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CARACTERIZAÇÃO ESTRUTURAL, TÉRMICA E MORFOLÓGICA DO GRÃO DE BICO (CICER ARIETINUM L.) SUBMETIDO À HIDRATAÇÃO

STRUCTURAL, THERMAL, AND MORPHOLOGICAL CHARACTERIZATION OF CHICKPEA (CICER ARIETINUM L.) SUBMITTED TO THE HYDRATION PROCESS

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ABSTRACT

Chickpea is a legume of great added value, and the hydration process is an essential step in its processing and improvement of cooking and consumption characteristics. In the present work, chickpeas were characterized in natura and hydrated at different temperatures to verify the structural and morphological changes caused by the hydration process. The in natura grains were subjected to hydration for 4 hours at temperatures of 50°C, 60°C, 70°C, and 80°C. Then, they were characterized for crystallinity (XRD), thermal stability (TG and DTG), and morphology (SEM). The results of the characterization by X-ray diffraction allowed us to verify that the samples of *in-natura* and hydrated grains presented diffractograms characteristic of legumes, with standard C starch, and percentages of crystallinities that reduced their values with increasing hydration temperature. The results of the characterization by thermogravimetric analysis (TGA) and its derivative (DTG) provided the temperature values at which the phenomenon of dehydration occurs, as well as the degradation events of the polysaccharides present in the different samples of *in-natura* and hydrated chickpeas. While the micrographs (SEM) identified that the increase in hydration temperature caused the gelatinization of the starch present in the chickpeas, making the grain structures with amorphous characteristics.

Palavras-chave: Starch; gelatinization; polysaccharides.

RESUMO

No presente trabalho teve por objetivos realizar a caracterização do grão-de-bico *in-natura* e hidratado em diferentes temperaturas com intuito de verificar as alterações provocadas pelo processo de hidratação nos grãos de bico. Os grãos de bico *in-natura* foram submetidos à hidratação durante 4 horas nas temperaturas de 50°C, 60°C, 70°C e 80°C. Em seguida, foram caracterizados quanto à cristalinidade (DRX), estabilidade térmica (TG e DTG) e morfologia (MEV). Os resultados da caracterização por difração de raios-X permitiram verificar que as amostras dos grãos *in-natura* e hidratados apresentaram difratogramas característicos das leguminosas, amido de padrão C, com percentuais de cristalinidades que reduziram os seus valores com o aumento da temperatura de hidratação. Já os resultados da caracterização por análise termogravimétrica (TG) e sua derivada (DTG) forneceram os valores de temperatura nos quais ocorrem o fenômeno de desidratação e os eventos de degradação dos polissacarídeos presente nas diferentes amostras do grão-de-bico *in-natura* e hidratado. Enquanto as micrografias (MEV) identificaram que o aumento da temperatura de hidratação provocou a gelatinização do amido presente no grão de bico, tornando as estruturas dos grãos com características amorfas.

Keywords: Starch; gelatinization; polysaccharides.

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INTRODUCTION

Chickpea (*Cicer arietinum L.*) is a legume considered a food of great nutritional potential to be explored. Considering that it is a good source of fiber, vitamins and minerals, it can minimize protein and mineral deficiencies in its consumers. It is an excellent source of carbohydrates and proteins that comprise about 80% of the total weight of dried seeds. Standing out as a food legume with the second most important crop in the world, behind only soybeans (OLIVEIRA et al., 2009; ONU, 2020).

This hydration technique depends on time, temperature and concentration of soluble solids. The amount of water generated increases with increasing temperature and hydration time (FRACASSO et al., 2014, JOHNNY et al. 2015). However, hydration at temperatures greater than 60ºC results in loss of total solids, nitrogenous compounds, sugar, oligosaccharides, minerals and vitamins (MALI et al, 2010, MARQUES et al. 2014).

