

## Study on rapid detection of irradiated rice based on RVA profile Postprint

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**Date:** 2023-06-18T00:00:00+00:00

### Abstract

In order to confirm whether the rice sample was exposed to gamma radiation, the changes in starch pasting viscosity of rice irradiated by different absorbed doses with additive acid solution and distilled water were investigated. The peak viscosity of irradiated rice sample was significantly decreased with the increment of absorbed doses even for the samples stored over 360 days. Irradiation samples can be distinguished from non-irradiated samples by their RVA profile as in the acid solution (pH=2.5), the starch pasting viscosities of irradiated rice samples were apparently higher while the starch pasting viscosities of non-irradiated rice samples were lower than that in distilled water (pH=7.0). The irradiation absorbed dose can be estimated accurately by the regression equation constructed with peak viscosity and absorbed doses.

### Full Text

### Preamble

**Nuclear Science and Techniques 24 (2013) 040301**

**Study on Rapid Detection of Irradiated Rice Based on RVA Profile**

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### Abstract

This study investigated changes in the starch pasting viscosity of rice exposed to different gamma radiation doses, using both acidic solution and distilled water as additives to confirm whether rice samples had been irradiated. The peak viscosity of irradiated rice decreased significantly with increasing absorbed dose,

even for samples stored over 360 days. Irradiated samples could be distinguished from non-irradiated samples by their RVA profile: in acid solution (pH=2.5), the starch pasting viscosities of irradiated rice samples were apparently higher, while those of non-irradiated samples were lower than in distilled water (pH=7.0). The irradiation absorbed dose could be estimated accurately using a regression equation constructed from peak viscosity and absorbed dose data.

**Keywords:** Rice, Starch, Irradiation, RVA profile

## Introduction

Ionizing radiations such as gamma rays, X-rays, and electron beams have been employed as convenient and efficient treatments for food preservation. The minimal physical, chemical, biological, and microbiological changes induced in irradiated foods can be detected to determine whether food has been irradiated. The international standard on “Irradiated Food Detection Methods” proposed by the EU was approved by 2004<sup>1–10</sup>. These methods can be classified into chemical, biological, and physical categories. Establishing detection methods for irradiated food provides standards for distinguishing irradiated from non-irradiated samples and for determining absorbed doses. Global research efforts have achieved significant progress in assessing irradiated food, with six European standards (GC/MS, TL, etc.) widely adopted and expected to remain primary methods for the foreseeable future. Four recommended standards (such as LAL/GNB, PSL, etc.) were approved by the European Committee for Standardization and can be used in preliminary screening due to their simple, convenient, and economical operations. Meanwhile, simple and economical methods for identifying irradiated food, such as ultraweak bioluminescence, have attracted considerable interest<sup>11</sup>.

Rice is one of the major cereals, with starch as its main component consisting of amylose and amylopectin. Rice storage methods are closely related to food safety and public health. Techniques for keeping rice fresh can protect it from insect infestation and microbial contamination during storage, processing, market circulation, and consumption. Gamma irradiation, with its high penetrating power and thorough insecticidal effect<sup>12</sup>, is an economical and effective method for rice preservation. Capable of killing worm eggs inside rice grains without leaving odor or residual toxicity, gamma irradiation is an economical and effective approach for preservation and has attracted increasing attention. However, few reports have addressed methods for assessing whether rice has been irradiated and for estimating absorbed doses.

In this paper, to distinguish irradiated rice from non-irradiated rice without reference samples, we investigated the pasting viscosity of rice starches (round-shaped rice) with different absorbed doses and different solution pH values using a Rapid Visco Analyser (RVA). This approach provides valuable information for detecting irradiated rice.

## 2.1 Sample Source

Round-shaped japonica rice was purchased from a local supermarket. The samples were divided into five equal portions of 50 g each, sealed in polyethylene (PE) bags, and stored in a desiccator until use.

## 2.2 Irradiation Treatment

Rice samples were exposed to a 60-Cobalt source at ambient temperature at the Irradiation Center of Zhejiang University, Hangzhou, China. The gamma radiation activity was  $2.96 \times 10^{15}$  Bq. Five irradiation doses were applied: 0, 1, 2, 3, and 5 kGy, with a dose rate of 0.8 kGy/h. All samples were placed at the same horizontal level as the irradiation source and irradiated at natural temperature.

