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BUCKWHEAT STARCH (*Fagopyrum esculentum*): AQUEOUS EXTRACTION, MODIFICATION BY HMT AND CHARACTERIZATION

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ABSTRACT

Buckwheat (*Fagopyrum esculentum*) is a pseudoceral. It grains are nutritionally rich and boast great technological potential for use in the food industry in infant and backed food as well as ingredient of functional products. Flour is considered as an alternative to celiacs because it does not contain gluten. It is an option for the growing appeal for a healthy diet, as well as containing starch as a major component. Starches in native form have restricted use in the industry. So modifications are made to increase their application. These modifications can be chemical or physical. Physical modification is preferable and accepted by consumers. Heat-moisture treatment (HMT) consists of heating starch above gelatinization point with insufficient moisture (<35%). The main objective was extract buckwheat starch, modify by HMT and assess its physico-chemical, thermal and morphological properties. The starch granules from buckwheat have spherical or polygonal shape, with an average size around 1 – 7.5 µm. Morphology of buckwheat starch granules was not altered. The XRD technique showed no signifficative differences between main peaks in diffractograms however the relative crystallinity decrease. DSC analysis allowed to observe that according HMT the enthalpy decrease and gelatinization occurs in higher temperatures.

1.Introduction

Starches are the most abundant carbohydrate reserve in plants. They are found in fruits, seeds, leaves, steems and roots as water-insoluble granules with several shape (oval, spherical, lenticular, etc.) and size (1 up to more than 100 µm). Starches are made up to biopolymers called amylose and amylopectin. There are formed by α-D-glucose units. Amylose is a linear polymer with α-1,4 linked glucose units and amylopectin is an highly branched polymer with α-1,4 linked glucose and with α-1,6 linkages at the branched points (Smith, 2001; Andrade et al. 2014; Alcázar-Alay and Meireles, 2015).

The buckwheat (*Fagopyrum esculentum* Moench) plant is a crop that belongs to the *Polygonaceae* family. It is a pseudo-cereal usually grouped with cereals due the similarity in cultivation and utilization. It is called the common buckwheat starch and is the main species of buckwheat which has been widely consumed and used around the world (Hung et al., 2009; Liu et al., 2015).

The hydrothermal treatment or heat-moisture treatment (HMT) is a physical and safe method of modification of starch. It involves incubation of starch granules at low levels (less than 35% water, w/w) by a certain period of time (15 min to 16 hours) in temperatures higher than the gelatinization temperature (84 to 120 °C). The HMT is important due to alter some physicochemical properties of starch without

destroying its granular structure. It is considered a technique with low cost and used in several food products (Vieira and Sarmento, 2008; Zavareze and Dias, 2011; Sun et al., 2013; Bet et al, 2018). The changes in properties of starches treated by HMT vary according to the conditions employed as starch-to-moisture ratio, temperature and heating time. Regardless of the starch origin, HMT promotes an increase in the gelatinization temperature with widening the gelatinization temperature range (Chung et al, 2009; Zavareze and Dias, 2011; Moraes, Branzani and Franco, 2014).

The main objectives of this study were: the extraction of buckwheat starch by aqueous method and investigation of thermal and pasting properties as well as the morphology and structure of buckwheat starch granules.

2. Materials and methods

2.1. Materials

2.2.1.Samples

The buckwheat seeds of (*Fagopyrum esculentum*, Moench) were supplied by Protecta Co., Ltd., Ponta Grossa-PR-Brazil.

The extraction and treatment of buckwheat starch was performed in the Food Engineering Department Laboratory and analyses in the Multiusuary Laboratories of the State University of Ponta Grossa.

2.2.2.Starch Extraction by Aqueous Method

It was performed in agreement procedure described (Andrade et al, 2014; Barros et al., 2020). The buckwheat seeds were milled vigorously and so obtained the buckwheat flour. An aliquot of this flour was suspended in distilled water (ratio 3:1, water:flour, v/m). A consistent dough was formed and maintained in stirring by 30 minutes. After, this suspension was passed through sieves 200 and 325 mesh, respectively (0.075 mm and 0.043 mm). The suspension was centrifuged (Hettish routine 420R Zentrifugen, Germany) at 8500 rpm and 4ºC by 10 minutes. The precipitate was recovered and dried in an oven with forced air circulation at 40ºC by 24 hours. So, the starch obtained was kept in a desiccator containing anhydrous calcium chloride until analysis and/or modification.

