International Journal of Biological Macromolecules 129 (2019) 460–470

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Irradiation of mung beans (*Vigna radiata*): A prospective study correlating the properties of starch and grains

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ARTICLE INFO

Article history:
Received 16 October 2018
Received in revised form 4 January 2019
Accepted 16 January 2019
Available online 11 February 2019

Keywords:
Vigna radiata
Structure
Properties
Emerging technologies

ABSTRACT

In this work, the effect of Gamma-irradiation was evaluated on the characteristics of mung bean (*Vigna radiata*) grains and starches, considering doses up to 5 kGy. For this purpose, the starch structure and properties were evaluated, as well as the grains' hydration, germination and cooking. The irradiation process was able to change the characteristics of both mung bean starches and grains. The starch structure was partially changed, presenting smaller molecules and small changes in the granule morphology. No alterations were observed in the starch X-ray diffraction pattern, while lower pH was achieved. Considering the starch properties, it was observed lower water retention ability at 75 °C, lower apparent viscosity, higher paste clarity and, in general, harder and less viscous gels. The ionizing radiation accelerated the hydration, reduced the germination capacity and improved cooking time of the mung bean grains. The results proved the efficacy of using ionizing radiation, at the doses applied in this work, to desirably modify the mung bean starch and grains.

1. Introduction

Ionizing radiation is a high-energy technology, pointed as an effective and emerging technology with different applications in food processing. It is a physical treatment which involves a direct exposure of the product to ionizing electromagnetic rays, aiming to guarantee its safety and also improve its properties [1–3]. The principle of action of this method is based on the generation of free radicals, which are capable of causing the modification on the exposed materials [3,4]. In fact, after decades of study, the food irradiation was proved to be save, being recognized as a food technology around the world [5].

However, although there is a vast literature regarding the use of irradiation for microbial inactivation, the effect of this technology on technological properties of foods still needs further studies. In special, Gamma-irradiation can be used to desirably modify food products and ingredients, although this approach needs further studies.

The hydration and germination of grains are two important steps of the grains industrialization. The ionizing radiation was reported to affect several of the seeds metabolics (growth and development), as well as its morphology (changing cells and tissues) [6]. The effect of the Gamma-irradiation on the hydration and germination of mung bean grains, however, is still unexplored. In fact, there is only one work on the literature evaluating the effect of irradiation on the hydration kinetics of grains (Ramaswamy et al. [7]). Even so, the work of Ramaswamy et al. [7] was carried out with fava beans, whose hydration behaviour is different of other typical pulses [8]. Furthermore, the correlation between the observed behaviour of the grain and the properties of its starch was not carried out.

Further, the modification of starches is important to obtain ingredients with specific functional properties for their innumerous industrial applications. Most of the methods, however, are complex, time consuming and/or generates undesirable wastes [9]. Several works reported the use of Gamma-irradiation on the modification of different starch sources, as rice [10,11], chickpea [4], potato [12,13] and maize [14], among others. The results, however, do not always follow the same behavior, probably due to the different starch sources and/or irradiation dose. Furthermore, the irradiation of mung bean starch has not been evaluated yet.

In this work, the effect of Gamma-irradiation was evaluated on the characteristics of mung bean (*Vigna radiata*) grains and starches. For this purpose, the grains' hydration, germination and texture after cooking were evaluated, as well as the starch structure and properties. Furthermore, this work correlated the observed behaviour of the grain with the structure of properties of its starch. To the best of our knowledge, the effect of this technology on the mung bean grains and starches is firstly described here.

2. Material and methods

2.1. Material

Mung beans (*Vigna radiata*) were obtained at a local market of Campinas, SP, Brazil. This pulse presented 10.52 ± 0.76% d.b (g water/100 g

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https://doi.org/10.1016/j.ijbiomac.2019.01.221
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of dry matter) of moisture content and average dimensions of 4.89 ± 0.42 mm length, 3.75 ± 0.34 mm width and 3.52 ± 0.11 mm thick.

For the starch extraction, the grains were hydrated at 5 ± 2 °C (to avoid germination) for 12 h and then milled with distilled water using a blender. The mixture was sieved using firstly a sieve of 60 mesh and them a sieve of 325 mesh, washing the supernatant with distilled water. The starch was separated from the water, protein and lipids by centrifugation (3200*g* for 5 min at 25 °C). Finally, the starch was dried at 35 °C until 10.3% of moisture content (~12 h) and then softly milled using a mortar and a pestle.

2.2. Irradiation process

The irradiation process was conducted at CENA/USP using a ^{60}Co radioisotope source. The samples were packed in 100 mL containers of polypropylene with a polyethylene screw cap and irradiated at ambient temperature (~25 °C). The grains (~60 g) and the starch (~40 g, 10.3% moisture content) samples were irradiated using different radiation dose up to 5 kGy. A non-irradiated sample (0 kGy) was used as control.

2.3. Starch evaluation

2.3.1. Granule morphology: scanning electron microscopy (SEM) and light microscopy

The surface and the morphology of the starch granules were evaluated using a scanning electron microscope (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England) with an acceleration voltage of 20 kV. The starch samples were sprinkled on double-sided adhesive tape placed over circular stubs and coated with a 30-nm gold layer.

The shape of the starch granules was evaluated using a light microscope (model L1000, Bioval, Curitiba, Brazil) with a 20-W halogen lamp. A 1% dispersion of starch in distilled water was mixed with a drop of Lugol solution (to better distinguish the granules). The dispersion was put onto a glass slide, which was covered by a glass cover slip, and observed in the microscope. The magnification used was of 400× and a portable camera of 1.3-megapixel was used to obtain the images.

2.3.2. Granule crystallinity: X-ray diffraction pattern and relative crystallinity

Firstly, to ensure a constant activity of water, the starch samples were maintained in a desiccator containing saturated $BaCl_2$ solution (25 °C, aw = 0.900) for 10 days. After this period, the diffraction pattern of the samples was obtained using an X-ray diffractometer (Shimadzu XRD 7000, Tokyo, Japan) with copper radiation at an angle 2θ ranging from 3 to 40°. The working conditions were: scan rate of 2°·min^{-1}, 40 kV and 30 mA. The curves obtained were smoothed using the Origin software, version 9.1 (Microcal Inc., Northampton, MA, USA), and the relative crystallinity (RC) of the starch granules was calculated as described by Nara e Komiya [15], using the same software, plotting the graphs between 2θ angles from 3 to 40°.

