine absorption spectrum of starch was measured according to [28], 100 mg of starch were suspended in 1 mL of ethanol then 9 mL of NaOH (1 M) were added, the mixture was heated in a boiling water bath for 10 min with continuous shaking, then adjusted to pH 6.5 with HCl (1 M) and diluted to 100 mL with distilled water, an aliquot of 5 mL of the solution was added to 1 mL of 0.2% iodine solution and made up to 100 mL with distilled water, the mixture was kept at room temperature for 15 min then the wavelength of maximum absorbance λ(max) from 450 to 800 nm was measured. Blue value (BV) of iodine–starch complexes was calculated with the absorbance measured at 680 nm according to the equation (4) where C is the concentration of starch in the solution in 1mg/100mL.

$$BV = 4x(Abs_{680}/C) \quad (4)$$

Light transmittance was measured according to [29], a 1% aqueous suspension of starch was heated in a water bath at 90 °C for 1 h with constant stirring, the suspension was cooled to 30 °C then stored for 5 days at 4 °C, light transmittance was determined every 24 h by measuring transmittance at 640 nm against a water blank.

II.4. Statistical analysis

The data analysis was performed and evaluated for significance with the SPSS software, V.17, the one way analysis of variance (ANOVA) was used to evaluate the difference between the two isolation methods, univariate analysis was used to obtain means, maximum and minimum values, standard deviations, ranges, and variances for each trait, correlations among traits and the chemical composition were analyzed using the multivariate analysis, and the hierarchical clustering was realized to classify the isolated starches into clusters.

III. Results and discussion

III.1. Pearl Millet grain quality and chemical composition

According to Munsell colour system, grains colour varied from different shades of yellow to yellowish brown (Table 1), some grains didn't show uniform colour which is reported to be controlled genetically and modified by environmental conditions during and after maturation [24]. Visual and microscopic examination of the endosperm texture showed variances in starchy fractions percentage from 33 to 71 % (Table 1), six of the seven samples had starchy endosperm. The thousand grains weight (TGW) varied between 5.11 and 8.23 g, and the grains bulk density ranged from 814.55 to 840.03 g/L (Table 1).

Table 1. *Phenotypic traits of Pearl Millet grains.*

Sample	Year	Locality	Grains colour		TGW (g)	Bulk density (g/L)	Starchy Endosperm Percentage
			Munsell notation	Colour			

							(%)
M1.07.Is	2007	Tidikelt	5Y 7/6	Yellow	7.3±0.0	840.0±2.1	71
M2.07.Is	2007	Tidikelt	2.5Y 7/4	Pale Yellow	7.8±0.0	814.7±0.6	59
M.07.Tam	2007	Hoggar	2.5Y 7/6	Yellow	5.1±0.1	831.5±1.3	33
M.09.Djf	2009	Tidikelt	10YR 5/6	Yellowish Brown	7.4±0.2	830.4±5.3	46
M.10.Djf	2010	Tidikelt	2.5Y 6/6	Olive Yellow	8.2±0.0	814.6±3.4	56
M1.11.Fe	2011	Tidikelt	2.5Y 7/6	Yellow	8.1±0.0	829.0±3.7	61
M.12.Amgl	2012	Hoggar	5Y 7/3	Pale Yellow	7.1±0.0	833.1±3.6	43

Y: Yellow, YR: Yellow-Red.

In this study, the pearl millet flour moisture content varied from 9.50 to 11.62 % (Table 2), it is higher than the results reported by Abdalla et al. [2] which didn't exceed 5.89%, whereas Abdalla et al. [30] have found nearly similar results with 9.1 – 11.7 %. For the protein content analysis, results in the present work varied in the range of 14.54 – 16.88 %, but Wankhede et al. [31] and Abdalla et al. [2] found lower results (7.8 - 9.6 %, and 12.25 – 13.09 % respectively), the latter reported that higher ratio of germ to endosperm is responsible for the higher protein content of pearl millet flour. Total Starch in pearl millet flour in this work varied between 59.25 and 69.40 %, similar results have been reported with ranges of 62.5 – 69.4 %, 67.5 – 68.7 %, 58.5 – 70 %, 60– 65 %, and 50 –75% [30,32,33,34,35].