2.2.3.Proximal Composition

This analysis was performed in triplicate according AOAC (2000), and were determined moisture, carbohydrate, protein, lipid and fiber.

2.2.4.Physical Modification of Starch (HMT)

After constant mass, the obtained starch was divided in four portions: the first, called (NAT) was the native or untreated sample. The other parts were treated after the moisture content determined in the proximal composition and TG/DTG. It was added distilled water with micropipette in such a way that samples reached the levels 15, 20 and 25 % of water and called samples (H15), (H20) and (H25). Each sample was homogenized with pistil and mortar to avoid points with higher concentration of water. After homogenized each sample was transferred to hermetically flasks and sealed. So, all samples were maintained by 24 hours. The modification by HMT was performed in an autoclave at 120 ºC for 1 hour (Bet et al., 2018; Barros et al., 2020).

2.2.5.Instrumental Analysis

2.2.5.1. Scanning Electron Microscopy-Field Emission Gun (SEM-FEG)

The morphology of starch granules was observed using a scanning electron microscope with field emission gun, model MIRA 3 (Czech Rep.). The voltage of the electron beam was 15 kV, generated by a tungsten lamp (Bet et al., 2018).

2.2.5.2. Color Analysis

It was used the colorimeter (Miniscan EZ 4500L, Reston, |USA) calibrated previously. The colour parameters, where L* gives us lightness L*=100 (white) and L*=0 (black); the a* value characterizes the redness region +a*= (red) and –a*= (green); the b* value indicates the color range from +b* (yellow) and –b* (blue) (Martins et al., 2020).

2.2.5.3. Pasting Properties (RVA)

The RVA instrument (Newport Scient., Australia) was used to evaluate the pasting properties. A dispersion of 8% of starch (dry basis) in 28 g of total mass. Each sample was heated to 95 ºC and cooled to 50 ºC cycle under

constant stirring (160 rpm) at a heating rate of 6 ^{o}C min^{-1} (Bet et al., 2018).

2.2.5.4. Differential Scanning Calorimetry (DSC)

The instrument DSC model Q-200 (TA Instr., USA) was previously calibrated and checked with standard Indium, purity 99.99%, m.p.= 156.6 ^{o}C, ΔH_m = 28.56 J g^{-1} was used to obtain DSC curves. A mass around 2.5 mg of each starch sample was weighed and mixed to proportional distilled water (≅ 10 μL). Each mixture was added to aluminum crucible and sealed with aluminum lid and so maintained during 60 min. The DSC analysis were performed as follows: air flow of 50 mL min^{-1}, heating rate of 10 ^{o}C min^{-1}, from 30 ^{o}C to 100 ^{o}C (Bet et al., 2018; Barros et al., 2020).

2.2.5.5. Thermogravimetry/Derivative Thermogravimetry (TG/DTG)

The TG/DTG curves were obtained using the TGA-50 microthermobalance (Shimadzu, Japan). Each mass sample was around 10 mg. It was used open α-alumina crucible and each sample was heated from room temperature to 650 ^{o}C. It was performed in air atmosphere with flow of 150 mL min^{-1}. The TG/DTG curves were obtained using TA-60-WS software and mass loss calculated (Bet et al., 2018; Martins et al., 2020).

2.2.5.6. X-Ray Diffractometry (XRD)

The X-ray diffractograms of each sample follow the methodology according Colman, Demiate, Schnitzler, 2014; Chen et al., 2015. The instrument model was Ultima 4 (Rigaku, Japan) under CuKα radiation, λ = 1.5418 Å, configured at 40 kV and 20 mA. The relative crystallinity (RC) was estimated. The diffraction pattern of each sample was determined by occurrence of peaks in angular range from 2 to 50 ^{o} in 2θ.

3.Results and discussions

3.1. Proximal Composition

The results of proximal composition of the native buckwheat starch are depicted in Table 1.

Table 1. Proximal composition of native buckwheat starch.

