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Starch Digestibility and Functional Properties of Rice Starch Subjected to Gamma Radiation

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Abstract: This study investigated the effect of gamma radiation on the digestibility and functional properties of rice starch. Rice cultivars IRGA417 and IAC202 were used for isolation of starch by the alkaline method. Starch samples were irradiated with 1, 2 and 5 kGy doses of ^{60}Co at a rate of 0.4 kGy/h. A control sample, which was not irradiated, was used for comparison. Irradiated and control starches were characterized by *in vitro* starch digestibility, total dietary fiber, color, water absorption index, water solubility index, syneresis, swelling factor, amylose leaching, pasting properties and gel firmness. Irradiations changed starch digestibility differently in either cultivar. Increasing radiation doses promoted increase in the color parameter *b** (yellow), elevation in the capacity to absorb water, and solubility in water as well as the amylose leached from granules for both cultivars. Pasting properties showed a decrease that was proportional to the dose applied, caused by the depolymerization of starch molecules. Gel firmness of the starch from IAC202 was inversely proportional to the radiation dose applied, whereas for IRGA417, there was a reduction at 5 kGy dose. Rice starches can be modified by irradiation to exhibit different functional characteristics and they can be used by the food industries in products such as soups, desserts, flans, puddings and others.

Key words: *Oryza sativa*; irradiation; viscosity; swelling; amylose; starch; digestibility

Rice is consumed by people in many countries and is the basis of diet for more than half of the world's population (Beinner et al, 2010). Brazil is the largest producer of rice among the Western countries (FAO, 2014), with the primary consumption of polished grains. During rice processing, about 14% of the grains are broken resulting in a by-product with a low commercial value, which can be used for starch extraction as an alternative to add value, thereby transforming it into an ingredient with an increased industrial and commercial interest (Zavareze et al, 2009).

Starch is used to bring consistency to food, but the regular starches can have limitations such as low resistance to shear and high tendency toward retrogradation and syneresis (Liu et al, 2012). Because of these limitations in the properties of starches, the concerned industries look for modified starches that can meet their needs. Thus, starches from different botanical sources are modified to obtain the desirable functional properties in the industries.

Gamma radiation is a physical method of modifying starch (Bao et al, 2005), with advantages such as not significantly raising the temperature of the product, requiring the minimal sample preparation, being fast, and not dependent on a catalyst (Bhat and Karim, 2009; Gani et al, 2012).

Ionizing radiation generates free radicals that are

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able to induce molecular changes and fragmentation of starch. During the irradiation treatment, glycosidic bonds are broken, promoting the decomposition of amylose and amylopectin into smaller molecules (Bhat and Karim, 2009; Chung and Liu, 2009).

Another factor that has been considered lately when selecting starch as an ingredient is its digestibility, due to health implications. Depending on the rate at which the starch is digested *in vitro*, it may be classified into rapidly digestible starch (RDS), digested between 0 and 20 min; slowly digestible starch (SDS), digested between 20 and 120 min; and resistant starch (RS), undigested even after 120 min (Englyst et al, 1992). The SDS and RS fractions have several health benefits; however, foods containing high levels of RDS, when ingested in large quantities, can cause health hazards, such as development of diabetes (Englyst et al, 2007; Zhang and Hamaker, 2009).

In general, starches subjected to gamma radiation present reductions in paste viscosity, molecular weights of amylose and amylopectin, and increases in solubility and carboxyl content (Bao et al, 2005; Chung and Liu, 2009, 2010; Gani et al, 2012; Liu et al, 2012; Falade and Kolawole, 2013; Othman et al, 2015; Reddy et al, 2015). However, other properties such as granule crystalline structure, granule morphology, amylose content, thermal properties and amylopectin fine structure show distinct behaviors depending on the botanical source of starch and irradiation conditions (Chung and Liu, 2009; Liu et al, 2012).

The irradiation can cause reduction in the starch digestibility due to the formation of β-bonds, carboxyl groups and structural modifications (Chung and Liu, 2009; Lee et al, 2013). The β-bonds reduce digestibility because they are partially digested by the amylolytic enzymes (Rombo et al, 2004), whereas the carboxyl groups of the irradiated starch inhibit the enzymatic attack, leading to an increase in RS content compared to the native starch (Chung et al, 2009). Structural modifications restrict the access of the enzymes to the starch molecules. However, other researchers have also observed increased starch digestibility with irradiation, attributing this fact to the loss of the granular structure and to the molecular fragmentation of the starch, which facilitates the access of the amylolytic enzymes (Bhat and Karim, 2009).

The objective of this study was to investigate the effect of gamma radiation on the digestibility and functional properties of rice starch.

MATERIALS AND METHODS

Raw materials

Polished rice grains of cultivar IAC202 were acquired from the Instituto Agronômico de Campinas (IAC) and IRGA417 was courtesy of Instituto Rio Grandense do Arroz (IRGA). These cultivars were selected because they are used on a large commercial scale.