Table 2. *Chemical composition of Pearl Millet grains.*

Sample	Moisture content %	Total Starch content %	Protein content %
M1.07.Is	11.32±0.04	63.73±1.06	15.63±0.07
M2.07.Is	11.62±0.02	64.91±0.95	15.95±0.12
M.07.Tam	09.50±0.25	65.16±0.81	16.88±1.63
M.09.Djf	11.27±0.18	63.98±1.03	16.79±1.27
M.10.Djf	09.70±0.25	69.40±1.05	14.54±0.29
M1.11.Fe	10.39±0.25	68.55±1.15	14.90±0.56
M.12.Amgl	11.43±0.34	59.25±0.73	14.86±0.95
Means	10.74	64.99	15.65
Maximum	11.62	69.40	16.88
Minimum	9.50	59.25	14.54
Range	2.12	10.15	2.34
SD	0.87	3.35	0.94
Variance	0.76	11.28	0.88
CV%	8.10	5.15	6.00

III.2. Starch isolation

III.2.1. The isolated starch chemical composition, isolation yield Y%, and starch recovery R%.

The isolated starch chemical composition for both isolation methods is given in table 3.

Table 3. *The isolated starch chemical composition.*

Sample	Wet Milling Method			Ultrasound-Assisted Isolation		
	Humidity %	Total Starch%	Protein %	Humidity %	Total Starch%	Protein %
M1.07.Is	12.02	84.45	00.84	12.47	94.58	00.46
M2.07.Is	11.85	84.00	00.81	12.45	93.32	00.66
M.07.Tam	12.38	85.27	01.03	12.50	92.35	00.42
M.09.Djf	11.93	89.02	01.14	12.55	91.31	00.89
M.10.Djf	11.88	90.50	00.82	12.54	92.98	00.60

M1.11.Fe	11.74	89.77	00.75	12.49	93.21	00.55
M.12.Amgl	11.97	91.75	00.80	12.48	92.52	00.46
Means	11.96	87.82	00.88	12.49	92.89	00.57
Maximum	12.38	91.75	01.14	12.55	94.58	00.89
Minimum	11.74	84.00	00.75	12.45	91.31	00.42
Range	0.64	7.75	00.39	0.10	3.27	00.47
SD	0.20	3.17	00.14	0.03	1.00	00.16
Variance	0.04	10.05	00.02	0.00	1.01	00.02
CV%	1.67	3.60	15.90	0.24	1.07	28.07

In the wet milling isolation, starch moisture varied from 11.74 to 12.38 %, these values are within the range of 10.8 – 14.1 given by Beleia et al., (1980) [34], starch protein content varied from 0.75 to 1.14 % but higher result has been reported by Abdalla et al. [2] reaching 1.70 %, whereas Beleia et al. [36] found lower content 0.44 – 0.77 %, this may be due to the presence of highly hydrated pentosans and insoluble protein which entrap starch granules in the matrix, the exact quantity of protein remaining in the starch fraction depends on the biological source, extraction and purification conditions, and probably on the affinity of these proteins for the starch granule surface [8, 37], total starch content which is also called starch purity varied in this first method from 84.00 to 91.75 %.
In the ultrasound-assisted isolation, starch moisture content was around 12.55 % , starch protein content varied from 0.42 to 0.89 %, thus, ultrasound treatment appears to disrupt the hydrophobic protein matrix surrounding starch granules and the amylose–lipid complex, this will free more starch granules and makes them available for added action of enzymes which is responsible for an increase in the starch recovery [38], total starch content varied in this second method from 91.31 to 94.58 %.
Starch isolation recoveries (R%) and yields (Y%) (equations 5 and 6) for both of the wet milling isolation method, and the ultrasound-assisted isolation are given in table 4.

$$R\% = Y\%/Total\ Starch \quad (5)$$

$$Y\% = (Starch\ weight/Grains\ weight)x100 \quad (6)$$

Table 4. *Starch isolation recoveries R%, yields Y%, and sieving and centrifugation residues analysis.*

Sample	Y%	R%	Sieving residues %	Centrifugation residues %	Total Starch % in sieving residues	Protein % in centrifugation residues
Wet Milling Method						
M1.07.Is	33.82	44.82	43.70	06.74	47.76±0.47	26.29±0.47
M2.07.Is	44.07	57.03	29.68	06.78	38.36±2.00	34.34±1.04
M.07.Tam	43.86	57.40	33.19	08.44	51.99±0.61	27.87±0.90
M.09.Djf	42.71	59.43	35.40	07.70	49.64±0.78	33.40±1.25
M.10.Djf	42.19	55.02	35.97	07.62	49.74±1.91	32.73±0.40
M1.11.Fe	38.21	50.04	30.31	07.70	48.14±1.31	33.86±0.46
M.12.Amgl	35.16	45.45	44.97	05.26	51.86±0.78	33.49±0.48
Means	40,00	52.74	36,17	7,17	48.21	31,71
Maximum	44,07	59.43	44,97	8,44	51,99	34.34
Minimum	33,82	44.82	29,68	5,26	38.36	26.29
Range	10,25	14.61	15,29	3,18	13,63	8.05
SD	4,25	5.96	6,05	1,02	4,64	3.23
Variance	18,07	35.53	36,68	1,06	21.53	10.45
CV%	10.62	11.30	16.72	14.22	9.62	10.19
Ultrasound-Assisted Isolation						
M1.07.Is	43.39	64.39	29.13	12.25	43.55±0.88	23.94±1.52