2.3.3. Molecular size distribution

The molecular size distribution profile of the starch molecules was determined by gel permeation chromatography (GPC). The methodology was based on the described by Song and Jane [16], with modifications. The samples were prepared as follow: 0.1 g of starch was mixed with 10 mL of Dimethylsulfoxide (DMSO; 90%, Labsynth, Brazil) and heated in a bath of boiling water for 1 h and then kept at 25 °C for 24 h under constant stirring. An aliquot of 3 mL of this solution was mixed with 10 mL of absolute ethanol and then centrifuged for 30 min at 3000*g*. The precipitated starch was mixed with 10 mL of boiling distilled water and heated until complete dissolution. 8 mL of this solution was then upwardly eluted in a glass chromatographic column (2.6 cm diameter and 70 cm high, packed with Sepharose CL-2B gel - Sigma, Sweden), with an eluent solution (25 mmol·L^{-1} of NaCl and 1 mmol·L^{-1} of NaOH), at a rate of 60 mL·h^{-1}. A fraction collector (Gilson, model FC203B, Middleton, England) was used to separate the sample into 4-mL portions. Each portion was mixed with 0.1 mL of Lugol solution (blue value method, according to Juliano [17]), and evaluated using a spectrometer (Femto, Model 600S, São Paulo, Brazil) at 620 nm.

2.3.4. pH

The pH was determined using a calibrated potentiometer (Tecnal, TEC-5 mode, Piracicaba, Brazil), according to the specified by the Adolfo Lutz Institute [18]. A 10% starch slurry was maintained under constant stirring, using a magnetic stirrer, and then evaluated.

2.3.5. Pasting properties

The starch pasting properties were evaluated using a Rapid Visco Analyser equipment (RVA-4, Newport Scientific Pvt. Ltd., Australia, with the software TCW3). A suspension of 3 g (in relation to 14% moisture basis) of starch was homogenized in 25 g of distilled water for 10 s at 960 RPM and then heated and cooled under a constant shear (160 RPM). The suspension was initially held at 50 °C for 1 min, then heated to 95 °C at a rate of 6 °C·min^{-1}, then kept at 95 °C for 5 min, followed by cooling to 50 °C at a rate of 6 °C·min^{-1}, and finally holding it at 50 °C for 2 min. The apparent viscosity was recorded over the procedure.

2.3.6. Gel strength

The strength of the starch gel was determined by a uniaxial compression procedure, using a Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490.3 N). The gels obtained after the RVA assays were stored in plastic cups (40 mm diameter × 20 mm height) for 24 h at 5 ± 2 °C inside a desiccator (to ensure uniform moisture of the samples and avoid drying). After the storage period, the gels were removed from the cups and compressed until the distance of 15 mm at 1 mm·s^{-1}, using a cylindrical probe with 100 mm of diameter (SMS P/100). A drop of mineral oil was placed above and below the gel cylinder to ensure that only normal forces were measured by the probe.

2.3.7. Water Absorption Index (WAI) and Water Solubility Index (WSI)

The starch water absorption (WAI) and solubility (WSI) indexes were evaluated as described by Anderson et al. [19], with modifications. 0.5 g of starch (dry basis) were mixed with 6 mL of distilled water in pre-weighed centrifuge tubes. The tubes were then brought to water bath with stirring for 30 min at different temperatures (25, 50 and 75 °C). After heating, the tubes were centrifuged at 3000*g* for 10 min, and two phases were obtained: the supernatant and the precipitated.

The supernatant was dried at 105 °C in glass plates, and the soluble starch fraction (SS) of the supernatant was determined (Eq. (1)). Therefore, the WSI represents the soluble portion (%) of the starch at the given temperature. The precipitated (PT) sample retained in the tube was also weighed. The WAI is an indicator of the starch swelling power and represents the amount of water that 1 g of starch (disregarding the soluble portion) was able to absorb and retain (Eq. (2)). For both equations, MS is the mass of the starch sample, in dry basis.

$$WSI\ (\%) = \frac{SS}{MS} \cdot 100 \tag{1}$$

$$WAI\left(\frac{g\ water}{g\ starch}\right) = \frac{PT - (MS - SS)}{(MS - SS)} \tag{2}$$

2.3.8. Paste clarity

The starch paste clarity was evaluated as described by Craig et al. [20] and modified by Aplevicz and Demiate [21], by transmittance

(T%) measurement. 0.2 g of starch was mixed with 19.8 mL of distilled water in test tubes with screw caps. The tubes were then placed in a thermal bath with boiling water for 30 min and stirred individually every 5 min. The tubes were then cooled to room temperature and evaluated in a spectrometer at a 650 nm wavelength (Femto, Model 600S, São Paulo, Brazil).

2.4. Grain hydration process

The hydration process was conducted immersing approximately 10 g of grains in 250 mL of distilled water at 25 ± 1 °C. The temperature was controlled using a water bath (Dubnoff MA 095 MARCONI, Brazil). The hydration kinetics was obtained following the methodology described by Miano and Augusto [8], where the grain moisture content during soaking time is obtained by mass balance.

Since the hydration kinetics of mung bean presents sigmoidal behaviour, the data of moisture content (M_t) against soaking time (t) was fitted to the equation proposed by Kaptso et al. [22] (Eq. (3)).

$$M_t = \frac{M_\infty}{1 + \ exp[-k\cdot(t-\tau)]} \tag{3}$$

where M_∞ is the equilibrium moisture content, k is the hydration rate and τ is the lag phase time of hydration (the required time to change the curve concavity).

2.5. Grain germination

The germination was studied by obtaining the germination courses (percentage of germination against time). For which, 50 seed were immersed in distilled water at 25 ± 1 °C using a Beaker. The temperature was controlled using a water bath (Dubnoff MA 095 MARCONI, Brazil). Every certain time, the germinated beans were counted. The germinated grain was considered when its radicle grows 3 mm outside the seed. The data of the percentage of germinated beans (G) against time (t) was tabulated and fitted to the sigmoidal function of Gompertz, modified by Zwietering et al. [23] (Eq. (4)).

$$G = G_{max}\cdot\ exp\left\{-\ exp\left[\frac{k_g\cdot e}{G_{max}}(\lambda - t) + 1\right]\right\} \tag{4}$$

where G_{max} is the maximum germination percentage, k_g is the germination rate, λ is the lag phase time of germination (the required time to start the germination) and e is the Euler number.

2.6. Grain cooking kinetics

Native and irradiated beans were cooked in distilled boiling water (98.8 °C) for different periods (0, 5, 10, 15 and 20 min). In each of that periods, the texture of the grains were immediately evaluated through a uniaxial compression assay using a Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490.3 N). The grains were compressed until half of their width at a velocity of 1 mm·s^{-1} using a cylinder probe of 35 mm of diameter (P/35). The force measured by the equipment as a function of the compression was recorded, being the maximum peak considering for cooking description. It should be mentioned that the moisture content of the beans before being cooked was the same for all samples, to isolate the effect of irradiation from the effect of the initial moisture content. For that, according to their respectively hydration kinetics, the beans of each treatment were hydrated enough time to reach the same equilibrium moisture content of the control sample (25 °C).