The hydration process in grains is present in the characterization of physiological quality, in the extraction of the constituent of interest, in cooking, in the reduction or elimination of anti-nutritional factors existing in the grains, and in the improvement of digestibility (FRACASSO et al., 2014; SHAFAEI et al., 2016). This technique depends on time, temperature, and concentration of soluble solids. The amount of water absorbed increases with increasing temperature and hydration time (MARQUES et al., 2014).

In studies by Johnny et al. (2015) evaluated the effect of temperature and hydration time of 360 minutes on the physical properties (main dimensions, average diameters, sphericity, area, density and electrical conductivity) of chickpeas. The results revealed that these physical properties were affected by water absorption especially at temperatures above the gelatinization temperature (45°C).

Ferreira et al. (2006) observed that the bioavailability of iron in the legume increases with its cooking and hydration. Furthermore, Benevides et al. (2015) highlight that hydration is a widely used method for reducing and/or inactivating undesirable substances in foods. Although studies show the efficiency of heat treatment in reducing nutritional factors, this process has some disadvantages, such as the loss of essential nutrients. Other methods used in food processing can also reduce the concentrations of anti-nutritional factors, such as the addition of water to the food, maceration in the presence of sulfites, grinding, stripping of grains, controlled atmosphere (N2, ethylene, and absolute ethanol), treatment enzyme, high hydrostatic pressure, among others.

According to Lamberts et al. (2009), the crystalline regions of the granules provide specific X-ray diffraction patterns, defined based on the interplanar spaces and the relative intensity of the diffraction lines, which vary according to the botanical source of the granule. Pattern A is characteristic of cereal starches, pattern B of tuber starches, and pattern C is intermediate between A and B, characteristic of legume starch.

The main commercial sources of starch are corn, potatoes, rice, wheat, and cassava (MALI et al., 2010), however, among other promising sources for obtaining starch are chickpeas with a percentage of iron and protein superior to that of carioca beans, legume most consumed by Brazilians (NASCIMENTO et al., 2016).

Several studies were carried out using X-ray diffraction analysis to identify the type of crystallinity for granules from different starch sources. These crystallinity patterns depend, in part, on the length of the amylopectin chains, the packing density within the granules, as well as the presence of water (CAPPA et al., 2016; DUTTA et al., 2015; MARTINS et al., 2020).

The thermogravimetric analysis (TGA) allowed the determination of the temperature values where the phenomenon of dehydration and the degradation events of the polysaccharides present in the different sources of starch occur (LIMA et al., 2012; PIGŁOWSKA et al., 2020). Scanning electron microscopy (SEM) analysis has also been explored in recent research on different sources of starch to obtain images referring to the surfaces of the particles of starch granules (ARNS et al., 2015; CAPPA et al., 2016; MARTINS et al., 2020).

In this context, the characterization of *in-natura* and hydrated chickpeas was carried out at different temperatures from 50°C to 80°C, evaluating their crystallinity, thermal stability, and micrography.

MATERIAL AND METHOD

The research was carried out at the Laboratory of Plant Physiology, belonging to the Academic Unit of Agronomy of the Federal University of Campina Grande, UAGRA, CCTA, UFCG. Analyzes for structural evaluation of chickpeas by X-ray diffraction and analyses to determine thermal stability through a thermogravimetric analysis were carried out at the

Nanotechnology Laboratory belonging to the Northeast Strategic Technologies Center – CETENE. The morphological analyzes by scanning electron microscopy (SEM) were performed at the Food Engineering Laboratories of the Federal University of Campina Grande, CTRN, UFCG.

Feedstock

Chickpeas acquired in the city of Pombal, hinterland region of the state of Paraiba, Brazil, were used for the tests. Being the sample field of 1kg marketed for cultivation.

Initially, a manual pre-benefiting of the material was carried out to eliminate defective grains. Then, the material was divided into equal 50 g samples stored in the absence of light at room temperature and packaged in the quantities necessary for each experiment. Weightings were performed on a Bel Engineering® digital analytical scale with a precision of 0.001g.