M2.07.Is	52.65	75.70	23.37	15.42	36.59±1.03	23.45±2.50
M.07.Tam	49.55	70.22	30.28	13.19	48.90±1.46	27.29±0.73
M.09.Djf	50.38	71.91	25.66	13.46	42.39±1.30	30.36±0.42
M.10.Djf	48.36	64.80	26.78	14.18	48.61±0.56	27.18±0.81
M1.11.Fe	48.43	65.85	28.22	13.69	45.70±1.53	28.75±0.73
M.12.Amgl	43.56	68.02	35.18	10.41	61.91±0.32	25.95±0.39
Means	48,04	68.69	28,37	13,22	46.80	26.70
Maximum	52,65	75.70	35,18	15,42	61.91	30.36
Minimum	43,39	64.39	23,37	10,41	36.59	23.45
Range	9,26	11.31	11,81	5,01	25.32	6.91
SD	3,43	4.16	3,77	1,57	7.86	2.48
Variance	11,82	17.33	14,24	2,48	61.91	6.15
CV%	07.13	6.05	13.28	11.87	16.81	9.28

For the wet milling isolation method, yields and recoveries vary from 33.82 to 44.07% and from 44.82 to 59.43% respectively, while for the ultrasound assisted isolation, they vary from 43.39 to 52.65 and from 64.39 to 75.70% respectively, these values are partially in conformity with the results reported by Jiang et al. [10]. L. Wang and Y-J. Wang [25] reported yields for ultrasound-assisted isolation varying in the range of 62.3 – 76.2 % for rice, and in the range of 63.8 – 64 % for corn (for treatment duration of 15 and 30 min), this method produced the highest starch yield and recovery.

III.2.1. Seiving and centrifugation residues analysis.

The results of the analysis of the final sieving residues and the centrifugation fraction (for starch and protein content respectively) are given in table 4. The final sieving residues starch content was in the range of 36.59 – 61.91 %, thus this residue was considered as starchy, starch can be lost in its fiber fraction because of the presence of some starch in the pericarp, some peripheral endosperm cells are not opened during the wet milling process, and kafirin proteins are associated with starch granules and make Pearl Millet (and even Sorghum) more difficult to fractionate. The centrifugation fraction protein content was in the range of 23.45 – 34.34 %, this made this fraction a valuable source of proteins which must be seriously considered.

III.3. Starch characterization

III.3.1. Swelling power SP, water solubility index WSI, and water holding capacity WHC.

Results for swelling power, water solubility index, and water holding capacity are shown in figures 1, 2 and 3, respectively.