The maximum force against the cooking time was plotted and fitted using Eq. (5) to estimate the maximum softening of the grains [24]. Where F_0 is the force before cooking process, F_t is the force as function of cooking time, F_∞ is the minimum force that beans reached during cooking and k_F is the softening rate during cooking.

$$F_t = F_\infty + (F_0 - F_\infty)\cdot e^{-k_F\cdot t} \tag{5}$$

2.7. Experimental design, mathematical fitting and statistical evaluation

A completely randomized design was applied in three replicates. For the nonlinear regressions, each replication datum was fitted using a generalized reduced gradient algorithm, which is implemented in the 'Solver' tool in the Excel 2016 software (Microsoft, USA). Different initial guesses of the three parameters were assessed to detect possible local convergence. Furthermore, when relevant, a statistical analysis was performed on the treatments through analysis of variance (ANOVA) and Tukey's test, using the Statistica 12.0 software (StatSoft, USA). A significance level of 5% was considered.

3. Results and discussion

3.1. Starch structure evaluation

3.1.1. Granule morphology

The light microscopy and SEM images of the starch granules are shown in Fig. 1. Both native and irradiated starch presented oval-shaped granules with "cavities" in the central region. Few changes were observed in the surfaces of the irradiated samples, as slight imperfections (as pores). These imperfections could only be seen through the SEM images. In the 5 kGy sample, it was possible to find some broken granules, but in a very small quantity (as shown by the red arrow in the Fig. 1).

Gani et al. [25] studied the effect of the irradiation process up to 20 kGy in starches from 4 different kidney bean samples. They observed some granule disruption by the irradiation process, with increasing surface fracturing as the irradiation dose increased. The authors observed that the changes were apparently dose-dependent and related these results with the highly energetic and penetrating effect of the radiation. Other authors observed similar results for rice [9], beans and potato starches [26], as surface cracking, deformation of the granular structure and size decreasing.

On the other hand, Liu et al. [14] did not observe any visible changes in the shape, size or surface of irradiated maize starch granules, even after extremely high doses of gamma irradiation (500 kGy). The authors discussed that, in this case, the irradiation must have affected only the internal region of the granules, damaging the structure of the molecules.

Those results indicate that not only the irradiation dose, but also the starch intrinsic characteristics, interfere in the irradiation process. Considering the mung bean starch samples, where the external structure of the granules was affected, it is possible to state that the irradiation affected both internal and external structures.

3.1.2. Molecular structure

Fig. 2 represents three different aspects of the starch internal structure: the size distribution of starch molecules (Fig. 2A), the pH of the starch suspensions (Fig. 2B) and the granule X-ray diffraction patterns and relative crystallinity (Fig. 2C and D). These properties would indicate, respectively, if there was hydrolysis of the glycosidic bonds of the molecules (leading to a formation of small-sized chains), if there was oxidation with possible formation of acid groups (which decreases the pH of the samples) and if there was any change in the crystalline structure of the starch samples.

Fig. 2A shows the starch molecular size distribution. In the gel permeation chromatography analysis, a gel with known porosity is used,

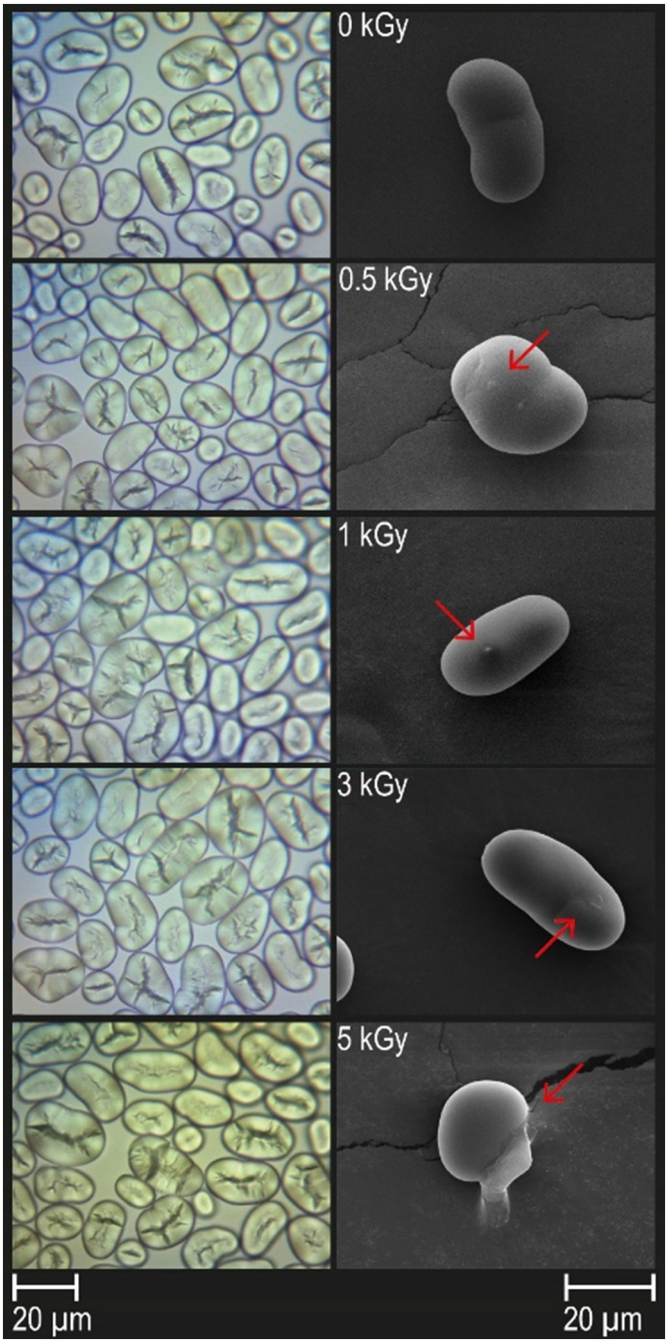

Fig. 1. Light microscopy (left column) and scanning electron microscopy (right column) of the native (0 kGy) and irradiated (0.5, 1, 3 and 5 kGy) mung bean starch. The white lines measure 20 μm. The red arrows indicate imperfections in the granules. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the smaller molecules can penetrate the pores and be retained longer in the chromatographic column. In this way, the larger and more branched molecules (as is the amylopectin) usually elute first, composing the first peak of the chromatogram, being followed by the small-sized fractions [27]. With increasing irradiation dose, both first and second peaks apparently "increased" in all samples (except the first peak of the 5 kGy sample). Also, it could be observed an "intermediate" peak in the 0.5 and 1 kGy samples (as a "shoulder" of the second peak). These results can indicate two main factors: the gradual size-decreasing of the starch molecules (eluting later in the chromatographic column) and the possible formation of molecular fractions with higher iodine affinity (i.e. less branched).

It is possible to say that the low-dose irradiated samples (0.5 and 1 kGy) suffered a light hydrolysis, which was able to generate molecules with smaller chain-length and higher iodine affinity. However, after a more intense irradiation (3 and 5 kGy), the molecules were eluting after the second peak or were not able to complex the iodine anymore due to their small chain length. It was illustrated by the displacement of the first and second peaks as the irradiation dose increases, with the rising and posterior "disappearance" of an intermediate "fraction".