Starch isolation

The rice starch was isolated according to the alkaline method of Patindol et al (2003) with modifications. Polished rice grains were soaked in 0.1% NaOH in the ratio of 1:2 for 24 h. After that, the grains were ground and sieved (63 μm). The sieved slurry was centrifuged at 1 500 × *g* for 15 min, the supernatant was discarded, and the protein upper layer (yellowish) carefully removed with a stainless steel spatula. The decanted material was suspended in 0.1% NaOH solution, centrifuged at 1 500 × *g* for 15 min, the supernatant was discarded and the upper layer removed carefully. The decanted starch was suspended in distilled water, and the pH was adjusted to 6.5 with 0.2 mol/L HCl, and then the sample was centrifuged at 1 500 × *g* for 15 min. The supernatant was discarded and decanted starch was suspended in distilled water and centrifuged at 1 500 × *g* for 15 min. This procedure of suspending the decanted starch in distilled water and centrifuging was repeated three times. Starch was dried in air circulating oven at 40 ºC, ground in mortar with pestle, sieved (150 μm) and stored in a sealed glass flask.

Starch irradiation

The starch samples (200 g, approximately 9% moisture) were packed in high density polyethylene bags and subjected to 1, 2 and 5 kGy gamma radiation at a dose rate of 0.4 kGy/h in ^{60}Co gamma irradiator (Gammacell, 220 Excel, GC-220E, Nordion Inc., Ottawa, Canada) at room temperature. The irradiation treatments were performed at the Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil. A non-irradiated sample (dose 0) was used as a control.

Starch digestibility

In vitro starch digestibility was analyzed in raw and cooked starch samples according to Englyst et al (1992) with modification. An enzyme solution was prepared immediately before use. Pancreatin from porcine pancreas (1.5 g, P1750, Sigma-Aldrich, St. Louis, USA) was dispersed in distilled water (10 mL) by magnetic stirring (TE-085, Tecnal, Piracicaba, Brazil) for 10 min, and the dispersion was centrifuged at 1 500 × *g* for 10 min. The supernatant (8 mL) was collected, and then amyloglucosidase (0.4 mL, A7095, Sigma-Aldrich, St. Louis, USA) and invertase (5 mg, I4504, Sigma-Aldrich, St. Louis, USA) were added.

For preparing raw starch, 0.5 g sample was added into 0.1 mol/L sodium acetate buffer (pH 5.2) containing 4 mmol/L $CaCl_2$ (20 mL), and kept in a shaking water bath at 37 ºC. After 5 min, 5 mL enzyme solution was added.

For preparing cooked starch, 0.5 g sample was added into 0.1 mol/L sodium acetate buffer (pH 5.2) containing 4 mmol/L $CaCl_2$ (20 mL) and kept in a boiling water bath for 20 min (for starch gelatinization). The gelatinized sample was then kept in a shaking water bath at 37 ºC for 10 min, after which 5 mL enzyme solution was added.

For both samples (raw and cooked), after the addition of the enzyme solution, aliquots (0.5 mL) were collected at intervals of 0, 20 and 120 min; put in centrifuge tubes (15 mL) containing 4 mL absolute ethanol; and centrifuged at 3 000 × *g* for 5 min. The remaining sample was boiled for 30 min, and 10 mL of 7 mol/L KOH was added and stirred for 30 min at 25 ºC, followed by treatment with amyloglucosidase (A7095, Sigma-Aldrich, St. Louis, USA). The amount of glucose released was quantified with the reagent oxidase-peroxidase glucose (Glicose PAP Liquiform, Labtest Diagnóstica, Lagoa Santa, Brazil). The contents of different fractions (RDS, SDS and RS) were obtained from the definition.

Total dietary fiber (TDF)

TDF content was determined using the enzymatic-gravimetric method AOAC 985.29 (AOAC, 2005). The samples were digested by thermostable α-amylase (97 ºC, 15 min, pH 6.0), pepsin (40 ºC, 30 min, pH 1.5) and amyloglucosidase (55 ºC, 30 min, pH 4.5). TDF was precipitated with four volumes of 95% ethanol and recovered through filtration in sintered crucible No. #2 with celite. TDF values were corrected by subtracting the indigestible protein (Kjeldahl method; 6.25 serving as the conversion factor of nitrogen to protein) and ash (incineration at 525 ºC, 5 h).

Color

The starch samples were placed in a Petri dish (Ø = 100 mm) to form a 10 mm thick layer, and *L* and *b** parameters of color were evaluated using a colorimeter (Chroma Meter CR-400, Minolta Co., Ltd., Osaka, Japan) by CIELAB system (color space being specified by the International Commission on Illumination).

Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were determined according to Anderson et al (1969) with modification. The sample (0.5 g) was mixed with 6 mL water in a centrifuge tube and continuously stirred for 30 min in a water bath at 30 ºC. The suspension was centrifuged at 3 000 × *g* for 10 min. The supernatant was dried at 105 ºC for 4 h to obtain the dry solid weight, and the wet sediment was weighed. The indices were determined as:

WAI (g/g) = Weight of wet sediment / (Weight of dry sample – Weight of dry solids in supernatant)

WSI (%) = (Weight of dry solids in supernatant / Weight of dry sample) × 100.

Syneresis

Syneresis of native and irradiated starches was determined using the method described by Sodhi and Singh (2003). A starch suspension (5%) was heated in a boiling water bath for 15 min with constant agitation. The resulting paste was transferred to a weighed centrifuge tube and stored at 4 ºC for 7 d. Syneresis was calculated as the percentage of water released after centrifugation at 3 000 × *g* for 10 min.