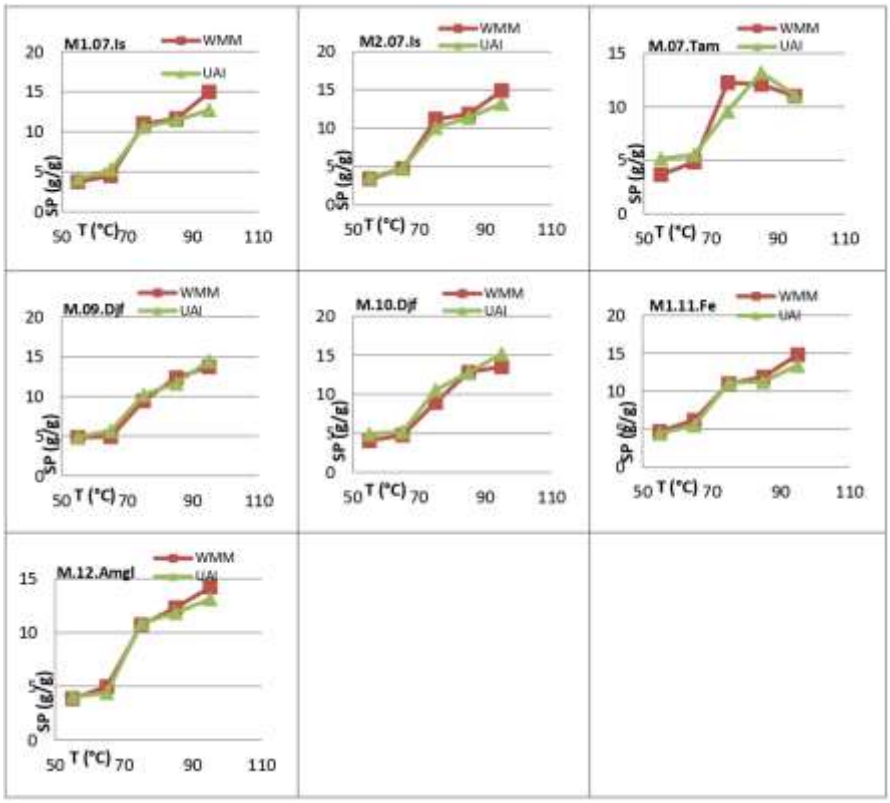

Figure 1. *Swelling power of the isolated starches (WMM: Wet Milling Method , UAI: Ultrasound-Assisted Isolation)..*

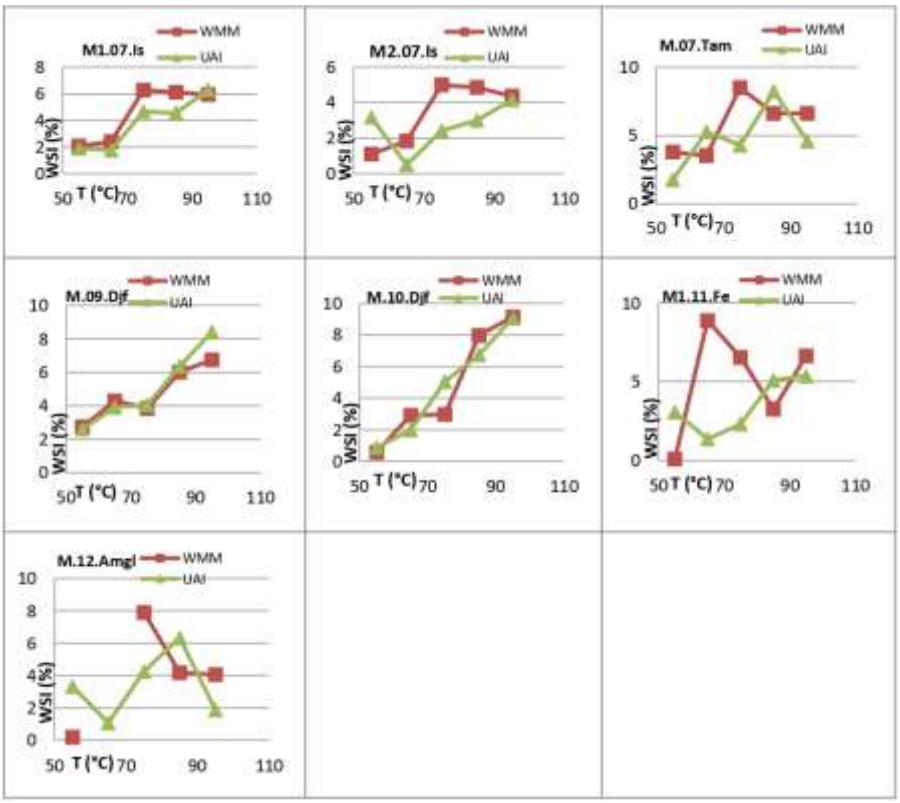

Figure 2. *Water solubility index of the isolated starches (WMM: Wet Milling Method , UAI: Ultrasound-Assisted Isolation)..*

