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Effect of γ-irradiation on the physicochemical and functional properties of rice protein

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Abstract

In this study, rice protein was used as raw material to explore the effects of γ-irradiation treatment doses (0, 0.5, 1, 2, 3, 5 kGy) on the physicochemical properties of rice protein (particle size, zeta potential, secondary structure, scanning electron microscope microstructure), surface hydrophobicity (H_0), thermal stability), functional properties (solubility, water and oil retention, emulsification) and sensory quality. The results show that when the γ-irradiation dose is 2 kGy, the average particle size of rice protein is the smallest, the absolute value of the potential is the highest 33.58 mV, the content of β-sheets in the secondary structure is at least 31.16 ± 0.16, and the content of random curl is at most 14.56 ± 0.06, the surface of the microstructure is rough and the degree of pore depression is the deepest, the highest H_0 is 160.45 ± 2.98, the minimum denaturation temperature (T_d) and enthalpy (△H) are 70.49 ± 0.05 °C and 1.30 ± 0.01 J/g, which shows that γ-irradiation treatment can be significant affect the physicochemical properties of rice protein. When the irradiation dose is 2 kGy, the highest solubility of rice protein is 69.18 ± 1.07%, and the highest water and oil holding capacity are 5.89 ± 0.08 g/g and 3.45 ± 0.04 g/g, respectively. The highest emulsification activity and emulsification stability are 45.65 ± 1.26 m^2/g and 208.33 ± 4.79 min, which shows that γ-irradiation treatment can improve the functional properties of protein. When the irradiation dose was less than 5 kGy, the sensory quality of rice protein was not significantly affected. The research results provide a theoretical basis for the deep processing and value-added utilization of rice protein by γ-irradiation technology.

Keywords: γ-irradiation; rice protein; physicochemical properties; functional properties.

Practical Application: To provide methodological and data analysis ideas for the application of γ-irradiation in the quality improvement of rice protein.

1 Introduction

Rice is one of the most important staple foods in the world and the main source of plant protein (Amagliani et al., 2017). Rice contains 6-8% protein. Compared with other major cereal proteins, rice protein is recognized as a protein with high nutritional value and low sensitization. It is very suitable for infants and special people as nutritional food (Muthayya et al., 2014). Rice protein is the plant protein closest to the FAO/WHO ideal model, with reasonable amino acid composition ratio, high raw price and low allergenicity, and is a kind of high-quality cereal protein (Hansen et al., 1981). Recent studies (Wongthaweewatana et al., 2021) have found that rice protein has important health care functions, such as anti-diabetes, anti-cholesterol, anti-cancer, etc. Although the nutritional value and health care value of rice protein has been recognized. However, the application of rice protein in food industry is limited due to its compact structure and poor solubility. In recent years, people have tried to improve the functional properties of rice protein through physical, chemical, enzymatic and genetic modification (Hou et al., 2017; Liu & Tang, 2013). Chemical treatment can effectively improve the performance of protein by changing the structure of protein, but the problems such as chemical reagent residue will reduce the nutritional value of protein products (Pereira et al., 2020; Ramkisson et al., 2020). Compared with chemical methods, physical methods such as microfluidization (Wan et al., 2015), microwave (Phongthai et al., 2016), ultrasonic (Yang et al., 2017), irradiation (Houée-Levin & Sicard-Roselli, 2001). Due to the environmental and economic characteristics, it is widely used to improve the functional properties of rice protein.

High energy γ-ray (γ-irradiation) has the characteristics of fast processing speed, no pollution to food and environment, strong penetration and improving food safety (Moon & Song, 2001; Guimarães et al., 2013). When the γ-irradiation dose is lower than 10 kGy, the nutritional properties of most foods are not affected by irradiation, and the shelf life of the food is significantly extended (Li et al., 2020). γ-irradiation technology can induce the unfolding and denaturation of protein structure to produce a new conformation (Wang et al., 2017b). Studies have found that γ-irradiation unfolds the molecular structure of peanut protein and exposes hydrophobic groups, thereby improving various

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functional properties. However, as the irradiation dose further increases, the antigenicity of the allergenic protein decreases (Luo et al., 2013). The combination of irradiation and Maillard reaction can improve the freeze-thaw stability of soybean protein emulsion (Wang et al., 2020). The study found that, γ-irradiation can change the structure of egg white protein and improve its degree of hydrolysis (Jin et al., 2017). Wheat germ protein was hydrolyzed by alkaline protease and then γ-irradiation, the results show that γ-irradiation treatment can improve the antioxidant capacity and functional properties of wheat germ protein hydrolysate (Wang et al., 2019). The above research shows that, γ-irradiation technology has broad application prospects in modifying protein structure and improving protein functionality.