Swelling factor (SF) and amylose leaching (AML)

SF was determined according to Tester and Morrison (1990). The starch sample (100 mg) was added into centrifuge tubes (15 mL) with water (5 mL) and heated at 55 ºC, 65 ºC, 75 ºC, 85 ºC and 95 ºC for 30 min. The tubes were then rapidly cooled to room temperature, and 0.5 mL of 0.5 mg/mL blue dextran solution was added. The tubes were gently inverted several times to homogenize the solution. After centrifugation at 1 500 × *g* for 5 min, the absorbance of the supernatant was measured at 620 nm.

The percentage of AML was quantified according to Chung et al (2008). The starch sample (20 mg) was added into centrifuge tubes (15 mL) with water (10 mL) and heated at 55 ºC, 65 ºC, 75 ºC, 85 ºC and 95 ºC for 30 min. The tubes were cooled at room temperature and centrifuged at 2 000 × *g* for 10 min. The amylose content was determined in the supernatant and AML was expressed as percentage.

Pasting properties

The pasting properties of starch slurries (3 g sample with 14% moisture, 25 mL distilled water) were determined using the Rapid Visco Analyzer (RVA-S4A, Newport Scientific, Warriewood, Australia). The slurry was held at 50 ºC for 1 min, heated to 95 ºC at a rate of 6 ºC/min, held at 95 ºC for 5 min, cooled to 50 ºC at a rate of 6 ºC/min, and held at 50 ºC for 2 min. The samples were also analyzed with the pH adjusted to 7. The pH adjustment was performed to eliminate the pH effect on the pasting properties since the reduction of the pH with the irradiation is detected in previous work (Polesi et al, 2016). To this end, the sample was weighed and distilled water was added to make up to 27 g (3 g sample + 24 g water). The pH was adjusted to 7.0 (0.01 mol/L NaOH) and distilled water was added to make up to 28 g, following the same analysis parameters without pH adjustment.

Gel firmness

The gel firmness of starch was analyzed using a texturometer (TA.XT Plus Texture Analyzer, Stable Micro Systems Ltd., Godalming, Surrey, United Kingdom) in the samples that were analyzed by RVA. The canister with the starch paste analyzed in RVA was covered with a parafilm wrap and stored at 4 ºC for 24 h. The gel was compressed using a cylindrical probe P0.5R (Ø = 12 mm) at a pre-test speed of 1.5 mm/s, test speed of 1.0 mm/s, and post-test speed of 1.0 mm/s to a distance of 10 mm. The peak force measured was reported as the gel firmness.

Experimental design and statistical analysis

The experiment was conducted as a 2 × 4 completely randomized factorial, with two rice varieties, four gamma radiation doses, and three replications of the irradiation process. The results were subjected to analysis of variance (ANOVA) and Tukey's test ($P < 0.05$) for comparison of mean values using the ASSISTAT software, version 7.7 beta (Silva, 2014).

RESULTS

Starch digestibility and total dietary fiber

The *in vitro* digestibility of raw starch (Table 1) was found to be higher in SDS fraction than in RDS and RS fractions in both cultivars. RDS showed no difference for IRGA417, but showed a reduction in digestibility at 2 kGy for IAC202. In general, irradiation promoted an increase in SDS with a reduction in RS for IAC202. For IRGA417, a nonlinear behavior of starch digestibility was observed, with a reduction in SDS and an increase in RS at 1 kGy, with no change at 2 kGy, and an increase in SDS with a reduction in RS at 5 kGy.

The cooking of starch increased RDS content and reduced SDS and RS contents (Table 1). IAC202 showed a decrease in RDS and RS with an increase in SDS at 1 kGy, a decrease in RDS with an increase in SDS and RS at 2 kGy, and an increase in RDS with a decrease in SDS and RS at 5 kGy. On the other hand, IRGA417 showed an increase in RDS with a decrease in RS at 1 kGy, a decrease in SDS with an increase in RS at 2 kGy, and an increase in RDS with a decrease in SDS at 5 kGy.

TDF content did not change with irradiation for IRGA417 (Table 2). TDF showed no significant difference between the control and the irradiated sample IAC202. However, when comparing only irradiated samples, the highest dose (5 kGy) resulted in the highest TDF content.

Table 1. Starch digestibility of raw and cooked samples of native and irradiated starch of two rice cultivars. %