Another important aspect is that the first peak, which is known as the "amylopectin peak" (as previously explained), was not completely degraded. This indicates that the irradiation process do not completely destroy the amylopectin chains at this conditions (up to 5 kGy), differently as occurs after severe oxidative process, for example [28].

In the literature, several authors [4,13,26,29] reported the hydrolysis of the glycosidic bonds as one of the consequences of the irradiation process of starches, leading to a decrease in their chain length. These results can be related to the formation of free radicals on the starch molecules, as discussed by Sokhey and Hanna [29]. Summarizing, the chromatographic results can prove the molecular depolymerization of the mung bean starch samples after the irradiation process, being in accordance with the reported in the literature. It can affect both starch and grain properties.

Fig. 2B indicates that the starch suspension pH value decreased with increasing irradiation process. The pH was used as an indirect measurement of acid groups' formation [12]. Similar trend was reported by other authors [12,25,30,31], which associated the pH decreasing with the formation of carbonyl and carboxyl groups in the irradiated starches, as well as the formation of different acids (acetic, formic, pyruvic and glucuronic). The results indicate, therefore, that the irradiation process contributed to the oxidation of the hydroxyl groups of the starch samples, especially into carbonyl and carboxyl groups. Besides, as the pH decreased, we can suppose that the presence of the acid groups was higher in the most irradiated dose.

The X-ray diffraction pattern of mung bean starch can be observed in Fig. 2C, and their respective Relative Crystallinity (RC) in Fig. 2D. The X-ray diffraction pattern indicate that the mung bean starch can be designated as C-type pattern, according to the classical literature [32,33].

However, a current approach is being used to evaluate the starch diffraction patterns, and it is based on the comparison of the X-ray results with a database of Powder Diffraction Files (PDF-4) [34,35]. This database is still under construction, and thus present only the amylose crystalline structure catalogued. According to this approach, we can observe that the peaks of the mung bean starch samples were located at ~15°, 17°, 18° and 23° in both native and irradiated samples. This indicates that the irradiation did not changed the crystalline pattern of the amylose and the amylopectin molecules.

The RC was not significant affected ($p < 0.05$) by the irradiation process (Fig. 2D), indicating that, despite the higher proportion of small molecules observed in the chromatography, the crystallinity of the samples was not affected by the irradiation (as the intensity of their X-ray diffraction pattern did not change). Besides, the amylose molecules seem to be as affected as the amylopectin molecules by the process, since the displacement of both peaks were similar in the chromatography. This can indicate that the irradiation process uniformly affects the molecules within the starch granules.

These results are in accordance with the literature, where the irradiation process is described to degrade the starch molecules through the generation and transformation of free radicals [9], that are capable of changing both internally and externally the molecules. This is also supported by the fact that the surface of the starch granules presented little or no changes (Fig. 1).

To sum up, the starch structural changes can be summarized as: smaller molecules (indicating the hydrolysis of the glycosidic bonds), lower pH (indicating acid groups formation), little changes in the granule morphology and no alterations in the diffraction pattern of the starch samples.

In the next section it will be discussed how the structural changes affected the starch technological properties.

3.2. Starch properties evaluation

Fig. 3 presents the starch Water Absorption Index (WAI, Fig. 3A), Water Solubility Index (WSI, Fig. 3B), pasting properties (RVA, Fig. 3C) and paste clarity (Fig. 3D) as a function of irradiation dose.

Regarding the WAI results it is possible to observe that, at 25 and 50 °C, no significant difference was observed among the samples. This result is of great importance to understand the hydration process behaviour (discussed later in this work), since the hydration behaviour of the grains at 25 °C was affected by the irradiation, and some authors hypothesized that this behaviour was related to the increase of the starch water absorption (which was not). On the other hand, the WAI of the irradiated starch were lower at 75 °C than the native one ($p < 0.05$).

These results indicate that the WAI of the starch granules below the gelatinization temperature was not affected by the irradiation – in fact because that values are very small, once the water absorption by the granule at that conditions is negligible. On the other hand, at 75 °C the structural changes undergone by the most irradiated samples (3 and 5 kGy) were sufficient to affect their water absorption and retention capacity. This can be explained by the lower molecular size of the irradiated starches and, especially, by the replacement of the hydroxyl groups by other less hydrophilic groups.

The WSI (Fig. 3B) did not differ significantly from the native starch sample, in any observed temperature. In this case, the structural changes undergone by the starch molecules after the irradiation was not sufficient to change their solubility, in conditions applied in this work.

Regarding the pasting properties (Fig. 3C), it is possible to observe that irradiation reduced the paste apparent viscosity, if compared to the native starch. The same behaviour was reported by Chung & Liu [26] while studying potato and beans starches irradiated up to 50 kGy, and by Gul et al. [11] while studying irradiated rice starch up to 10 kGy. The decrease in the apparent viscosity indicates that, under the RVA analysis conditions (i.e. stirring and heating), the modified starch presented a reduced capacity to maintain their integrity, if compared to the native sample. The granule is broken in a lower temperature. This result can be explained by the cleavage of the glycosidic bonds of the starch molecules, which decreases the size of their chains, resulting in "weaker" granules.

The paste clarity (Fig. 3D) increased with increasing the irradiation dose. The 5 kGy sample reached almost the double of the transmittance observed for the native sample. These results are in accordance with the structural changes undergone by the irradiated samples. The carboxylic groups are electronegative and cause an electrostatic repulsion between the molecules, impairing their association [36]. The less intra and intermolecular associations, the higher will be the paste clarity [20]. It is worth mention that this is a very positive change, considering industrial application.

Fig. 4 illustrates the mechanical properties of the mung bean starch at different irradiation dose. The starch gels were evaluated through a compression test, until their complete disruption. The obtained results can be discussed using the relative strain that the samples reached before their disruption (Fig. 4B), as well as by comparing the peak force that the samples were able to reach before their disruption (Fig. 4C). It is possible to observe that the native sample was able to deform almost ~65% of its height before its disruption, a value much higher than the 5 kGy irradiated sample (which reached ~50% of its height before disrupting). The 0.5, 1 and 3 kGy samples were statistically ($p > 0.05$) equals to the native sample, besides presenting lower values of relative