At present, the food industry is developing innovative technologies to meet consumers' demand for safer and higher quality food. In this study, rice protein was used as raw material to explore the effects of γ-irradiation doses (0, 0.5, 1, 2, 3, 5 kGy) on the physicochemical effects of rice protein (particle size, zeta potential, secondary structure, scanning electron microscope microstructure, surface hydrophobicity (H_0), thermal stability) and function (solubility, water and oil retention, emulsification) characteristics, so as to provide a theoretical basis for the deep processing and value-added utilization of rice protein by γ-irradiation technology .

2 Materials and methods

2.1 Materials and reagents

Rice protein (Wuxi Jinnong Biotechnology Co., Ltd.), soybean oil (COFCO Fulinmen Food Marketing Co., Ltd.), potassium bromide, 1-aniline-8-naphthalenesulfonate (ANS) and bovine serum albumin (American sigma company).

2.2 Rice protein γ-irradiation treatment

The irradiation test was carried out at the cobalt source device No.2 of the Heilongjiang Institute of Atomic Energy. Rice protein samples were packed in plastic bags with a thickness of about 4 mm with rice protein as raw material γ-ray pretreatment. The γ-irradiation dose is 0.5 kGy, 1 kGy, 2 kGy, 3 kGy and 5 kGy respectively. After irradiation, the samples were stored at -20 °C until further experimental use.

2.3 Determination of physical and chemical properties of rice protein

Determination of particle size

Referring to the research method of Xi et al. (2020), the particle size of rice protein was determined by Zetasizer Nano ZS 90 particle size analyzer (Malvin Instrument Co., Ltd., UK). The sample was diluted to 1 mg/10 mL, and the measurement parameters were: the observation angle, solution refractive index and viscosity were set to 173°, 1.333 and 0.00088 Pa•s, respectively, corresponding to the value of pure water at 25 °C. Measure the particle size distribution and volume average particle size of rice protein.

Determination of zeta potential

Referring to the research method of Lin et al. (2016), dilute 1% (w/v) rice protein sample solution to clear and transparent, and then use Zetasizer Nano ZS 90 particle size analyzer (Malvin Instrument Co., Ltd., UK) to determine the zeta potential value of rice protein. The wavelength and scattering angle are fixed at 632 nm and 90° respectively, and each measurement is controlled at 25 °C and repeated three times.

Determination of secondary structure

The secondary structure of rice protein was determined by slightly modifying the research method of Wang et al. (2015). The infrared spectrum of rice protein was recorded by scimitar 2000 Fourier transform infrared spectrometer (Agilent company of the United States). The rice protein sample was mixed with potassium bromide (KBr) and pressed into tablets. The measurement is carried out at room temperature (25 °C) and in a dry environment. The spectral wavelength of each sample is 4000~400 cm^{-1}, the resolution is 4 cm^{-1}, and the scanning times are 32. Results peakfit version 4.12 software was used for fitting analysis.

Determination of scanning electron microscopy

The microstructure of rice protein was determined with reference to the research method of Li et al. (2019b). About 1 mg of rice protein samples treated with different irradiation doses were placed on the tape attached to the short joint of circular aluminum sample, and coated with gold Palladium for 90 s under 15 mA current. The microstructure of rice protein samples was observed by su8010 field emission scanning electron microscope (Hitachi, Japan). It was amplified 6000 times at an accelerating voltage of 10.0 kV.

Determination of surface hydrophobicity

The determination of surface hydrophobicity (H_0) refers to the research method of Kato & Nakai (1980). Gradient dilute 1 mg/ml rice protein solution to 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL. Take 2 mL of sample solution and 25 μL 1-aniline-8-naphthalene sulfonate (ANS) (10 mmol/L, pH 7.0) was fully mixed and protected from light. The fluorescence intensity was measured at wavelengths of 365 nm (excitation) and 484 nm (emission) using a FL8500 fluorescence spectrophotometer (Perkin Elmer, USA). The surface hydrophobicity index (H_0) is the initial slope of fluorescence intensity with protein concentration.

Determination of thermal stability

With reference to Ma et al. (1996), the method was slightly modified and determined by differential scanning calorimetry thermal stability of rice protein after γ-irradiation. Seal 10-15 mg rice protein solution (20% w/v) in an aluminum plate, and use a thermogravimetric analyzer (Perkin Elmer, USA) to determine the samples. The measurement parameters are: 90% nitrogen and 10% oxygen form a pyrolysis mixture, scan at the rate of 10 °C/min in the temperature range of 20-120 °C, and determine

the denaturation temperature (T_d) and enthalpy (△H) of each sample.