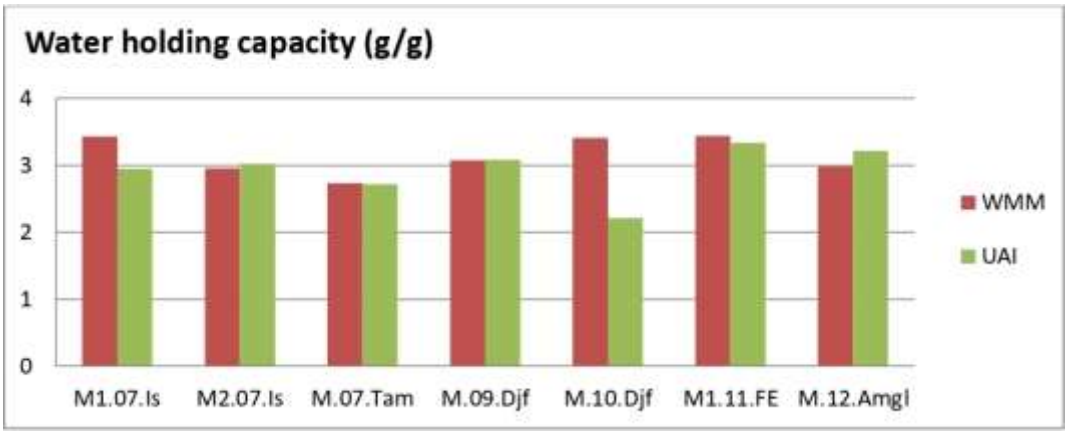

Figure 3. *Water holding capacity of the isolated starches (WMM: Wet Milling Method , UAI: Ultrasound-Assisted Isolation).*

Starch swelling power increased when it was heated in excess water (Figure 1), this reflects the ability of interacting with water molecules, the slight differences between the isolated starches might be due to the effect of protein and lipid content [38], the granules swelling could be related to the two stage relaxation of hydrogen bonding forces within the starch granules between the crystalline structure and the groups of amylose and amylopectin [7], Bangoura et al. [6] and Bhupender et al. [7] reported starch swelling power values that are within the range found in the present study. Starch water solubility index showed the highest values at 85 and 95°C (Figure 2), it is an indicator of the degree of molecule in starch granules dispersion after cooking [39], it could imply to the amount of amylose leaching out from the starch granule after the swelling, therefore the solubility increases the amylose leaching [7]. Starch water holding capacity is an important functionnal attribute of all flours andstarches used in food preparations [40], it varied in the ranges of 2.73 – 3.44, and of 2.21 – 3.33 g(H_2O)/g(starch) in the wet milling method and the ultrasound-assisted isolation respectively (Figure 3), this variation could be due to the difference in the engagement degree of hydroxyl groups to form hydrogen and covalent bonds between starch chains [27].

III.3.2. Starch iodine absorbance spectrum, blue value and light transmittance.

The blue value and the wavelength of maximum absorbance (λ_{max}) are given in table 5, ranges were nearly similar in both of wet milling method and ultrasound-assisted isolation, the wavelength of maximum absorbance was reported to relate to degree of polymerization and average chain length of amylose and amylopectin [28].

Table 5. *Blue value and wavelength of maximum absorbance (λ_{max}).*

Sample	Wet Milling Method		Ultrasound-Assisted Isolation	
	Blue Value	λ_{max} (nm)	Blue Value	λ_{max} (nm)
M1.07.Is	0.288	590	0.316	590
M2.07.Is	0.286	595	0.289	595
M.07.Tam	0.264	590	0.293	585
M.09.Djf	0.272	585	0.309	585
M.10.Djf	0.282	585	0.344	585
M1.11.Fe	0.302	590	0.330	585
M.12.Amgl	0.301	590-595-600	0.309	590-595

Light transmittance decreased with increase in storage duration (Table 6), the decrease rate was nearly similar for all the starches from both isolation methods, the difference may be due to the variation in amount of swollen granule remaining in the starches that refract light to different extent [29], light transmittance results reported by Bhupender et al. [7] which vary between 2.6 and 1.10 are within the range found in the present study 2.80 – 0.99.

Table 6. *Starch light transmittance.*

Sample	Wet Milling Method						Ultrasound-Assisted Isolation					
	0h	24h	48h	72h	96h	120h	0h	24h	48h	72h	96h	120h
M1.07.Is	2.46	2.18	1.94	1.67	1.27	1.18	2.19	1.94	1.56	1.47	1.32	1.25
M2.07.Is	3.01	2.59	1.67	1.58	1.04	0.96	2.11	2.05	1.72	1.50	1.31	1.22
M.07.Tam	2.17	1.91	1.85	1.65	1.60	1.28	1.93	1.89	1.45	1.30	1.10	1.03
M.09.Djf	2.53	2.44	1.81	1.71	1.40	1.33	2.40	1.85	1.70	1.54	1.27	1.19
M.10.Djf	2.69	2.63	2.02	1.75	1.70	1.25	2.23	2.22	1.47	1.42	1.10	0.99
M1.11.Fe	2.56	2.08	1.75	1.71	1.31	1.28	2.78	2.41	1.57	1.56	1.18	1.10
M.12.Amgl	2.80	2.30	1.78	1.73	1.41	1.31	2.32	2.27	2.07	1.81	1.88	1.54

III.4. Statistical analysis

The one way analysis of variance (ANOVA) didn't show any significant difference between the two isolation methods, univariate analysis results are given in tables 2, 3, and 4, the correlations matrix (Table 7) reported that isolation recovery, moisture and total starch content showed a significant correlation among themselves ($0.558 < r < 0.790$), but only total starch showed a significant negative correlation with the protein content ($r = -0.653$).