Native buckwheat starch	Content (%)
Carbohydrate	84.36
Moisture	9.97
Protein	2.09
Lipid	0.24
Fiber	0.61
Ash	2.73

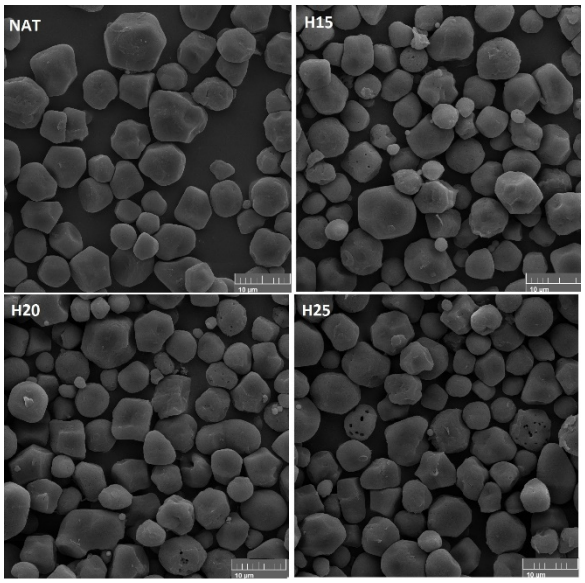
(*) The results are the triplicate average.

These results were determined by the AOAC (2000) methodology. Authors found values from water and ash content (calculated by TG/DTG) that were 10.6 and 2.4%, respectively.

Liu et al., found values from moisture 8,9%, and 2.1% of ash.

3.2. Scanning Electron Microscopy-Field Emission Gun (SEM-FEG)

The microimages of buckwheat starch granules obtained by SEM-FEG are shown in Figure 1. It can be observed that buckwheat starch granules has a bimodal shape (spheric and polygonal) which was not altered after applied modifications. The average diameter of granules was from 1.19 to 7.54 μm. Some cavities appeared on the surfaces, mainly in those that were treated with major moisture content. In the same way, fissures and holes can be observed. In ageement with literature (Liu et al., 2015), the results showed that the effect of HMT on the morphologic structure was moisture dependent. According Watcharatewinkul et al., 2009 and Liu et al, 2015., the cavities and holes were due to recombination of amylose and amylopectin chains. This recombination is result from the termal force given by HMT and contribute to more compact amorphous regions.



F**igure 1.** MEV-FEG microimages of buckwheat starch granules. (magnification 2kx)

3.3. Color Analysis Pasting Properties (RVA)

The color is a criterion for assess starch quality. The obtained values of L*, a* and b* are shown in Table 2.

Table 2. Color Analysis

Sample	L*	a*	b*
NAT	91.61±0.15	0.49±0.03	4.62±0.09
H 15	86.45±0.27	2.75±0.02	8.69±0.14
H 20	85.52±0.08	3.34±0.05	9.3±0.06
H 25	84.24±0.07	3.63±0.02	9.99±0.05

Results presented as mean±standard deviation

According to Sira and Amaiz, 2004, the pure starch present the L* value higher than 90. In this work the found value was 91.61, which decrease according the treatment performed. The a* and b* values increase with increasing amount of water.

In Figure 2, are depicted the pasting obtained after gelatinization of samples.

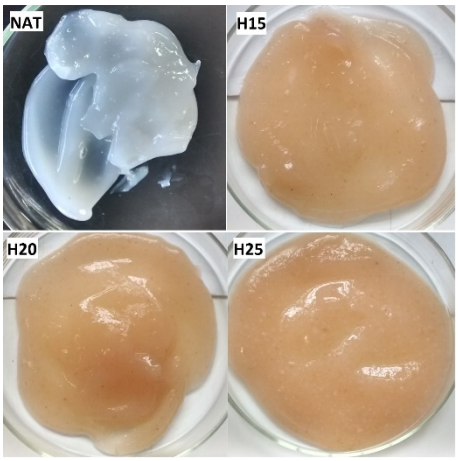


Figure 2. Gelatinized starches: (NAT) native and (H15, H20 and H25) modified starches

It can be observed alterations in color after gelatinization process, which values are in Table 2. The L* value show a decrease in brightness after HMT. The results show that the cromaticity a* and b* were significantly altered.

3.4.Pasting Properties (RVA)

A viscous paste of untreated and modified starches is obtained during heating of granules under excess of water, due to hydration and swelling. An important property, the viscosity, is the main factor for applicability in food processing. The pasting parameters obtained for native and HMT starches are summarized in Table 3.

Higher pasting temperature was presented by starch with 25% water (H25).

The breakdown inicially increase when compared with native starch and show considerably decrease according HMT.

The retrogradation show considerable decrease according were treated the samples.