The initial water content of the chickpea samples was determined by the standard oven method at 105°C±1°C, for a period of 24 hours, using five subsamples of 10 g of chickpeas, according to the Rules of Analysis of Seeds (Brasil. Ministério da Agricultura, 2009). The water content was calculated on a wet basis (Xbu) applying Equation (1):

$$X_{bu} = \frac{(M_i - M_f)}{(M_f - t)} \cdot 100$$

Where:
Mi is the initial mass, the mass of the crucible plus the mass of wet beans;
Mf is the final mass, crucible mass, and dry seed mass;
t is the tare, mass of the empty crucible.

The moisture content on a wet basis was transformed into a dry basis (Xbs) by Equation (2)

$$X_{bs} = \frac{(X_{bu})}{(100 - X_{bu})} \cdot 100$$

The hydration process was studied using 50 g of chickpeas immersed in 250 mL of distilled water inside a 500 mL Becker, placed inside a Quimis® thermostatic bath for a period of 4 hours, with three repetitions for temperatures. The temperatures of 50 °C, 60 °C, 70 °C, and 80 °C were those in which the hydration of chickpeas was evaluated.

After the hydration period, the chickpeas samples were dried in a Marqlabor® oven with air circulation at 50°C for 24 hours until reaching a water content low than 10% on a wet basis.

The chickpea powder samples submitted to crystallinity analysis (XRD) and thermal analysis (TG/DTG) were macerated in a pistil and subjected to sieving in 25 mesh sieves. On the other hand, the samples of chickpeas submitted for morphological analysis were analyzed in their grains.

X-ray diffraction analysis

The X-ray diffractograms of the chickpea samples were obtained in an X-ray Diffractometer – Bruker model D2 Phaser, operated with Cu Kα tube radiation, with a voltage of 40 kV and a current of 40 mA with a filter of nickel, the scan rate of 1°.min^{-1} at room temperature. The diffraction scan range was adjusted to angles from 3° to 70° (2θ).

The calculations of the index or degree of crystallinity of *in-natura* chickpeas were determined using the method used by Hulleman et al (1999), through Equation 3:

$$I_{cr} = \frac{H_c}{H_c + H_a}$$

Where, Icr is the crystallinity index; Ha is the height corresponding to the amorphous phase concerning the baseline and Hc is the height corresponding to the crystalline peak.

Thermogravimetric analysis (TGA/DTG)

The TGA/DTG analyzes were performed in a TGA/DTG simultaneous thermogravimetric analyzer, model Netzsch STA 449F3 - Jupiter. Approximately 15 mg of grain sample in N_2 atmosphere, the flow of 20 mL.min^{-1} and in Al_2O_3 sample port, with a range from 40 °C to 600 °C and heating rate of 10 °C.min^{-1}

Scanning Electron Microscopy Analysis (SEM)

The images of the chickpeas were obtained by scanning electron microscopy at 1000x magnification, performed in a scanning electron microscope, model Tescan Vega 3 and voltage 10kV. One grain of each sample was placed on carbon adhesive tape and observed in SEM.

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RESULTS AND DISCUSSION

X-ray Diffraction Analysis (XRD)

Figures 1 contain the X-ray diffractograms corresponding to the samples of *in-natura* (A) and hydrated at temperatures of 50°C (B), 60°C (C), 70°C (D), and 80°C (E).

The crystallinity pattern contained in Figures 1 (A-E) showed peaks of greater intensity at 15°; 17° and 22° for *in-natura* chickpeas and chickpeas hydrated at different temperatures (50°C to 80°C). According to Lima et al. (2012), the type A pattern presents peaks of greater intensity in 2θ of 15, 17, 18, and 23, and the type B in 5.6; 15, 17, 22, and 23(LIMA et al., 2012). Oliveira et. al (2009) reported a type C diffraction pattern for chickpeas, with peaks corresponding to the 2θ angle between 14-16°, 16-17.5°, 17.5-19°, and 21.5- 25th (OLIVEIRA at al., 2009). Subpatterns Ca and Cb are also designated depending on the proximity of the C pattern to types A and B. Thus, it can be said that the chickpea samples analyzed to follow the type C crystallinity pattern, common for legumes. It can be said that the chickpea samples analyzed also resembled type A characteristics, so the chickpea samples contain characteristics of the C-a pattern, as can be seen in the diffractogram patterns in Figure 1.