Table 7. *Correlations matrix.*

	Recovery	Moisture	TotalStarch	Protein	SP	WSI	WHC	BlueValue	LamdaMax	LightTransmit
Recovery	1.000									
Moisture	,790**	1.000								
TotalStarch	,577*	,558*	1.000							
Protein			-,653*	1.000						
SP					1.000					
WSI						1.000				
WHC							1.000			
BlueValue			,640*	-,647*				1.000		
LamdaMax						-,596*			1.000	
LightTransmit	-,568*	-,588*					,623*			1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

In the hierarchical clustering analysis (Figure 4), the obtained dendrogram allowed classifying the isolated starches into two distinct clusters at the 10 distance scale, the totality of starches from each isolation method accumulated in a distinct cluster, this is in accordance with the ANOVA results.

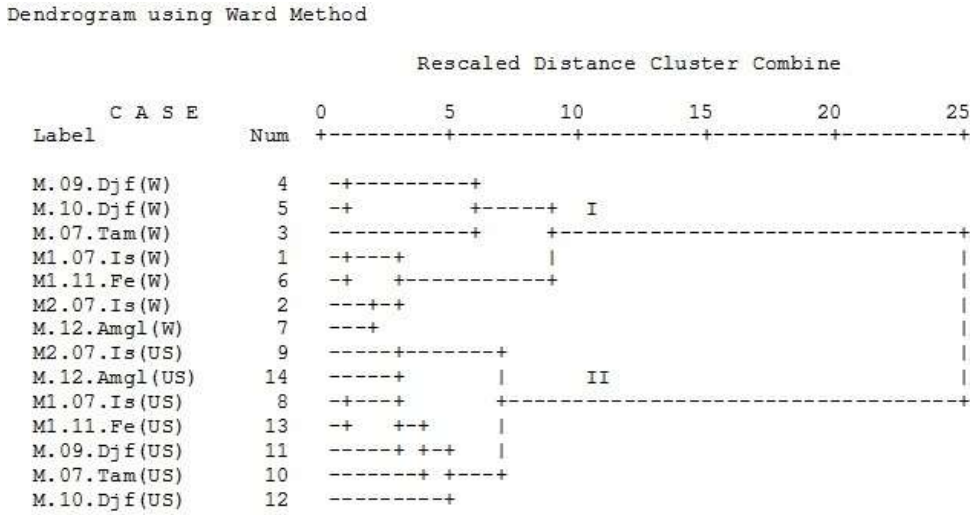

Figure 4. *Hierarchical clustering analysis dendrogram (W:Wet Milling Method, US: Ultrasound-Assisted Isolation)*

IV. Conclusion

The highest yield of the pearl millet grains starch isolation was obtained with the ultrasound-assisted isolation, the residual protein content in the wet milling method was higher, and this is an obvious indication that the ultrasound-assisted isolation starches were purer, on the basis of these results, it could be considered that:

- Large variation for grains phenotypic traits was observed in Algerian pearl millet landraces, probably due to environmental conditions.
- Pearl millet grains gave acceptable starch isolation yields, which could be useful especially in this country, where pearl millet could resurge as an important cereal crop.
- The protein layer removed during starch isolation could be easily recovered as value-added product such as pearl millet protein concentrates because no chemical were used.
- The ultrasound-assisted isolation eliminated the steeping and the chemicals use, therefore the cleaning steps were simplified and the waste water was significantly reduced.
- The isolation method affects the pearl millet starch properties.

The ultrasound-assisted isolation is an effective, fast and easy method to isolate starch from pearl millet, and it seems to better preserve the isolate properties, the use of ultrasound creates new and interesting methodologies which are often complementary to conventional techniques, reducing processing time and improving efficiency. These studies are expected to be useful in the production of starch from highly nutritious, low cost and underutilized pearl millet grains.