## 2.3 Determination of Starch Pasting Viscosity

After irradiation, samples were ground into powders and passed through a 100-mesh sieve. Starch pasting viscosity was determined in duplicate using a Rapid Visco Analyser (RVA, Model-3D, Newport Scientific Inc., Australia) controlled by Thermo Cycle for Windows (TCW) software (American Association of Cereal Chemists (AACC), 1998)<sup>13</sup>. Rice starch (3.0 g, 14% moisture content) was weighed into the sample cup, and 25 mL distilled water was added just before analysis. The temperature profile while stirring was as follows: maintained at 50°C for 1 minute, then raised to 95°C at 12°C/min, held at 95°C for 2.5 min, cooled to 50°C, and maintained at 50°C for 1.4 min. The paddle rotated at 960 rpm for the first 10 seconds, then at 160 rpm until completion.

Starch pasting viscosity parameters were expressed in Rapid Visco Units (RVU). The characteristic values of the RVA profile included peak viscosity (PV), hot viscosity (HV), cool viscosity (CV), breakdown (PV minus HV), and setback (CV minus PV).

## 2.4 Detection Method

Two parallel samples were selected from the starch samples without gelatinization: one was mixed with acid solution (pH=2.5), and the other with distilled water (blank experiment). All samples were stirred uniformly under identical conditions, and starch pasting viscosity was measured using the Rapid Visco Analyser while monitoring dynamic changes in the RVA profile. Samples were determined to be irradiated if the RVA profile at pH=2.5 was higher than that of samples with distilled water. Samples were determined to be non-irradiated if the RVA profile at pH=2.5 was lower than that of samples with distilled water.

### 3.1 Effect of Different Absorbed Doses on Starch Pasting Viscosity

The major RVA profile parameters—PV, HV, and CV—decreased considerably with increasing absorbed doses when rice starch was irradiated at different levels, as shown in Table 1. The viscosity of the non-irradiated sample was significantly higher than that of irradiated samples. With increasing absorbed doses, all viscosity values including PV, HV, and CV decreased when samples were mixed with distilled water at pH 7.

When acid solution (pH 2.5) was added, PV was higher than that with distilled water at pH 7, and PV still decreased with absorbed dose as listed in Table 2. The reduction in starch pasting viscosity values can serve as a reference for determining whether samples have been irradiated. Some reports suggest that starch degradation reactions resulting in decreased molecular weight are responsible for the decline in starch viscosity when amylose and amylopectin undergo radiation degradation<sup>14</sup>. Radiation can cause cleavage of polysaccharide chains, generating degradable dextrin fragments, which reduces starch content<sup>15</sup>.

### 3.2 Effect of Solution pH on RVA Profile

According to the method described in Section 2.4, samples were divided into two equal portions: one mixed with distilled water (pH 7) and the other with acid solution (pH 2.5). The RVA profile was determined by the Rapid Visco Analyser. As shown in Fig. 1 [Figure 1: see original paper], RVA profiles at pH=2.5 were higher than those at pH=7 for non-irradiated rice.

However, for irradiated samples, starch pasting viscosity values at pH=7 were lower than those at pH=2.5, which were still less than those of non-irradiated counterparts. Higher irradiation doses resulted in lower starch pasting viscosity. This can be explained by hydrogen ions cleaving small polysaccharide chains caused by irradiation, and as a result, cleavage of large molecular weight compounds compensates for the viscosity reduction. Hence, we can easily and rapidly inspect whether rice starch has been exposed to irradiation treatment by adding acid solution to markedly alter the RVA profile.

### 3.3 Effect of Storage Time After Irradiation on RVA Profile

To maintain rice breathability, both irradiated and non-irradiated samples were placed in nylon bags on an experimental desk and stored for 360 days to investigate changes in starch pasting viscosity. It was found that PV values decreased by 1.56% after 360 days of storage in non-irradiated samples, while reductions of 6.58% were observed in samples irradiated at 1 kGy, 3.25% at 3 kGy, and 10.69% at 5 kGy, as listed in Table 3. The decrease in RVA profile was also clearly observed after 360 days of storage (Fig. 2 [Figure 2: see original paper]), indicating that fragmentation of molecular chains continued to occur in irradiated macromolecules of amylose and amylopectin during storage.