As reported by Collado and Corke, 1999, hydrothermally treated starches could be utilized in infant and backed foods.

3.5.Thermal Analysis

3.5.1.Thermogravimetry/Derivative Thermogravimetry (TG/DTG)

The obtained TG/DTG curves are shown in Figure 3. There were performed in air atmosphere and heated from room temperature to 650ºC. All curves display mass loss in three steps: the first due to dehydration followed by a stability. After, the second and third mass losses are due to oxidation and decomposition of organic matter with formation of ash as final product (Colman et al., 2014; Lacerda et al., 2015).

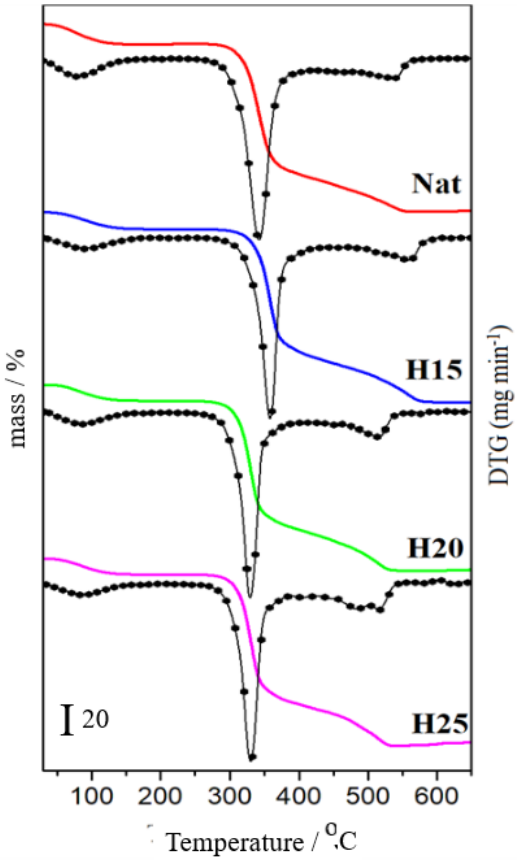


Figure 3. TG/DTG curves

The obtained results of thermal decomposition of native and modified starches are summarized in Table 4.

The ash content after thermal decomposition of untreated and modified starches were 2.4; 1.6; 3.6 and 3.0 %, respectively.

3.5.2.Differential Scanning Calorimetry (DSC)

The DSC technique was used to determine the gelatinization parameters of buckwheat starches. The curves are depicted in Figure 4 and values of T_o, T_p, T_c and enthalpy in Table 5.

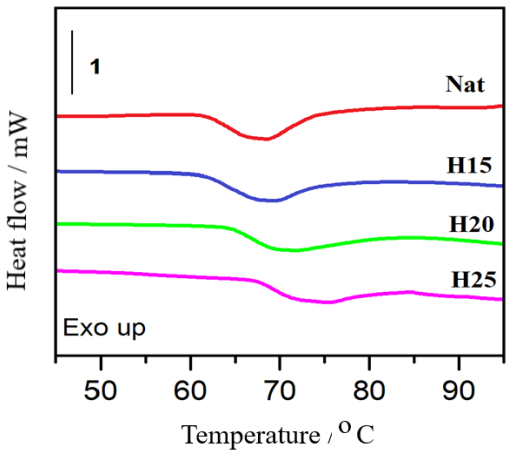


Figure 4. DSC curves of native and modified buckwheat starch

According to literature (Gunaratne and Hoover, 2002; Zavareze and Dias, 2011), these effects are dependent on the moisture level of the treatment, the starch source and the amylose content.

In this work, we observe a displacement of peak temperatures (T_p) to higher values as well as an enlargement in peaks between T_o and T_c.

3.5.3. X-ray difractometry (XRD)

According to literature (Andrade et al., 2014), the main differences in crystallinity between starches can be attributed to some factors: the crystal size, the number of crystalline regions that are influenced by amylopectin content and chain length, the orientation of double helices within the crystalline area and the extent of interactions between the double helices. Other factors that can be considered are the HMT conditions and the starch source.

In Figure 5 are the diffractograms of each sample from 2-50º in 2θ.