V. References

1. Zhu, F. Structure, physicochemical properties, and uses of millet starch-Review. *Food Research International* 64 (2014) 200-211.
2. Abdalla, A.A.; Ahmed, U.M.; Ahmed, A.R.; El Tinay, A.H.; Ibrahim, K.A. Physicochemical characterization of traditionally extracted pearl millet starch (Jir). J*ournal of Applied Sciences Research* 5(11) (2009) 2016-2027.
3. Bryden, W. Prospects to enhance Pearl Millet production and its uptake by the poultry industry. *Rural Industries Research And Development Corporation* (2013) publication number 12/135.
4. Pérez, S.; Bertoft, E. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Starke* 62 (2010) 389-420.
5. Zuo, Y.Y.J.; Hébraud, P.; Hemar, Y.; Ashokkumar, M. Quantification of high-power ultrasound induced damage on potato starch granules using light microscopy. *Ultrasonics Sonochemistry* 19 (2012) 421-426.
6. Bangoura, M.L.; Nsor-Atindana, J.; Ming, Z.H.; Wei, P.; Mothibe, K.J.; Xue, Z.K. Starch functional properties and resistant starch from Foxtail Millet [Setaria italica (L.) P. Beauv] species. *Pakistan Journal of Nutrition* 11(10) (2012) 821-830.
7. Bhupender, S.K.; Rajneesh, B.; Baljeet, S.Y. Physicochemical, functional, thermal, and pasting properties of starches isolated from pearl millet cultivars. *International Food Research Journal* 20(4) (2013) 1555-1561.
8. Baldwin, P.M. Starch granule-associated proteins and polypeptides: A Review. *Starch/Starke* 53 (2001) 475-503.
9. Wrolstad, R.E. Handbook of food analytical chemistry. Volume 1: water, protein, enzymes, lipids and carbohydrates. *Current protocols in Food Analytical Chemistry* (2001) E2.1.1-E2.1.6.
10. Jiang, C.; Li, X.; Jiao, Y.; Jiang, D.; Zhang, L.; Fan, B.; Zhang, Q. Optimization for ultrasound-assisted extraction of polysaccharides with antioxidant activity in vitro from the aerial root of Ficus microcarpa. *Carbohydrate Polymers* (2014) http://dx.doi.org/10.1016/j.carbpol.2014.03.027.