Type	Dose	RDS		SDS		RS	
		IAC202	IRGA417	IAC202	IRGA417	IAC202	IRGA417
Raw starch	0 kGy	31.3 ± 0.4 bA	40.5 ± 0.4 aA	51.4 ± 1.0 aC	52.1 ± 0.8 aB	16.5 ± 1.0 aA	6.1 ± 0.3 bB
	1 kGy	31.8 ± 0.6 bA	40.4 ± 0.8 aA	62.6 ± 0.8 aA	47.4 ± 1.0 bC	4.7 ± 0.8 bD	10.8 ± 0.6 aA
	2 kGy	29.2 ± 1.0 bB	40.1 ± 0.5 aA	59.4 ± 0.9 aB	51.6 ± 0.9 bB	10.5 ± 0.4 aB	7.0 ± 0.6 bB
	5 kGy	31.3 ± 0.2 bA	40.7 ± 0.4 aA	60.6 ± 0.9 aB	55.0 ± 0.8 bA	7.2 ± 1.0 aC	3.0 ± 0.4 bC
Cooked starch	0 kGy	87.6 ± 1.2 aB	88.7 ± 1.1 aB	7.8 ± 0.7 aB	6.5 ± 1.0 aA	3.8 ± 0.7 aB	3.4 ± 0.7 aB
	1 kGy	83.0 ± 0.6 bC	92.6 ± 1.1 aA	15.1 ± 1.4 aA	5.1 ± 0.7 bAB	1.1 ± 0.4 aC	1.0 ± 0.7 aC
	2 kGy	76.6 ± 0.8 bD	88.6 ± 1.6 aB	15.5 ± 0.5 aA	4.5 ± 0.5 bB	7.0 ± 0.8 aA	5.7 ± 0.5 bA
	5 kGy	95.2 ± 0.8 aA	92.6 ± 0.4 bA	2.6 ± 0.6 aC	2.2 ± 0.3 aC	1.4 ± 0.4 bC	3.9 ± 0.4 aB

RDS, Rapidly digestible starch; SDS, Slowly digestible starch; RS, Resistant starch.

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a column for the same starch type and the same lowercase letter within a row for each parameter do not differ significantly according to Tukey's test ($P < 0.05$).

Table 2. Total dietary fiber content (% dry basis) and instrumental color of native and irradiated starch of two rice cultivars.

Dose	TDF		*L*		*b**	
	IAC202	IRGA417	IAC202	IRGA417	IAC202	IRGA417
0 kGy	0.6 ± 0.2 aAB	0.7 ± 0.2 aA	99.4 ± 0.3 aA	98.7 ± 0.3 bB	6.0 ± 0.1 aD	5.9 ± 0.0 bD
1 kGy	0.3 ± 0.1 bB	0.7 ± 0.2 aA	99.6 ± 0.2 aA	99.5 ± 0.5 aA	6.2 ± 0.0 aC	6.2 ± 0.0 aC
2 kGy	0.4 ± 0.3 aB	0.5 ± 0.1 aA	99.2 ± 0.2 aA	99.6 ± 0.2 aA	6.4 ± 0.1 aB	6.4 ± 0.1 aB
5 kGy	1.0 ± 0.2 aA	0.6 ± 0.2 bA	99.6 ± 0.3 aA	99.4 ± 0.2 aA	6.8 ± 0.1 aA	6.9 ± 0.1 aA

TDF, Total dietary fiber; *L*, Lightness; *b**, Yellow/blue coordinate.

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a column and the same lowercase letter within a row for each parameter do not differ significantly according to Tukey's test ($P < 0.05$).

Color, WAI, WSI and syneresis

The effect of gamma radiation on parameter *L* occurred in different ways for the cultivars (Table 2). IAC202 showed no difference between the radiation doses, while IRGA417 showed slight whitening, considering that the original samples were already quite white in color (values near 100). Both cultivars showed an increase in value of *b** (Table 2), which was proportional to the radiation dose. WAI and WSI increased with increasing gamma radiation doses in both cultivars (Table 3). The cultivars showed a different behavior regarding syneresis with increasing doses of gamma radiation (Table 3). IAC202 showed less syneresis, without any variation between the samples that received the different doses of gamma radiation. IRGA417 showed an increase in syneresis with up to 2 kGy and a reduction at 5 kGy.

Swelling factor (SF) and amylose leaching (AML)

SF showed variability with the different temperatures, cultivars and radiation doses (Table 4 and Fig. 1). The increase in the heating temperatures caused an increase in swelling of the granules. Samples of both cultivars irradiated at 1 and 5 kGy doses showed higher expansion of starch at 55 ºC. IRGA417 at 5 kGy showed higher resistance to expansion for up to 85 ºC; however, at 95 ºC, it showed the highest expansion. At 95 ºC, there was a decrease in SF with irradiation for IAC202 and an increase for IRGA417. Irradiation as well as the temperature increased amylose leaching for both cultivars (Table 4 and Fig. 1).

Pasting properties and gel firmness

The pasting properties of the rice starches from both cultivars were evaluated in samples with only distilled

Table 3. Water absorption index (WAI), water solubility index (WSI) and syneresis of native and irradiated starch of two rice cultivars.

Dose	WAI (g/g)		WSI (%)		Syneresis (%)	
	IAC202	IRGA417	IAC202	IRGA417	IAC202	IRGA417
0 kGy	2.08 ± 0.01 bB	2.11 ± 0.02 aB	0.05 ± 0.01 aD	0.06 ± 0.04 aD	0.28 ± 0.01 bA	2.38 ± 0.09 aC
1 kGy	2.10 ± 0.02 aB	2.12 ± 0.01 aAB	0.11 ± 0.01 aC	0.12 ± 0.01 aC	0.33 ± 0.01 bA	2.77 ± 0.21 aB
2 kGy	2.10 ± 0.02 bB	2.15 ± 0.01 aA	0.15 ± 0.01 aB	0.16 ± 0.01 aB	0.38 ± 0.02 bA	3.93 ± 0.12 aA
5 kGy	2.17 ± 0.01 aA	2.15 ± 0.01 aA	0.27 ± 0.01 aA	0.24 ± 0.02 bA	0.42 ± 0.02 bA	2.44 ± 0.06 aC

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a column and the same lowercase letter within a row for each parameter do not differ significantly according to Tukey's test ($P < 0.05$).