Figure 1. X-ray diffractograms of *in-natura* chickpeas (A) and hydrated chickpeas at 50 °C (B), 60 °C (C), 70 °C (D), and 80 °C (E)

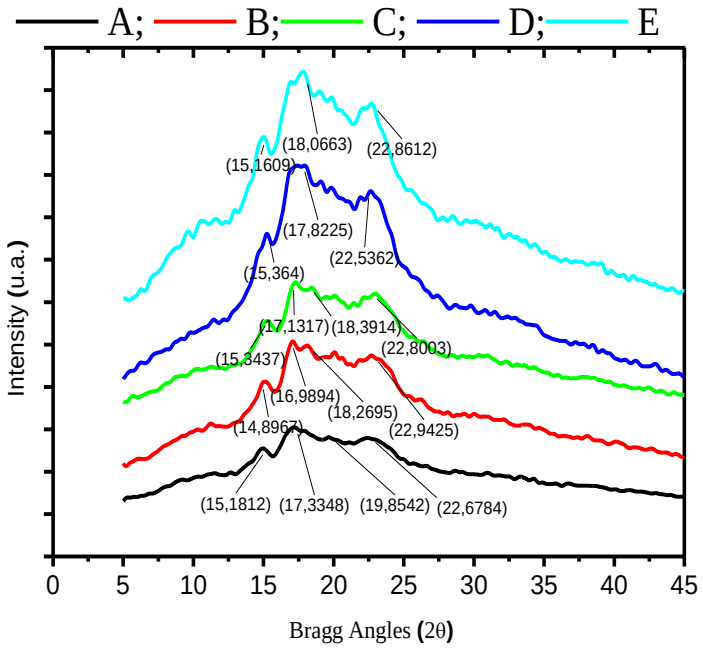

Table 1 shows the crystallinity calculated for *in-natura* and hydrated chickpeas at temperatures from 50°C to 80°C. According to Lima & Andrade (2010), the crystallinity of starches is proportional to their composition, regular starches are composed of 20 to 30% of amylose and 70 to 80% of amylopectin. The crystallinity observed in the present study is between 37.12 and 64.33% of amylopectin, values below the crystallinity standards for regular starches, which must be between 70% and 80%. This result indicates that the hydration process at temperatures above 60°C considerably reduces the crystallinity of the grains, evidencing rupture of the amylose and amylopectin granules, since chickpeas hydrated above 60°C have a structure with amorphous characteristics.

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Table 1. The crystallinity of chickpea samples

Chickpea samples	Crystallinity (%)
In-natura	64,33
Hydrated at 50°C	58,87
Hydrated at 60°C	58,04
Hydrated at 70°C	52,54
Hydrated at 80°C	37,12