11. Balasubramanian, S.; Sharma, R.; Kaur, J.; Bhardwaj, N. Characterization of modified pearl millet (Pennisetum thyphoides) starch. *Journal of Food Science and Technology* (2011) DOI 10.1007/s13197-011-0490-1.
12. Chemat, F.; Huma, Z.E.; Khan, M.K. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry* 18 (2011) 813-835.
13. Chen, D.; Sharma, S.K.; Mudhoo, A. Handbook on applications of ultrasound, sonochemistry for sustainability. *Cereal Chemistry* (2014).
14. Vilkhu, K.; Mawson, R.; Simons, L.; Bates, D. Applications and opportunities for ultrasound assisted extraction in the food industry – A review. *Innovative Food Science and Emerging Technologies* 9 (2008) 161-169.
15. Sujka, M.; Jamroz, J. Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food Hydrocolloids* 31 (2013) 413-419.
16. Boudries, N.; Belhaneche, N.; Nadjemi, B.; Deroanne, C.; Mathlouthi, M.; Roger, B.; Sindic, M. Physicochemical and functional properties of starches from sorghum cultivated in the Sahara of Algeria. *Carbohydrates Polymers* 78 (2009) 475-480.
17. Djabali, D.; Belhaneche, N.; Nadjemi, B.; Dulong, V.; Picton, L. Relationship between potato starch isolation methods and kinetic parameters of hydrolysis by free and immobilised alpha-amylase on alginate (from Laminaria digitata algae). *Journal of Food Composition and Analysis* 22 (2009) 563-570.
18. Belhadi, B.; Djabali, D.; Souilah, R.; Yousfi, M.; Nadjemi, B.Three small-scale laboratory steeping and wet-milling procedures for isolation of starch from Sorghum grains cultivated in Sahara of Algeria. *Food and Bioproducts Processing* (2012) DOI: 10.1016/j.fbp.2012.09.008.
19. IBPGR, ICRISAT. Descripteurs du mil penicillaire [pennisetum glaucum (L.) R. Br.]. Conseil international des ressources phytogenetiques, Rome, Italie ; Institut International de Recherche sur les Cultures des Zones Tropicales Semi-arides, Patancheru, Inde, 1993.
20. Taylor, J.R.N.; Taylor, J. Five simple methods for the determination of sorghum grain end-use quality. Department of Food Science, University of Pretoria, South Africa, 2008.
21. Mwithiga, G.; Sifuna, M.M. Effect of moisture content on the physical properties of three varieties of sorghum seeds. *Journal of Food Engineering* 75 (2006) 480-486.
22. AACC, (2000). Approved Methods, 10th ed. American Association of Cereal Chemists, St. Paul.
23. Goni, I.; Garcia-Alonso, A.; Saura-Calixto, F. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research* 17(3) (1997) 427-437.
24. Souilah, R.; Djabali, D.; Belhadi, B.; Mokrane, H.; Boudries, N.; Nadjemi, N.In vitro starch digestion in sorghum flour from Algerian cultivars. *Food Science and Nutrition* (2014) DOI: 10.1002/fsn3.104.
25. Wang, L.; Wang, Y.J. Application of high-intensity ultrasound and surfactants in rice starch isolation. *Cereal Chemistry* 81(1) (2004) 140-144.
26. Li, J.Y.; Yeh**,** A.I. Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Food Engineering* 50 (2001) 141-148.
27. Mishra, S.; Rai, T. Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocolloids* 20 (2006) 557-566.
28. Shifeng, Y.; Ma, Y.; Menager, L.; Sun, D.W. Physicochemical properties of starch and flour from different rice cultivars. *Food and Bioprocess Technology* (2010) DOI: 10.1007/s11947-010-0330-8.
29. Singh, N.; Kaur, L.; Singh Sandhu, K.; Kaur, J.; Nishinari, K. Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloids*. xx (2005) 1-11.
30. Abdalla, A.A.; El Tinay, A.H.; Mohamed, B.E.; Abdalla, A.H. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. *Food Chemistry* 63(2) (1997) 243-246.
31. Wankhede, D.B.; Shehnaj, A.; Raghavendra Rao, M.R. Carbohydrates composition of finger millet (Eleusine coracana) and foxtail millet (Setaria italica). *Plant Food for Human Nutrition* 28(4) (1979) 293-303.
32. Krishna Kumari, S.; Thayumanvan, B. Content of starch and sugars and in vitro digestion of starch by alpha-amylase in five minor millets. *Plants Food for Human Nutrition* 53 (1995) 47-56.
33. Kim, S.K.; Sohn, E.Y.; Lee, I.J. Starch properties of native foxtail millet, Setaria italica Beauv. *Journal of Crop Science and Biotechnology* 12(1) (2009) 59-62.
34. Verma, V.C.; Kumar, A; Zaidi, M.G.H.; Verma, A.K.; Jaiswal, J.P.; Singh, D.K.; Singh, A.; Agrawal, S. Starch isolation from different cereals with variable Amylose/Amylopectin ratio and its morphological study using SEMand FT-IR. *International Journal of Current Microbiology and Applied Sciences* 7(10) (2018) 211-228.
35. Aruna, M; Parimalavalli, R. Properties of Pearl Millet: native and modified starch. *South-Asian Journal of Multidisciplinary Studies* 4(5) (2017) 31-44.
36. Beleia, A.; Varriano-Marston, E.; Hoseney, R.C. Characterization of starch from pearl millets. *Cereal Chemistry* 57(5) (1980) 300-303.
37. Wankhede, D.B.; Rathi, S.S.; Gunjal, B.B.; Patil, H.B.; Walde, S.G.; Rodge, A.B.; Sawate, A.R. Studies on isolation and characterization of starch from Pearl Millet (Pennisetum americanum (L.) Leeke) grains. *Carbohydrate Polymers* 13 (1990) 17-28.
38. Shewale, S.D.; Pandit, A.B. Enzymatic production of glucose from different qualities of grain sorghum and application of ultrasound to enhance the yield. *Carbohydrate Research* 344 (2009) 52-60.
39. Chanapamokkhot, H.; Thongngam, M. The chemical and physico-chemical properties of sorghum starch and flour. *Kasetsart Journal (Natural Science)* 41 (2007) 343-349.
40. Suma, F.P.; Urooj, A. Isolation and characterization of starch from Pearl Millet (Pennisetum typhoidium) flours. *International Journal of Food Properties* 18:12 (2015) 2675-2687.

Please cite this Article as:
Bouhallel S., Belhadi B., Souilah R., Djabali D., Nadjemi B., Isolation of starch from seven Pearl Millet grain landraces by two processes; wet milling and Ultrasound application, ***Algerian J. Env. Sc. Technology, 7*:1 (2021) 1687-1697**