### 3.4 Relationship Between Viscosity and Absorbed Doses

Based on the relationships between PV, HV, CV and absorbed doses, the correlation between the logarithm of starch pasting viscosity (Y) and absorbed doses can be expressed as follows:  $Y_{pv}=3.5012-0.1277X$  ( $R^2=0.9920$ ),  $Y_{hv}=3.3207-0.2139X$  ( $R^2=0.9916$ ),  $Y_{cv}=3.5003-0.1575X$  ( $R^2=0.9868$ ), allowing calculation of absorbed dosage<sup>16</sup>. Here,  $Y_{pv}$ ,  $Y_{hv}$ , and  $Y_{cv}$  represent the logarithm of starch pasting viscosity for PV, HV, and CV respectively, and X is the absorbed dose (kGy). Thus, the absorbed doses for irradiated rice samples can be calculated. When PV is used, it is relatively reasonable to estimate actual absorbed doses based on the PV exponential correlation, though the theoretical calculation is slightly lower than the determined value, as shown in Table 4 .

Additionally, as shown in Table 3, the PV of irradiated rice samples with added acid aqueous solution should be higher than with distilled water, while the PV of non-irradiated rice samples with added acid solution should be lower than with distilled water. Therefore, in practical application, we can distinguish whether samples were irradiated: if PV of rice samples is higher under neutral conditions than under acidic conditions, the samples were not irradiated.

### 4.1 Mechanism for the Decrement of Starch Pasting Viscosity Caused by Irradiation

Starch consists primarily of amylose and amylopectin. Amylose, commonly called linear starch, is composed of D-anhydroglucose units linked by  $\alpha$ -1,4 glycosidic bonds, while amylopectin is composed of D-anhydroglucose units connected by  $\alpha$ -1,6 and  $\alpha$ -1,4 glycosidic bonds, with branching occurring at  $\alpha$ -1,6 glycosidic bonds<sup>17</sup>. Starch pasting viscosity decreased with increasing absorbed doses (Tables 1 and 2). Gamma irradiation degrades starch through cleavage of glycosidic linkages and peptide bonds into smaller fragments such as short-chain aldehydes, ketones, acids, monosaccharides (glucose, maltose, and dextrin), peptide derivatives, amino acids, and other low molecular weight fragments, thereby reducing starch pasting viscosity<sup>18</sup>.

Water in irradiated samples can generate free radicals and hydrated electrons, which react with groups in glycosidic bonds and peptides, as well as with radicals generated from proteins after irradiation. Starch can be further cleaved through a chain reaction that accelerates the decrease in starch pasting viscosity<sup>19</sup>. The  $H\cdot$  in water can also react with groups on aldehydes, ketones, and acids, leading to polymerization of short chains, which can compensate for starch pasting viscosity to a certain extent.

### 4.2 Error Analysis for the Estimated Irradiation Dosage

There was relative error between theoretically calculated values and actual irradiation doses according to the regression equations (Table 3). The relative error between theoretically calculated values and actual irradiation doses ranged from

2.74% to 7.03% when estimated by PV exponential correlation, 0.73% to 10.53% by HV, and 4.28% to 10.93% by CV. The main sources of error can be described as follows:

First, error is caused by irradiation geometry. The absorbed dose designed in the experiment may not match the actual dose received by the sample since samples have volume rather than being a single point. Second, static irradiation processes introduce inaccuracy when the actual distance between samples and the irradiation source differs from the calculated distance. Third, it is necessary to correct for radioactivity decay when samples are being irradiated, ensuring samples maintain the same horizontal level as the irradiation source. Fourth, error is caused by sampling time for detection. Finally, system error arises from measurement of starch pasting viscosity in the samples. Based on analysis of experimental data, lower absorbed doses correspond to larger errors, while higher absorbed doses yield smaller errors.

## 5 Conclusion

The change in rice starch pasting viscosity (RVA) was significant after rice was treated with irradiation, with RVA values decreasing as irradiation doses increased. In this study, we compared RVA changes of the same samples tested in solutions with different pH values and found that for irradiated samples, the RVA profile was higher at pH=2.5 than at pH=7.0, while the opposite was true for non-irradiated samples. Therefore, the difference in RVA profiles between different testing solutions could be a promising method to identify whether starchy foods have been irradiated. Additionally, absorbed doses can be calculated according to the regression equation based on PV:  $Y_{pv}=3.5012-0.1277X$  ( $R^2=0.9920$ ).

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