Table 4. Swelling factor and amylose leaching of native and irradiated starch of two rice cultivars.

T (°C)	Cultivar	Swelling factor				Amylose leaching (%)			
		0 kGy	1 kGy	2 kGy	5 kGy	0 kGy	1 kGy	2 kGy	5 kGy
55	IAC202	2.8 ± 0.2 bB	4.0 ± 0.4 bA	3.3 ± 0.5 aAB	4.4 ± 0.6 bA	0.02 ± 0.01 bB	0.05 ± 0.02 bB	0.14 ± 0.05 bA	0.20 ± 0.03 bA
	IRGA417	3.8 ± 0.4 aC	5.7 ± 0.4 aB	4.0 ± 0.8 aC	7.7 ± 0.4 aA	0.22 ± 0.02 aC	0.27 ± 0.03 aBC	0.32 ± 0.01 aB	0.49 ± 0.02 aA
65	IAC202	4.8 ± 0.4 bC	6.0 ± 0.2 bA	5.8 ± 0.2 aAB	5.2 ± 0.5 bBC	1.55 ± 0.02 bD	1.96 ± 0.02 bC	2.37 ± 0.10 bB	3.38 ± 0.16 bA
	IRGA417	7.6 ± 0.1 aA	8.2 ± 0.1 aA	6.1 ± 0.2 aB	7.9 ± 0.1 aA	2.13 ± 0.06 aD	2.72 ± 0.10 aC	3.43 ± 0.07 aB	5.13 ± 0.14 aA
75	IAC202	10.2 ± 0.6 aA	10.4 ± 0.1 aA	10.1 ± 0.3 aA	7.8 ± 0.3 aB	8.00 ± 0.32 aD	9.79 ± 0.29 aC	10.98 ± 0.09 aB	13.92 ± 0.02 aA
	IRGA417	9.2 ± 0.6 bA	8.2 ± 0.4 bAB	7.9 ± 0.7 bB	7.6 ± 0.4 aB	4.09 ± 0.08 bD	5.14 ± 0.17 bC	6.49 ± 0.31 bB	8.99 ± 0.14 bA
85	IAC202	10.7 ± 0.3 aB	11.7 ± 0.5 aA	11.5 ± 0.1 aA	10.8 ± 0.4 aB	19.89 ± 0.29 aC	23.23 ± 0.59 aB	23.82 ± 0.58 aB	27.46 ± 0.61 aA
	IRGA417	10.5 ± 0.1 aA	9.8 ± 0.1 bB	10.3 ± 0.2 bAB	8.3 ± 0.2 bC	11.44 ± 0.45 bD	14.38 ± 0.18 bC	15.64 ± 0.45 bB	19.78 ± 0.03 bA
95	IAC202	25.7 ± 0.5 aA	23.8 ± 0.2 aC	24.9 ± 0.1 aB	24.4 ± 0.1 bB	33.56 ± 0.22 aC	36.46 ± 0.55 aB	36.74 ± 0.78 aB	42.29 ± 0.54 aA
	IRGA417	18.7 ± 0.1 bC	18.8 ± 0.3 bBC	19.3 ± 0.1 bB	24.8 ± 0.2 aA	24.82 ± 0.15 bD	27.99 ± 0.55 bC	29.77 ± 0.26 bB	33.75 ± 0.79 bA

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a row for each parameter and the same lowercase letter within a column for each temperature do not differ significantly according to Tukey's test ($P < 0.05$).