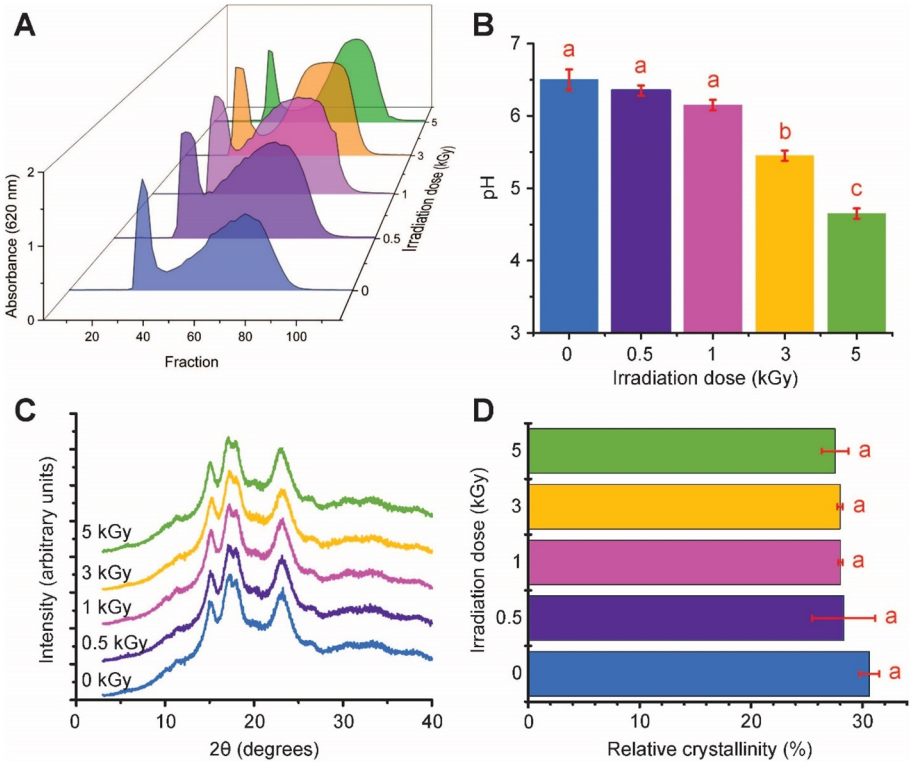

Fig. 2. (A) Molecular size distribution profile (blue value method), (B) pH values, (C) X-ray diffraction patterns and (D) relative crystallinity of the native (0 kGy) and irradiated (0.5, 1, 3 and 5 kGy) mung bean starch. Red bars indicate the standard deviations. Variations followed by the same small letters do not differ significantly ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

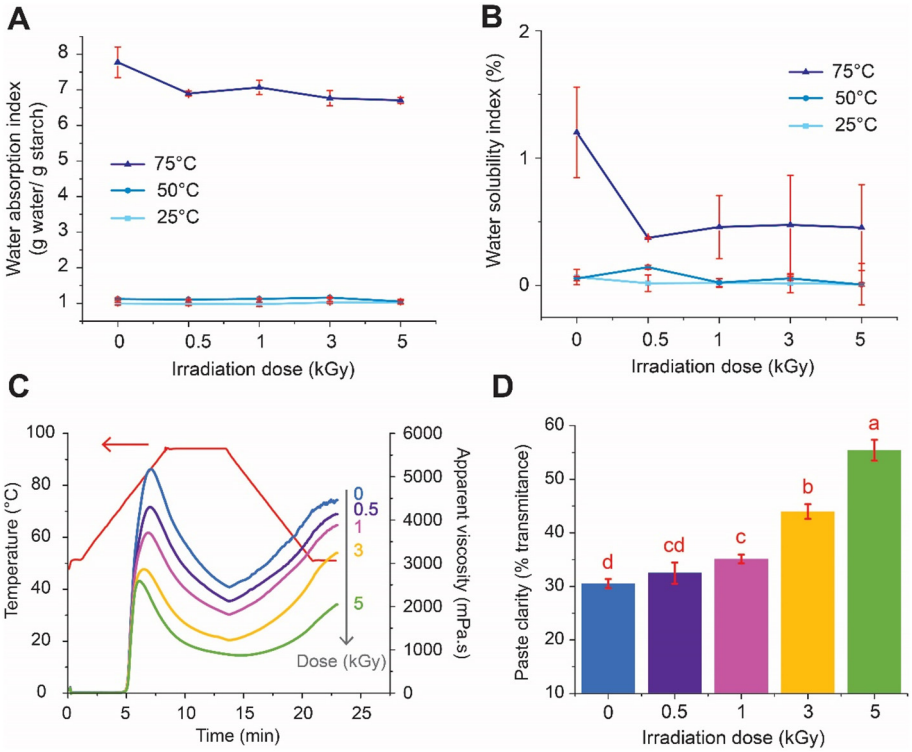

Fig. 3. (A) Water absorption index, (B) water solubility index, (C) pasting properties and (D) paste clarity of the native (0 kGy) and irradiated (0.5, 1, 3 and 5 kGy) mung bean starch. Red bars indicate the standard deviations. Variations in the properties due to irradiation dose, when followed by the same small letters, do not differ significantly ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

strain. By comparing the peak force, the results show that the 1 and 3 kGy samples presented a stronger gel if compared to the native and to the 0.5 and 5 kGy samples. It is worth mention that a stronger gel, considering the same starch concentration, is a characteristic of high industrial relevance.

These results can be explained by the cleavage of the glycosidic bonds of the starch molecules. The lower chain-length molecules can present a size that is more suitable for re-association after cooling, thus constituting harder gels (that need more energy to disintegrate). However, these same molecules lead to gels that are not resistant to compression forces, being disrupted in a lower temperature if compared to the native starch gels.

Summarizing, the irradiation process led to starches with lower water retention ability, lower apparent viscosity, higher paste clarity and, in general, harder and less elastic gels. These characteristics can be very promising from an industrial point of view, especially industries like food, paper and textile, were a high paste clarity and a lower apparent viscosity are desirable.

3.3. Hydration process

The hydration kinetics of mung beans showed a sigmoidal behaviour (Fig. 5), as expected [37]. This behaviour is usually found on grains from *Phabaceae* family, which it is characterized by a lag phase caused by the seed coat impermeability to water when at small activity of water values [8]. In fact, the hydration kinetics of seeds during all germination process produces a triphasic curve [38]: the first phase consists of the actual hydration process; in the second phase the moisture content of the seed keeps constant and the nutrients catabolism takes place; in the third phase the radicle grows to form the seedling and the moisture content is increased due to the cell reproduction (Fig. 5).