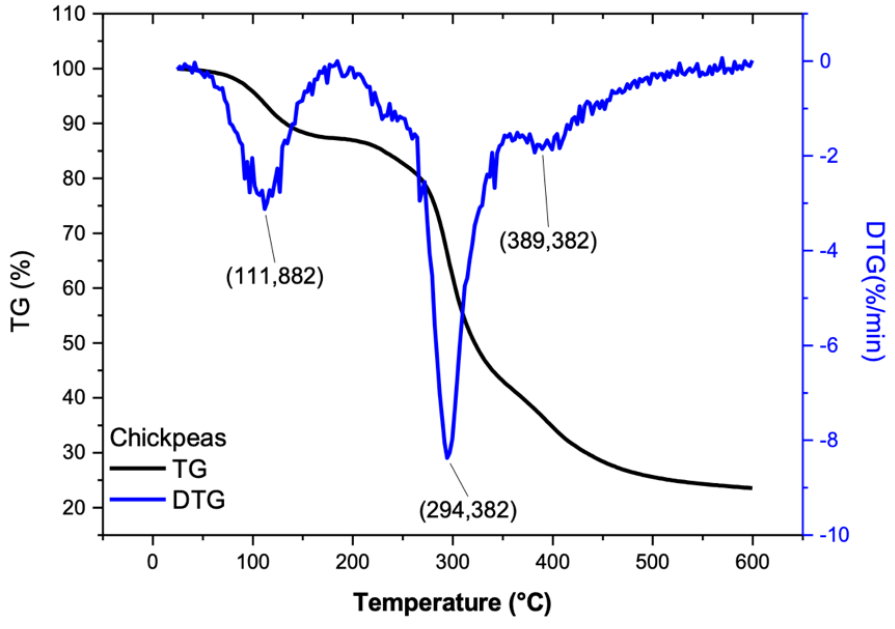
Thermogravimetric analysis

Figure 2 (A-E) contains the TGA/DTG curves for samples of *in-natura* chickpeas (A) and hydrated at temperatures of 50°C (B), 60°C (C), 70°C (D), and 80°C (E).

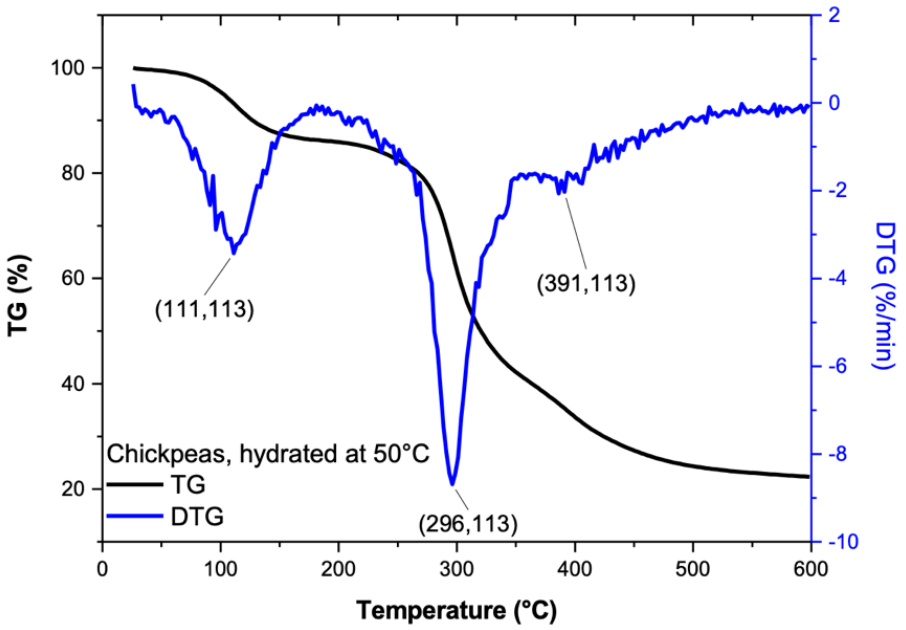
From the analysis of mass loss (TGA) and its derivative (DTG), it can be observed that *in-natura* and hydrated chickpeas at temperatures of 50 °C, 60 °C, 70 °C, and 80 °C show three thermal events. It is observed that the first thermal event, the one corresponding to the elimination of water molecules, occurred in the temperature range of approximately 99.321 °C to 111.882 °C. In the main thermal event occurs the elimination of polyhydroxyl groups, decomposition, and depolymerization of polymeric chains starch present in *in-natura* and hydrated chickpeas in the temperature range of 293.622 °C to 296.821 °C. Volatile gases, mainly bound water, carbon monoxide, and carbon dioxide are eliminated in the temperature range of 383.622 °C to 396.881 °C for *in-natura* and hydrated samples at temperatures from 50 °C to 80 °C.

Unlike the studies by Lima et al. (2012) the polysaccharide degradation event occurs by depolymerization when the applied temperature is lower than 300 °C. Caused by the hydration process of chickpeas.