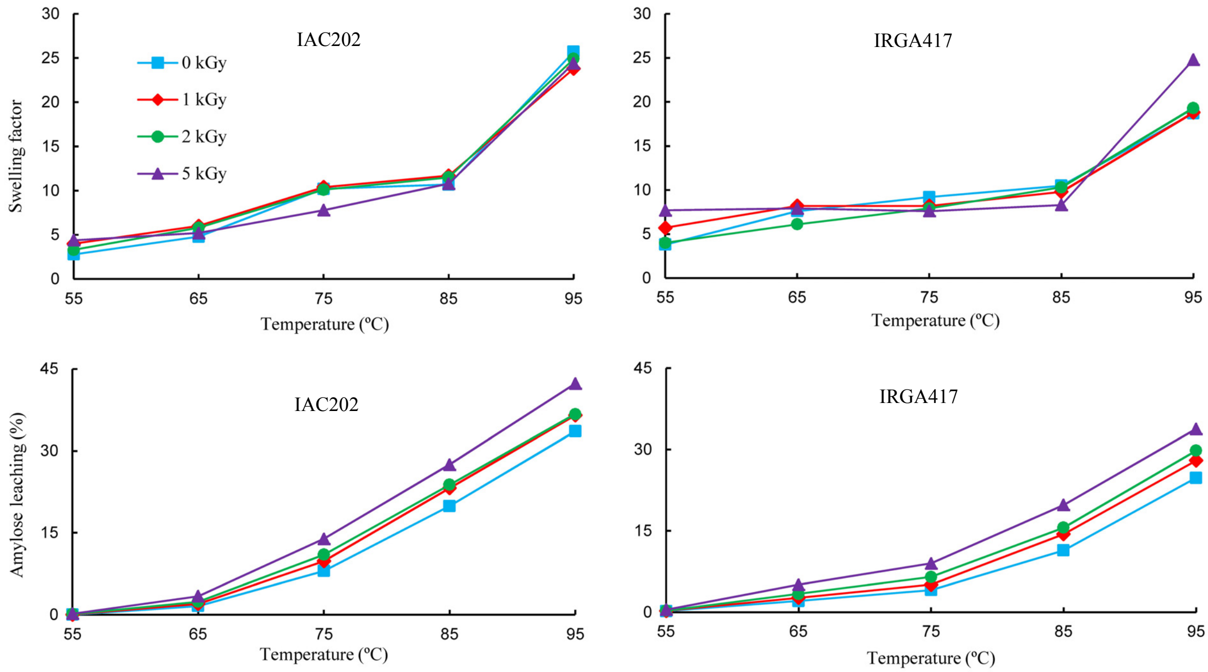

Fig. 1. Swelling factor of granules in the aqueous slurry (2%) and amylose leaching of granules in the aqueous slurry (2%) of native and irradiated starch of two rice cultivars.

water and in samples with distilled water with the pH adjusted to 7. Viscosity profiles of the samples with pH unadjusted did not differ in relation to the profiles of the samples with pH adjusted (Fig. 2).

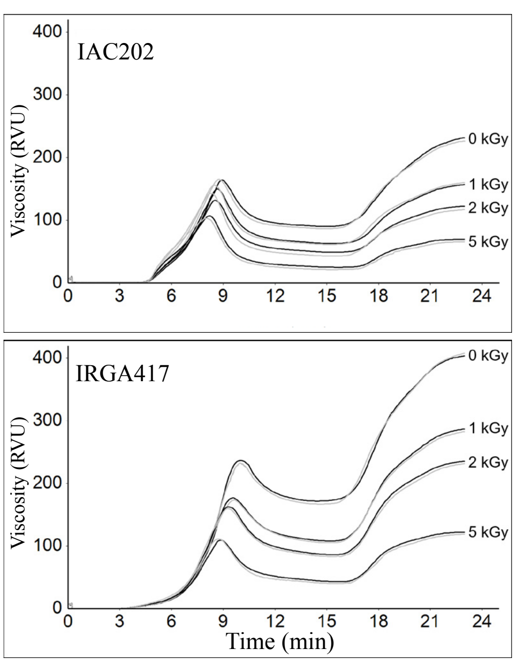

Fig. 2. RVA pasting profile of aqueous slurry (9.2%) of native and irradiated starch of two rice cultivars.

Black line shows data with pH unadjusted. Gray line shows data with pH adjusted to 7.

Irradiation caused a decrease in the viscosity of the starch pastes (Fig. 2, Tables 5 and 6) in both cultivars, which was proportional to the dose. Peak viscosity showed reduction with increasing doses of gamma radiation. Breakdown viscosity showed the highest values at 1 kGy for IAC202 and 2 kGy for IRGA417. Nevertheless, when the breakdown is compared to the peak viscosity, it is possible to observe an increase of the breakdown viscosity with the increasing doses. The ratio of breakdown viscosity to peak viscosity was 45%, 58%, 63% and 75% for IAC202, and 27%, 39%, 48% and 60% for IRGA417, at 0, 1, 2 and 5 kGy, respectively. Pasting temperature reduced with irradiation for IAC202 and increased for IRGA417.

The starch paste obtained by the RVA was stored under refrigeration for 24 h and the resulting gels were analyzed for firmness using a texturometer (Tables 5 and 6). For IAC202, the firmness increased with increasing radiation doses, while for IRGA417, there was a significant decrease at 5 kGy.

DISCUSSION

Starch digestibility and total dietary fiber

IRGA417 showed higher RS content and relative crystallinity at 1 kGy, as demonstrated recently (Polesi et al, 2016). This increase in RS is probably

Table 5. Peak viscosity, breakdown and final viscosity of native and irradiated starch of two rice cultivars.

Dose	Peak viscosity (RVU)		Breakdown (RVU)		Final viscosity (RVU)	
	IAC202	IRGA417	IAC202	IRGA417	IAC202	IRGA417
0 kGy	164 ± 1 bA	236 ± 1 aA	73 ± 1 aC	63 ± 1 bC	230 ± 4 bA	418 ± 2 aA
1 kGy	150 ± 1 bB	177 ± 2 aB	87 ± 3 aA	69 ± 1 bB	158 ± 5 bB	289 ± 3 aB
2 kGy	131 ± 1 bC	159 ± 4 aC	82 ± 1 aB	76 ± 1 bA	122 ± 1 bC	233 ± 3 aC
5 kGy	112 ± 4 aD	111 ± 1 aD	84 ± 2 aAB	67 ± 0 bBC	76 ± 6 bD	127 ± 4 aD

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a column and the same lowercase letter within a row for each parameter do not differ significantly according to Tukey's test (P < 0.05).

Table 6. Setback, pasting temperature and gel firmness of native and irradiated starch of two rice cultivars.