The model proposed by Kaptso et al. [22] was used to describe the hydration kinetics. As mung beans have a very rapid germination, the second phase of the curve is very short, and the phase 1 and phase 3 seems to be joined (Fig. 5, Miano et al. [37]). Consequently, it is difficult to determine the equilibrium moisture content during the hydration process of mung beans. Therefore, the equilibrium moisture content

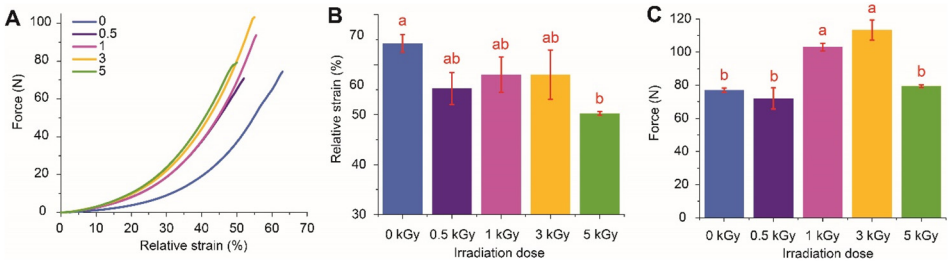

Fig. 4. (A) Gel compression force vs. relative strain curves until the peak force; (B) Relative strain of the peak force and (C) peak force of the native (0 kGy) and irradiated (0.5, 1, 3 and 5 kGy) mung bean starch. Red bars indicate the standard deviations. Variations followed by the same small letters do not differ significantly ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

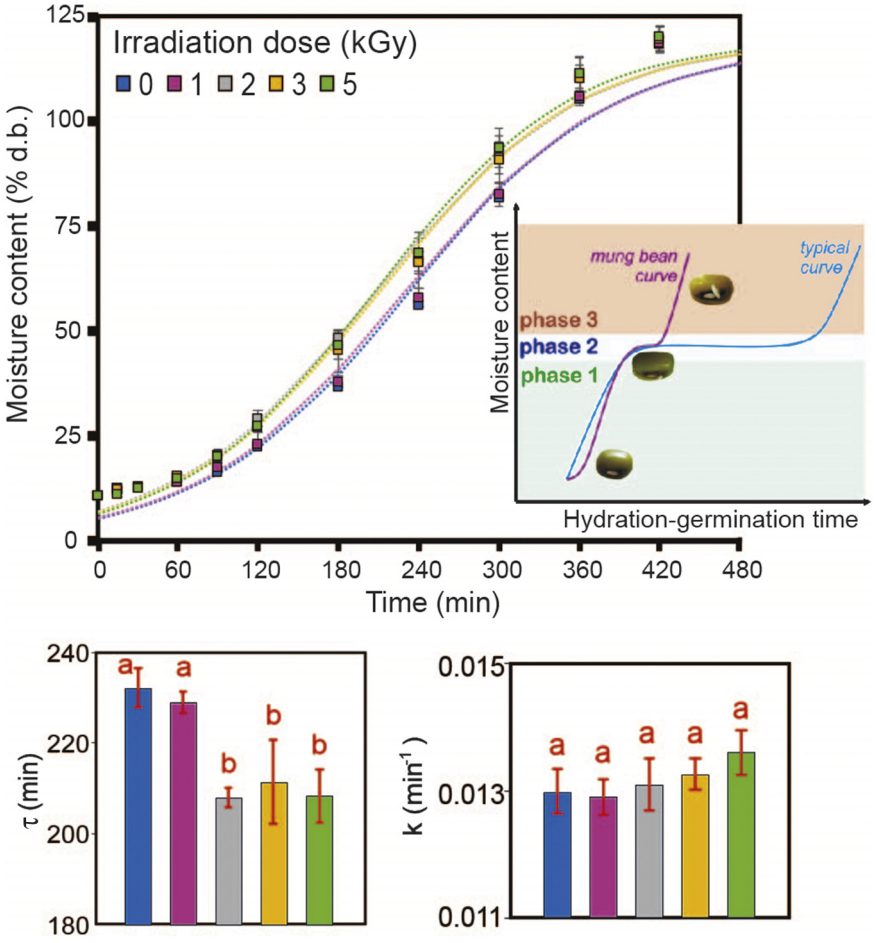

Fig. 5. Up: Hydration kinetics and the relation between moisture content and hydration-germination process of a typical seed and of mung bean seeds. Note the short phase 2 of mung bean due to its fast germination. Down: Effect of gamma radiation on the hydration kinetics of mung bean: Effect on the lag phase time (τ) and hydration rate (k). The dots represent the mean of the experimental data, the lines represent the model (Kaptso et al., 2008) and the vertical bars represent the standard deviation. Lowercase letters represent Tukey mean comparison test (95% of confidence).

(M_∞), was considered the same for all the treatment, as the average value of all treatments, as 119 ± 1%d.b. The hydration rate (k) and the phase lag time (τ) were determined individually for each treatment. As shown in Fig. 5, this approach successfully fit the data for all the used irradiation doses ($R^2 > 0.99$).

The ionizing radiation affected significantly ($p < 0.05$) the hydration kinetics of mung beans. According to Fig. 5, when 2, 3 and 5 kGy were applied, the hydration process was faster. In fact, the acceleration was due to the reduction of the lag phase (represented by τ), since the hydration rate (k) was not significantly affected (Fig. 5).

The parameter τ is related to the necessary time to increase the seed coat water activity in order to change its permeability to water [39]. At low moisture contents, the seed coat of many pulses is impermeable to water. Therefore, water enters the grain by the hilum and/or micropyle, hydrating the seed coat from inside until increasing its permeability to water [8].

In fact, the most probably cause of the lag phase reduction would be changes in the seed coat components. The seed coat is composed by carbohydrates and some components such as phytic acid, tannins and phenolics compounds [40]. These components could be affected by ionizing radiation, facilitating the seed coat hydration and changing its permeability faster. For instance, some works reported reduction of tannins and phytic acid on common beans using irradiation doses up to 10 kGy [41] and on fava beans using 1 kGy [42]. Therefore, the modification of these components may affect the permeability of the seed coat.

On the other hand, the hydration rate (k) was not affected by irradiation. This parameter is related to the velocity of hydration of the cotyledon and germ and represent a global water transfer constant, considering both diffusion and capillarity mechanisms [8]. Furthermore, the curve shape keeps very similar, without big differences. This probably means that irradiation did not significantly affect the cotyledon composition, and that its main effects take place on the seed coat.

In fact, despite the ionizing irradiation improved the hydration process, this acceleration was short. Therefore, this technology would not be used for this purpose, since there are other more efficient technologies which accelerate hydration process, such as using high temperatures [43,44], high pressures [45] or ultrasound technology [37,46].

On the other hand, this prove that ionizing irradiation can be used for disinfestation and/or disinfection purposes on this grain without affecting negatively the hydration process. This is interesting and important considering different applications, such as for cooking and starch extraction.

Ramaswamy et al. [7] evaluated the effect of irradiation on the hydration kinetics of fava beans, whose hydration kinetics showed downward concave shape behaviour. For the best of our knowledge, this is the only study regarding irradiation and the hydration kinetics of grains. They found that the hydration rate was increased by using ionizing irradiation at doses of 2 and 5 kGy. The authors hypothesized that this behaviour was related to the increase of the starch water absorption. However, contrary to the present work, this property was not evaluated there. In the present work, we demonstrated that the water absorption of starch at 25 °C was not affected by irradiation (please verify the next sections), being important only at 75 °C.

Therefore, the acceleration of fava beans hydration [7] should be caused by other reason.

Further than the isolated components, it is important to evaluate the grain as complex material, with tissues and cells. Therefore, another probable explanation for the effect of irradiation can be the grain metabolism.