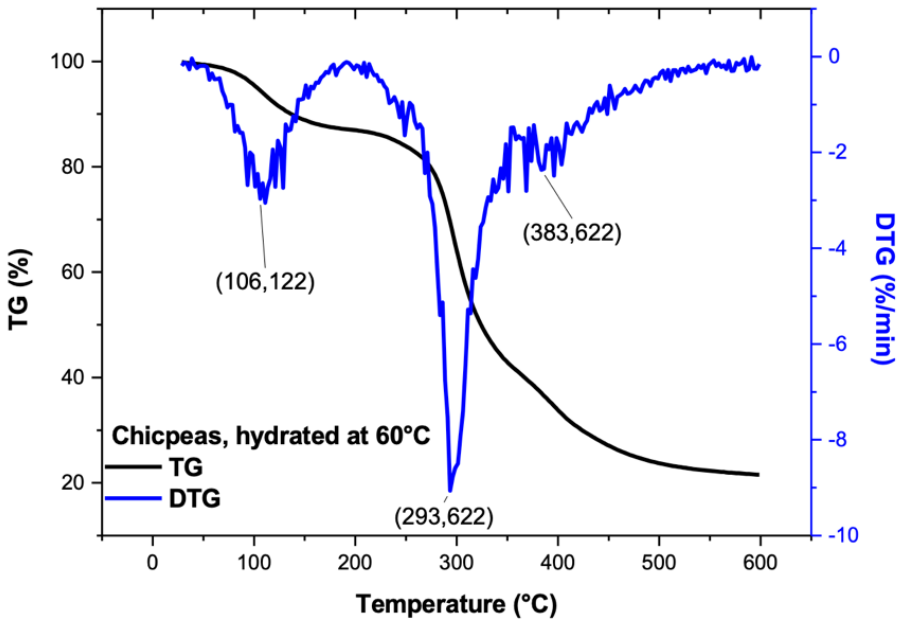
Figure 2. Thermograms for *in-natura* chickpea samples (A); and hydrated at 50°C (B), 60°C (C); 70°C (D); and 80°C (E).



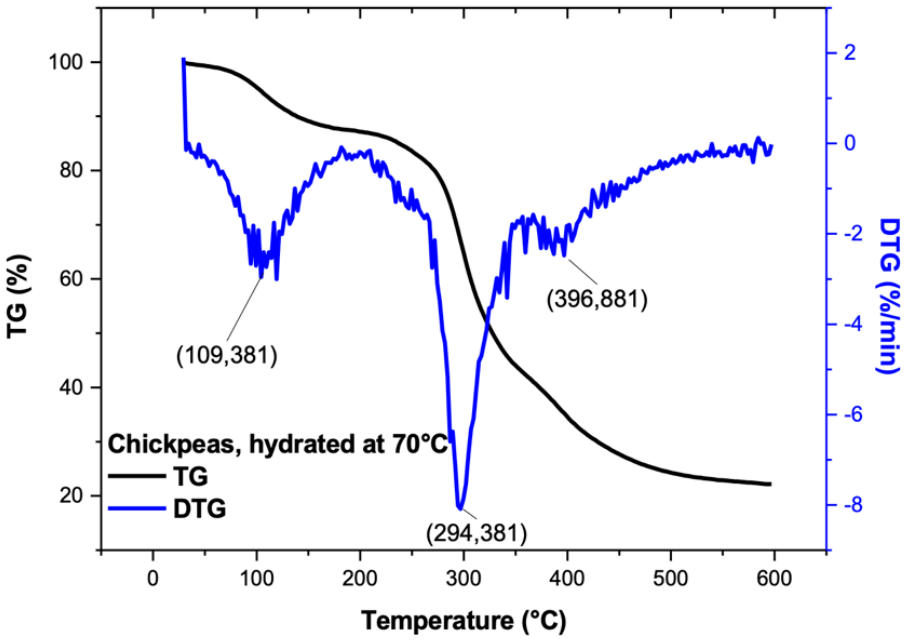
(**A**)


TG (%)
DTG (%/min)
Temperature (°C)
Chickpeas, hydrated at 50°C
TG
DTG
(111,113)
(391,113)
(296,113)