Dose	Setback (RVU)		Pasting temperature (°C)		Gel firmness (g)	
	IAC202	IRGA417	IAC202	IRGA417	IAC202	IRGA417
0 kGy	140 ± 3 bA	245 ± 1 aA	76.6 ± 0.2 bA	83.3 ± 0.0 aC	184 ± 3 bD	225 ± 2 aA
1 kGy	95 ± 3 bB	180 ± 2 aB	74.1 ± 0.4 bC	84.1 ± 0.0 aB	194 ± 2 bC	229 ± 3 aA
2 kGy	74 ± 1 bC	150 ± 1 aC	74.6 ± 0.2 bB	83.5 ± 0.2 aC	218 ± 2 aB	223 ± 3 aA
5 kGy	49 ± 3 bD	83 ± 3 aD	72.8 ± 0.0 bD	84.9 ± 0.0 aA	228 ± 3 aA	120 ± 5 bB

Values are mean ± SD (n = 3). Values followed by the same uppercase letter within a column and the same lowercase letter within a row for each parameter do not differ significantly according to Tukey's test (P < 0.05).

related to the crystallinity, because the crystalline regions are more resistant to enzymatic hydrolysis. For IAC202, the relationship between RS and crystallinity is not clear. The behavior of starch upon irradiation is not always similar, because their structures are different. Bao et al (2005) also found an increase in the crystallinity of irradiated rice starch at 1 kGy.

Studies using application of gamma radiation up to 50 kGy in starch of corn (A-type polymorphs), potato (B-type polymorphs) and bean (C-type polymorphs) concluded that irradiation increases the RS content, irrespective of the polymorph (Chung and Liu, 2009, 2010). However, the rice starch that has A-type polymorphs (Polesi et al, 2016), in the present study, showed no increase in RS with increasing irradiation doses.

The increase in RDS after starch cooking is maybe because the granular structure is lost and amylose is leached when the starch is gelatinized and more starch molecules are readily available for the enzyme, making it more susceptible. According to Chung and Liu (2009), amylose and amylopectin degradation caused by irradiation, with a consequent decrease in crystallinity and ordered structure of the granules, increases the starch digestibility. They also concluded that the formation of carboxyl groups and crosslinking inhibits the enzymatic attack, thereby reducing the starch digestibility. Thus, the combination of these factors makes it possible to increase or decrease the starch digestibility, depending on the factor that predominates.

TDF showed no relationship with RS content, indicating that the structures resistant to physiological digestion (37 ºC) were not resistant to digestion at the high temperature (97 ºC) of TDF analysis. This is because the temperature provides energy and breaks the hydrogen bonds that stabilize the starch structure, which causes the chains to become more open and accessible to enzymes.

Instrumental color, WAI, WSI and syneresis

The positive values of b* denoted a tendency toward yellow color. Therefore, there was a yellowing of the samples with irradiation. The yellowing showed by irradiated starches was also observed by Kang et al (1999), who attribute it to the caramelization reaction of monosaccharides generated by cleavage of the starch macromolecules. Other researchers (Lee et al, 2007; Falade and Kolawole, 2013) considered the Maillard reaction, another reaction of non-enzymatic browning, as a cause of yellowing in irradiated foods. According to these authors, irradiation produces free radicals and radiolysis products, reducing sugars and amino acids, which may condense and produce colored compounds.

Increase in WAI by irradiation was also observed in a study of irradiated bean starch (Abu et al, 2006; Gani et al, 2012), which is attributed to the damages that gamma radiation causes in the starch granules. Water absorption by the starch granules at room temperature occurs due to diffusion and absorption of water molecules in the amorphous regions (Bello-Pérez at al, 2006). Irradiation could disrupt both the crystalline and amorphous regions depending on the radiation dose (Chung and Liu, 2009). The probable reason for the increase in the WAI of the samples is the internal disruption of the starch granule, which facilitates the entry of water, without necessary relation to the crystallinity, since IRGA417 showed increased relative crystallinity by irradiation (Polesi et al, 2016). The reduction of the molecular weight of amylose and amylopectin (Polesi et al, 2016) confirms this internal disruption of the starch granules.

Increase in WSI with increasing gamma radiation doses was also observed in starches from beans, potatoes and corn subjected to irradiation (Lee et al, 2006; Henry at al, 2010; Gani et al, 2012; Liu et al, 2012). The increase in water solubility can result from an increase in the starch polarity due to the breakdown of glycosidic bonds and formation of carboxyl groups, as well as the reduction of hydrogen bonds between the starch chains (Henry et al, 2010; Liu et al, 2012). In an earlier study with these cultivars, we verified the reduction in starch chain size and an increase in the carboxyl content with irradiation (Polesi et al, 2016), which corroborates the increase in solubility.

Syneresis is the process during which water is released by starch gel stored under refrigeration. Syneresis depends on the re-association of starch molecules after gelatinization, and therefore, it is directly related to the retrogradation of starch gel (Karim et al, 2000). The increase in syneresis for IRGA417 may be due to the molecular fragmentation. Smaller molecules are easier to re-associate and form a stronger gel, which drive out more water from the gel. At 5 kGy dose, the break may have been too intense, generating very short chains, which are not able to re-associate.

Swelling factor (SF) and amylose leaching (AML)

The increase in the heating temperatures caused an increase in swelling of the granules because the higher the temperature, the more energy delivered to promote disruption of hydrogen bonds and opening up of the granules to water absorption.