The ionizing radiation can change the cells metabolism, from membrane damage to the cell dead. Therefore, the irradiated cells could lose their biological control of water transfer, letting a faster water entrance. This hypothesis can explain the hydration enhancement due to the irradiation process. In fact, the changes on the grain metabolism can be demonstrated though its germination.

3.4. Germination process

Fig. 6A shows the effect of irradiation on the germination courses of mung beans. Ionizing irradiation affected significantly ($p < 0.05$) the germination. In fact, this pulse manages to germinate even at doses up to 5 kGy, demonstrating to be a resistant organism. Even so, it was perceived that the seedling has difficulties to develop when they were let to grow (results did not register). In general, the higher the irradiation dose was, the lower the germination capacity was. This was because ionizing irradiation causes mutation on mung bean cells [47]. Therefore, this probable avoids some enzyme formation for reserve catabolism and seedling development.

As the germination kinetics presents a sigmoidal behaviour, the model of Gompertz was used to fit the data. Among different parametrizations, that one modified by Zwietering et al. [23] was adopted (Eq. (4)). This equation provided three parameters that helped to study the effect of irradiation on the germination process: the maximum germination (G_{max}), the germination rate (k_g), and the lag phase time (λ).

Fig. 6B shows that irradiation affected significantly the maximum grain germination (G_{max}). In fact, the maximum germination percentage significantly decreased ($p < 0.05$) only when 5 kGy were applied, from ~95% to ~72%. Despite this result, mung bean has shown a high resistance to ionizing irradiation comparing to other seeds. Many other seeds have demonstrated a great reduction on their maximum germination count when ionizing irradiation was applied. For instance, the germination capacity of wheat seed was null when 0.6 kGy was applied [48]; the germination capacity of *Lathyrus chrysanthus* was reduced to the half when 0.25 kGy were applied [6]; and the germination capacity of chick peas was reduced 40% when 1 kGy was applied [49].

The germination rate (k_g) was also significantly affected by ionizing radiation (Fig. 6C). In this case, the germination rate was reduced approximately 40% when 1, 2 or 3 kGy were applied, and approximately 75% when 5 kGy were applied. Therefore, despite having a high maximum germination, the velocity of germination was sharply reduced. This was probably caused by the cell mutation and/or enzyme inactivation that causes the reduction of metabolism and reserve catabolism for the radicle to grow. On the other hand, the lag phase time (λ) was not significantly affected by irradiation, despite there is a slightly increment when applying 5 kGy (Fig. 6C). It should be mentioned that the value of the lag phase of germination (λ) is very similar to value of the lag phase of hydration (τ). In fact, the lag phase of hydration is related to the time when the seed coat components suffer glassy state transition (pass from glassy to rubbery state) [39,50]. Therefore, there is an increase of water mobility, which would help enzymes to be activated promoting the radicle growth. Consequently, both parameters are related.

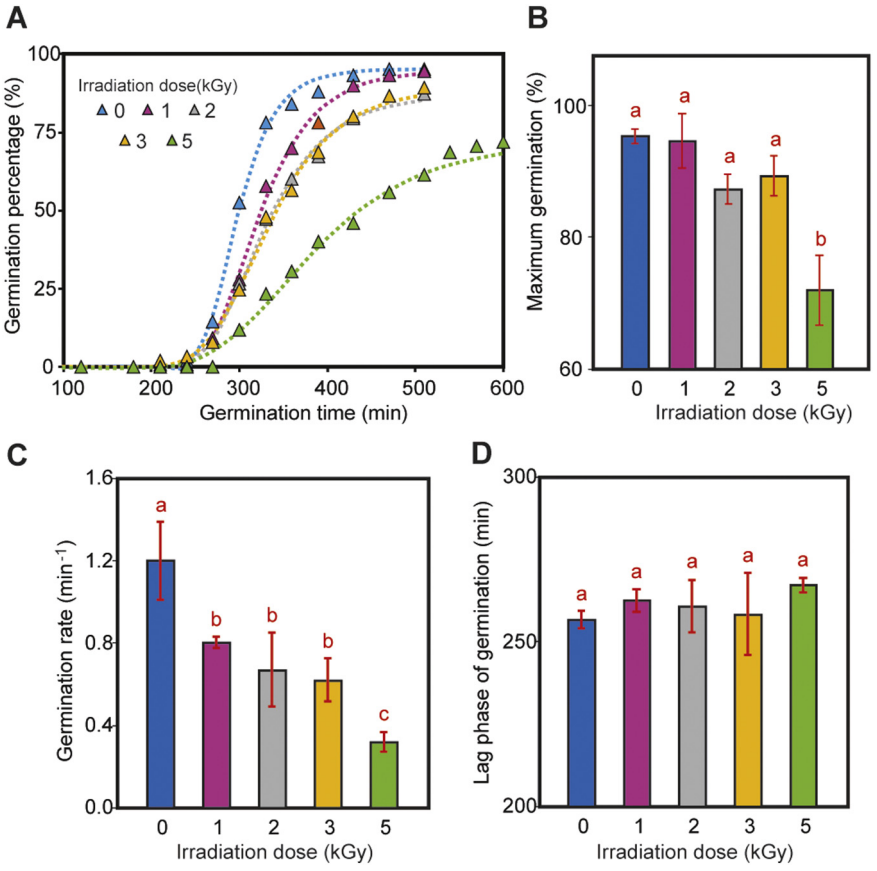

Fig. 6. Effect of gamma radiation on: (A) germination course of mung bean, (B) maximum germination percentage (G_{max}), (C) germination rate (k_g) and (D) lag phase of germination (λ). The triangles represent the mean of the experimental data, the lines represent the model of Gompertz (Eq. (4)) and the vertical bars represent the standard deviation. Lowercase letters represent Tukey mean comparison test (95% of confidence).

Summarizing, if the purpose of mung bean is sprouting, the irradiation would not be useful. However, if the purpose is to avoid the germination, besides de disinfestation and disinfection, for cooking and starch extraction, irradiation would be an excellent approach.

3.5. Grain cooking kinetics

The cooking kinetics of mung bean was evaluated through compression assays during cooking. The results are shown in Fig. 7, including the parameters of the kinetics model. The initial and equilibrium forces (Fig. 7B) of the mung bean samples did not present a statistical difference ($p < 0.05$). Similarly, the cooking kinetics parameter did not present a statistical difference ($p < 0.05$, Fig. 7C), although a clear trend can be observed (Fig. 7A and C).

After 5 min of cooking time (Fig. 7A), it is possible to see a difference among the values of force presented by the irradiated (2.3 kGy = 8.2 ± 0.3 N; 3.8 kGy = 7.6 ± 0.8 N) and the control (0 kGy = 11.1 ± 0.4 N) samples. In fact, the irradiated samples reached values of force close to the equilibrium force (~3.5 N) 5 min before the control sample, that reached the value of 5.5 ± 1.6 N only after 15 min of cooking.