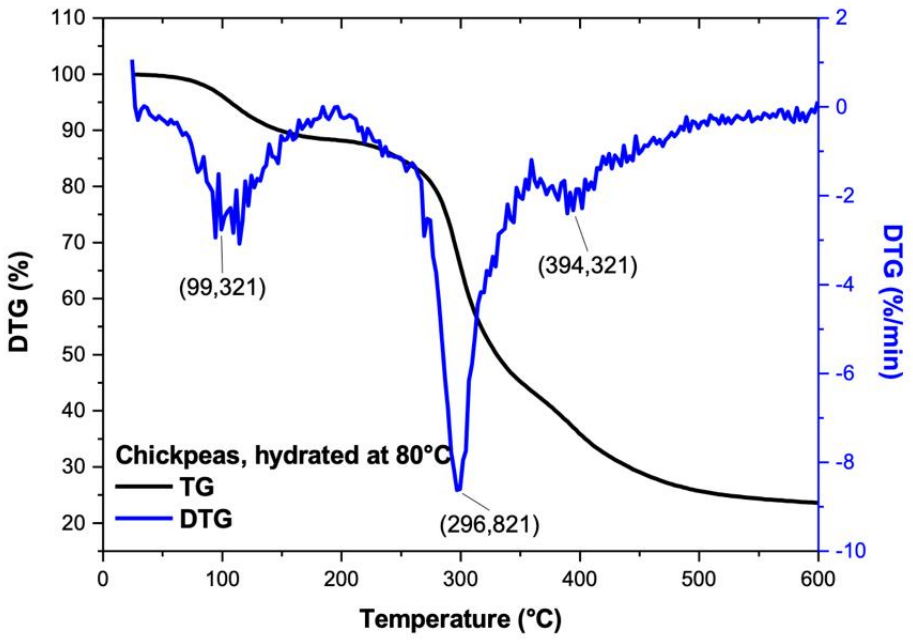
(B)


TG (%)
DTG (%/min)
Temperature (°C)
Chicpeas, hydrated at 60°C
TG
DTG
(106,122)
(383,622)
(293,622)

(C)


TG (%)
DTG (%/min)
Temperature (°C)
Chickpeas, hydrated at 70°C
TG
DTG
(109,381)
(294,381)
(396,881)

(D)