The increase in the expansion of the starch granules at 55 ºC can be caused by the breaking of the molecules, which probably caused disruption of the crystalline regions, and at temperatures below the gelatinization temperature, further expansion occurred.

The resistance to expansion for up to 85 ºC and the great increase in expansion at 95 ºC showed by IRGA417 at 5 kGy can indicate that irradiation promoted new molecular interactions in the starch, which required more energy to expand. However, these results also indicate the breakdown of starch molecules with weakening of the granular structure, because once the energy barrier for expansion is broken, the granules swell more.

Some studies have shown a reduction in SF with increasing doses of gamma radiation (Abu et al, 2006; Chung and Liu, 2009, 2010; Darfour et al, 2012; Gani et al, 2012). Swelling of the starch granules reveals the ability to absorb and retain water within the structure. Considering that amylopectin is the primary macromolecule responsible for granular expansion, irradiation can cause ruptures in amylopectin, reducing the expansion of the granules (Tester and Morrison, 1990; Gani et al, 2012).

A decrease in SF is related to the degradation of amylopectin by gamma rays; on the other hand, an increase in SF may be related to better restructuring of amylopectin. According to Chung and Liu (2009), molecular rearrangement associated with the formation of new double helical structure followed by dense packing (crystalline order) may occur when a longer irradiation time (low dose rate) is used.

The increase in amylose leaching by irradiation was observed in the starches of corn, bean and potato (Chung and Liu, 2009, 2010), which is attributed to the production of fractions of lower molecular weight and degradation of starch structure. Gel permeation chromatography confirms the breakdown of molecules with irradiation, which justifies the increase in amylose leaching (Polesi et al, 2016).

The temperature provides energy for the breakdown of the hydrogen bonds with consequent expansion of the granules, therefore, the higher the temperature, the greater the expansion. The greater the expansion of the granules, the easier the leaching of the amylose. Being a soluble molecule, the amylose is drawn out of the starch granule.

Pasting properties and gel firmness

The irradiation reduces the pH of the starch samples (Polesi et al, 2016). The pH correction was performed to verify the influence of lower pH caused by irradiation. However, there was no interference of pH in the pasting properties of the samples.

Irradiation caused a decrease in the paste viscosity proportional to the dose, in both cultivars. Reports on irradiated starches often show a reduction in paste viscosity (Bao et al, 2005; Abu et al, 2006; Chung and

Liu, 2009, 2010; Gani et al, 2012; Falade and Kolawole, 2013). The reduction in viscosity may be related to the reduction of the molecular weight of the starch macromolecules, which is caused by the breakdown of the glycosidic bonds (Chung and Liu, 2009). According to Falade and Kolawole (2013), starch degradation is the result of high energy rays that are capable of hydrolyzing chemical bonds, thereby cleaving the starch macromolecules into smaller fragments of dextrin and sugars, and thus, the pasting properties are decreased. IAC202 and IRGA417 starches showed reduction of molecular weight of the starch molecules in previously published data (Polesi et al, 2016), which supports the reduction of viscosity.

Pasting temperature is the temperature at which noticeable increase in viscosity occurs and is usually higher than the gelatinization temperature (Falade and Kolawole, 2013), because it is related to swelling of the starch granules that occurs after rupture of the crystalline regions. Chung and Liu (2010) observed a decrease in pasting temperature with irradiation in bean and potato starches and attributed the decrease to the degradation of the molecular structure, which may cause expansion of the granules at lower temperatures. On the other hand, the increase in pasting temperature may be related to the reorganization of starch molecules, requiring more energy for gelatinization (Polesi et al, 2016).

Gel firmness is primarily caused by retrogradation and it depends on factors such as amylose chain length, entanglement and rate of aggregation of amylose chains that provide elasticity to the retrograded gels (Singh et al, 2011; Oladebeye et al, 2013). The gel texture properties are also related to the leached amylose during gelatinization (Jiang et al, 2011). The increase in gel firmness in IAC202 may be due to the breakdown of starch molecules that favored leaching and association in an ordered structure, since hydrolyzed chains have a size more favorable to the formation of crystalline structure. The reduction in firmness in IRGA417 at 5 kGy may be due to an excessive breakdown of amylose molecules during irradiation, which hampered or prevented the association of molecules under refrigeration.

CONCLUSIONS

Irradiation was effective in modifying the starch. It caused an increase in SDS content with a reduction in RS content for IAC202. For IRGA417, a nonlinear behavior of starch digestibility was observed, with a decrease in SDS content and an increase in RS content at 1 kGy, no change at 2 kGy, and an increase in SDS content with a decrease in RS content at 5 kGy. Irradiation also caused yellowing of the starch of both rice cultivars, as well as increased water absorption capacity, water solubility and amylose leaching. There was a reduction in paste viscosity proportional due to the increasing of the radiation dose. IAC202 rice starch showed higher gel firmness and IRGA417 showed lower gel firmness at 5 kGy. Thus, IAC202 could be a good ingredient for applications that require lower paste viscosity and higher gel firmness, with low occurrence of syneresis. On the other hand, IRGA417 can be used in applications requiring lower hot viscosity and equal or less cold firmness.

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