Considering the results, it is possible to say that the irradiation led to a decrease in the time needed to cook the grains (i.e., the time needed until they reach the equilibrium force on Fig. 7A). It is worth to mention that this is a highly desirable result from an economic point of view, once reducing cooking time is one of the main objectives on bean development. Similar results (reduction of cooking time) were reported by Iyer et al. [51] using 5 kGy to irradiate Great Northern kidney and pinto beans.

Since the irradiation process is highly penetrating [52], the results can be explained by the partial degradation of both internal and external major components of the grains, as the starches and proteins, or even parts of the grain tissues and cells. In fact, the starch of the mung bean was highly affected by the irradiation process, as previously discussed in the present work. It is important to point out, however, that the observed changes in the cooking kinetics of the grains are also related to the higher temperatures applied for their cooking, since at lower temperatures (as demonstrated for water absorption capacity at 25 °C, Fig. 3B) the possible changes caused by the irradiation process were not measurable.

Considering the effect of the irradiation on other components of the grains, two main reactions can be associated with the observed changes: the partial denaturation of the proteins, especially the ones presented in the grains' coats [53], and the changes in the phytate molecules (reduction of phytic acid by irradiation was reported by several authors, as Brigide & Canniatti-Brazaca [41], Osman et al. [42] and Villavicencio et al. [54]).

Both changes can be associated with a structure disruption and/or with the phytate bonds with other components, causing a further reduced cooking time of the mung bean grains.

The decrease in the cooking time achieved after the irradiation process can be useful in cases when the cooking of the grains is the limiting of a process.

4. Conclusions

The irradiation process, at the doses applied in this work, was able to change the characteristics of both mung bean grains and starches.

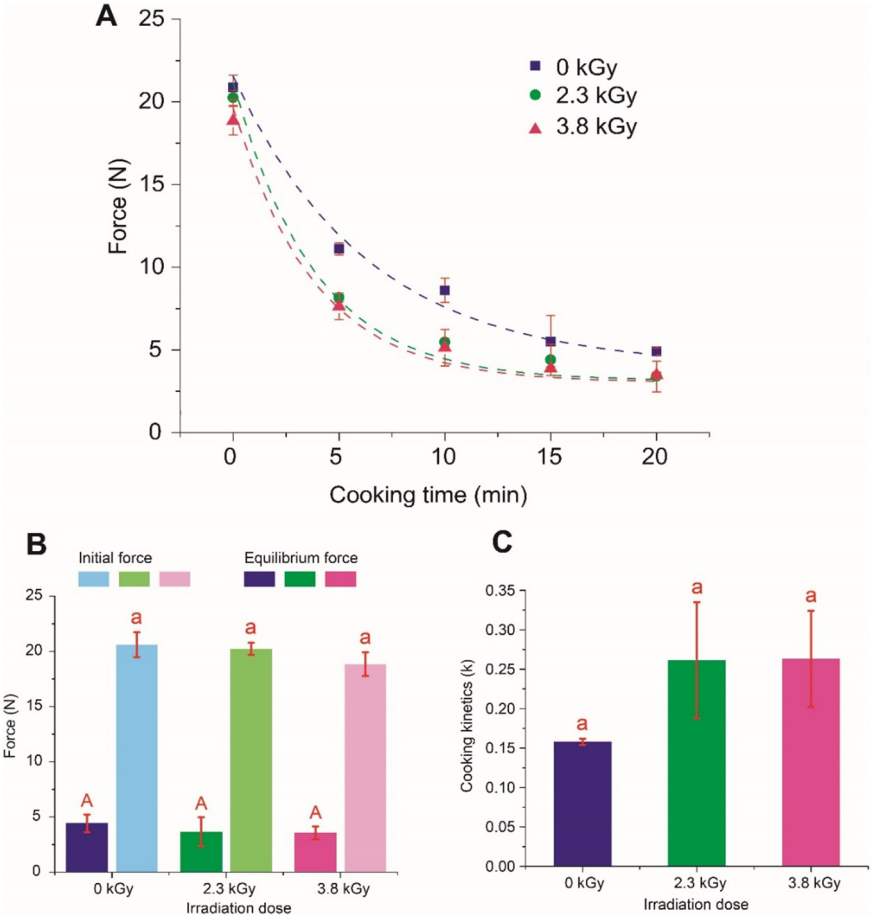

Fig. 7. Effect of gamma radiation on beans (A) cooking kinetics curves, (B) initial and equilibrium force of compression and (C) cooking kinetics (k). The symbols represent the mean of the experimental data, the dot lines represent the model of Eq. (5), and the vertical red bars represent the standard deviations. Same letters represent Tukey mean comparison test (95% of confidence). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Considering the starch samples' characteristics, their structure was partially changed, presenting smaller molecules, lower pH and little changes in the granule morphology. However, no alterations were observed in the X-ray diffraction pattern of the starch samples. Considering the starch properties, almost all of them underwent significant changes, except for the WAI at 25 and 50 °C and the WSI. It was observed lower water retention ability at 75 °C, lower apparent viscosity, higher paste clarity and, in general, harder and less elastic gels. The results proved the efficacy of using ionizing radiation on the modification of the mung bean starch, with possible industrial applications.

Considering the grains characteristics, ionizing radiation slightly accelerated the hydration of mung beans, reducing the lag phase time using doses of up to 5 kGy. These changes could not be attributed to the starch, being thus related with changes on the grain structure and/or metabolism. On the other hand, germination capacity was reduced up to 72% of germinated seeds using 5 kGy, proving a great resistance of mung bean to irradiation. The cooking time of the irradiated grains was also improved. Therefore, irradiation could be used for insects and microorganisms' control without affecting negatively the hydration process. Nevertheless, if sprouting is the main purpose, irradiation would not be useful.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

The authors are grateful to:

- the São Paulo Research Foundation (FAPESP, Brazil) for funding the project n° 2016/18052-5 and for the V. D. Sabadoti B.*Sc.* scholarship (2016/23908-6);
- the National Council for Scientific and Technological Development (CNPq, Brazil) for funding the project n° 401004/2014-7 and the productivity grant of P.E.D. Augusto (306557/2017-7).
- this study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)" - Finance Code 001, through the N. Castanha Ph.D. scholarship.
- Cienciactiva from the "Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica" (CONCYTEC, Peru) for the A.C. Miano Ph.D. scholarship (Contract 272-2015 - FONDECYT);
- the "Laboratório de Irradiação de Alimentos e Radioentomologia (LIARE)" and "Laboratório de Melhoramento de Plantas", of CENA/USP, for the irradiation of the samples.
- the Laboratory of Soil Mineralogy (LSO-ESALQ/USP), in the name of Prof. Dr. Antonio Carlos Azevedo and Leandro Luís Góia, for the X-ray analysis;
- Dr. Manoel D. Matta Junior and Mr. Caio C. L. Silva, for general support.

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