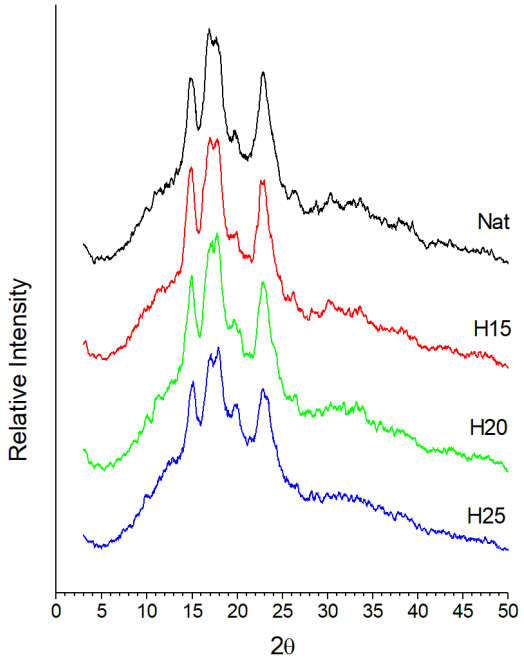


Figure 5. X-ray Diffractograms of of native and modified buckwheat starch

No significative displacements occurs between the main peaks. The relative crystallinity was calculated according Colman et al., 2014.

Table 6. Main results obtained by XRD

Sample	Relative Crystallinity (%)
Nat	21.04 ± 0.45[a]
H15	21.90 ± 0.55[a]
H20	19.56 ± 0.30[b]
H25	16.20 ± 0.21[c]

Averages followed by the same letters in the same column do not differ statistically by Tukey's test (P<0.05).

Table 3. Viscoamylographic (RVA) values of native and modified buckwheat starches

Sample	Pasting Temperature/ °C	Peak Viscosity/ mPa s^{-1}	Retrogradation/ mPa s^{-1}	Breakdown/ mPa s^{-1}	Final Viscosity/ mPa s^{-1}	Peak Time/ s
NAT	83.7	1899	1366	286	2969	600
H15	81.5	2657	1080	703	3034	544
H20	84.4	2384	628	530	2482	552
H25	90.0	1060	63	17	1106	643

Table 4. Results of TG/DTG curves.

Sample	Step	1st mass loss	Stability	2nd mass loss	3rd mass loss
NAT	Ti – Tf/°C	30-160	160-272	272-421	421-573
	Tp/°C	83		340	497
	Δ m/%	10.6		69.7	17.3
H15	Ti – Tf/°C	30-156	156-254	254-403	403-561
	Tp/°C	87		330	488
	Δ m/%	8.7		67.9	21.8
H20	Ti – Tf/°C	30-166	166-256	256-405	405-553
	Tp/°C	89		328	486
	Δ m/%	8.1		67.1	21.2
H25	Ti – Tf/°C	30-151	151-262	262-394	394-550
	Tp/°C	87		329	485
	Δ m/%	8.2		66.6	22.2

Table 5. DSC results of gelatinization of starches

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH_{gel} (J g^{-1})
NAT	61.8±0.1^d	68.6±0.0^c	74.0±0.3^d	12.2±0.2^d	6.2±0.2^b
H15	62.0±0.0^c	68.5±0.0^c	75.5±0.1^c	13.5±0.0^c	5.5±0.0^c
H20	63.6±0.0^b	70.8±0.2^b	81.3±0.4^b	17.7±0.2^a	7.1±0.2^a
H25	65.4±0.0^a	74.4±0.0^a	83.8±0.1^a	18.4±0.1^b	5.5±0.1^c

Averages followed by the same letters in the same column do not differ statistically by Tukey's test ($P<0.05$).

T_o = onset temperature; T_p = peak temperature; T_c = conclusion temperature; ΔT = difference between T_c and T_o; ΔH_{gel} = gelatinization enthalpy.

4. Conclusions

The buckwheat starch was extracted by aqueous process in a satisfactory manner. The heat-moisture treatment (HMT) of starches is an effective technology for change their pasting properties. The TG/DTG curves show similar profile, however it was possible to stablish the main steps of mass loss as well as the stability. Color analysis show proportional darkening according increase the moisture content. SEM-FEG images allowed to observe the spheric and polygonal shape of buckwheat starch with average diameter between 1.19 to 7.54 μm. The DSC analysis show displacement of gelatinization to higher temperatures with decrease of enthalpy. The viscosity of starch is an important fator for applicability to food processing. The HMT of buckwheat starch suggest application in infant and baked foods.

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