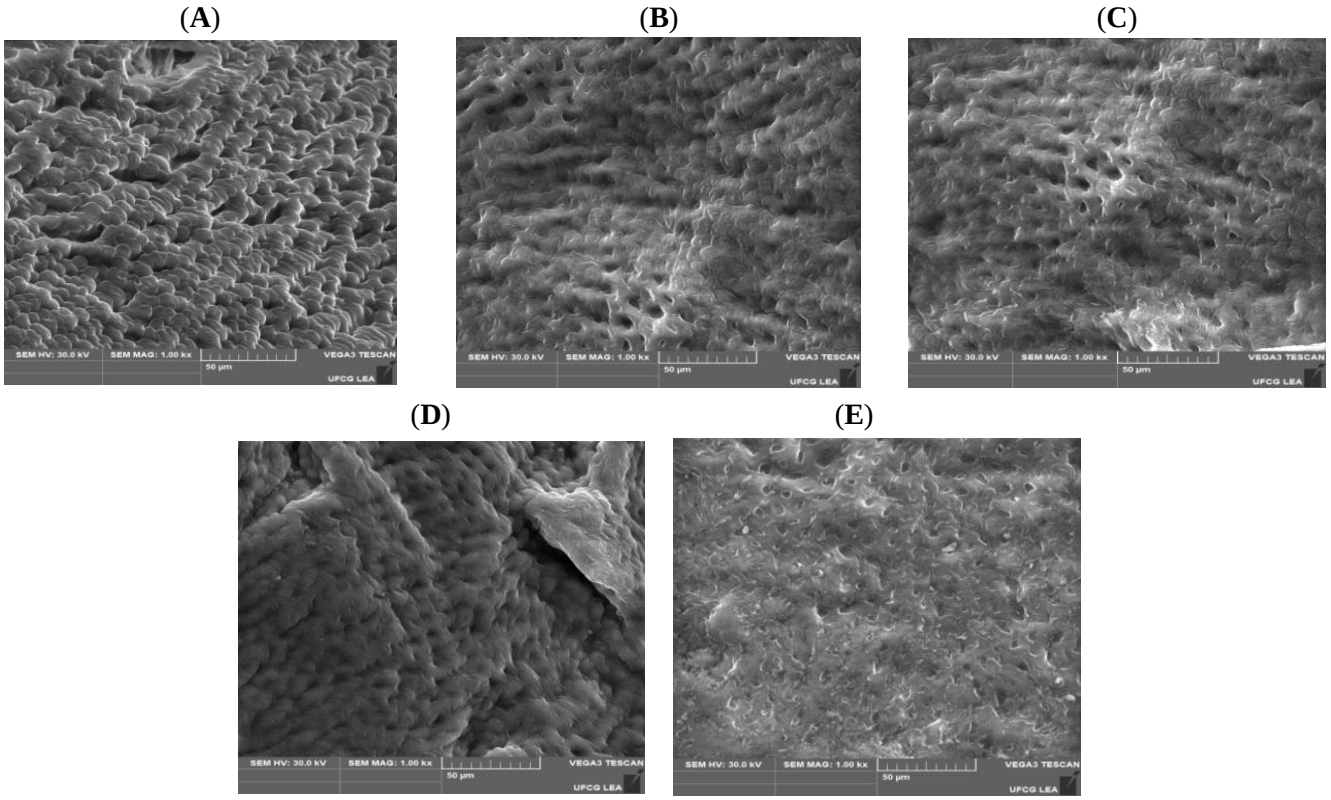

DTG (%)
DTG (%/min)
Temperature (°C)
Chickpeas, hydrated at 80°C
TG
DTG
(99,321)
(296,821)
(394,321)

(E)

Scanning Electron Microscopy (SEM)

Figures 3 (A-E) contain surface micrographs of *in-natura* and hydrated chickpeas at temperatures of 50°C, 60°C, 70°C, and 80°C. The analyzes show that the hydration of chickpeas at temperatures of 50°C, 60°C, 70°C, and 80°C compared to *in-natura* promoted changes in their morphological structure. This characterizes a condition with reduced crystallinity since in the hydration process there is a rupture of the molecules and starch complexes, consequently, adopting a random orientation, allowing cracks and intermolecular spaces in the grains to be filled, and consequently allowing a solid structure and high degree of compression. This modification of the crystal structure pattern to an amorphous structure with the presence of starch gelatinization was also obtained in the studies by Wu et al. (2010) and Martins et al. (2020)(MARTINS et al., 2020; WU et al., 2010).

Figure 3. Microscopic analysis of chickpeas: *in natura* (A); hydrated at 50°C (B); hydrated at 60°C (C); hydrated at 70°C (D); hydrated at 80°C (E).



CONCLUSION

The characterization of *in-natura* and hydrated chickpeas at temperatures of 50°C and 60°C by the XRD technique allowed us to observe an intermediate pattern of crystallinity (type C), typical of legumes, but with a clear tendency to type A (structure with crystalline features). However, it was observed that as the hydration occurred at temperatures of 70°C and 80°C, a reduction of the crystalline was observed, as well as a tendency towards a structure with amorphous characteristics. Thermogravimetric analysis (TGA) and its derivative (DTG) allowed the determination of the temperature of degradation of thermal events. These analyzes provided the temperature values at which the phenomenon of dehydration and the degradation events of the polysaccharides present in the different samples of *in-natura* and hydrated chickpeas occur. Finally, the scanning electron microscopy (SEM) analysis showed changes in the morphological structure of samples of

chickpeas hydrated at temperatures above 60°C, confirmed by obtaining a solid structure with a high degree of compaction, typical of amorphous structures.

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