Sensory evaluation

Each irradiated rice protein sample was divided into 3 parts. The samples were numbered with γ- irradiation dose, and the subjects were asked to describe their color, smell and taste.

2.4 Determination of functional properties of rice protein

Determination of solubility

The solubility of rice protein was determined by referring to the research method of Li et al. (2019a). The solution sample with rice protein content of 1% (w/v) was centrifuged at room temperature for 10 min at 10000 r/min, and the supernatant was taken. Bradford The crude protein content in the supernatant was determined by Bradford (1976), and the standard curve was drawn with bovine serum albumin as the standard sample. The solubility of rice protein is calculated as follows (Equation 1):

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content}} \times 100\% \quad (1)$$

Determination of water and oil holding capacity

For the determination of water and oil holding capacity of rice protein, refer to the method of Huang et al. (2020). With slight modification, dilute 1 g (m_0) of rice protein in deionized water to 10 mL, and then centrifuge the rice protein solution at 3000 r/min for 15 min. Pour out the supernatant and record the weight of the residue as m_1. The water holding capacity (WHC) is calculated by the following formula (Equation 2):

$$\text{WHC(g/g)} = \frac{m1 - m0}{m0} \quad (2)$$

Where, m_0 and m_1 are the weight of rice protein sample before and after water absorption (g).

Mix 1 g (M_0) rice protein sample with 5 mL soybean oil and let it stand in a plastic centrifuge tube for 24 h. then centrifuge the sample at 4500 r/min and 20 min, discard the supernatant, weigh and record the weight of the residue as M_1. The calculation formula of Oil Holding Capacity (OHC) is as follows (Equation 3):

$$\text{OHC}\ (\text{g/g}) = \frac{\text{M1-M0}}{\text{M0}} \quad (3)$$

Where, M_0 and M_1 are the weight of rice protein sample before and after oil absorption (g).

Determination of emulsifying activity and emulsifying stability

The emulsifying activity (EAI) and stability (ESI) of rice protein were determined with reference to the method of Wang et al. (2017b). Mix 9 mL of 1% (w/v) rice protein sample with 3 mL of soybean oil and homogenize at 10,000 r/min for 1 min to obtain an emulsion. Take 50μL of the homogenized emulsion after 0 and 30 min from the bottom of the emulsion, and dilute it 100 times with 5 mL of 1% SDS.The absorbance of the mixture was measured at 500 nm with a UV-2700 ultraviolet-visible spectrophotometer (Shimadzu Corporation, Japan). The calculation formulas of Emulsification Activity Index (EAI) and Emulsification Stability Index (ESI) are as follows (Equations 4-5):

$$\text{EAI}(m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times m} \quad (4)$$

$$\text{ESI}(\text{min}) = \frac{A_0}{A_0 - A_{30}} \times 30\text{min} \quad (5)$$

Where A_0 is the absorbance of the sample at 0 min, A_{30} is the absorbance of the sample at 30 min, and m is the weight of rice protein (g).

2.5 Statistical analysis

All samples in this experiment were measured in 3 parallel experiments. Origin 9.0 software was used to analyze the data and graphed, Peakfit Version 4.12 software was used to enter and exit the fitting analysis, and SPSS 22.0 software was used to perform one-way (ANOVA) analysis of variance on the data, and the data results were averaged Value ± SD expression, $p < 0.05$ indicates significant difference.

3 Results and analysis

3.1 Analysis of particle size

It can be seen from Figure 1 that, compared with the untreated rice protein sample, the particle size of the rice protein after γ-irradiation treatment shifts to the left, and the main peak appears, and there is a miscellaneous peak between 10-100 nm. When the γ-irradiation dose is 2 kGy, the rice protein particle size distribution has a main peak at 100-1000 nm, and an impurity peak at 10-100 nm, and the average particle size is the smallest; when the γ-irradiation treatment dose is greater than 2 kGy, The particle size distribution of rice protein showed miscellaneous peaks on the right side of 10-100 nm and 1000 nm, and the average particle size also increased. This may be because γ-irradiation treatment breaks the intermolecular bonds, thereby reducing the particle size of rice protein (Li et al., 2020). When the γ-irradiation dose is too large, cross-linking, electrostatic interaction, hydrophobic interaction and disulfide bond formation will occur between protein and protein to aggregate into higher molecular weight proteins (Cho & Song, 2000), increase the particle size. The above results indicate that the γ-irradiation treatment changes the intermolecular bonds and intermolecular forces of the protein, thereby changing the particle size of the protein molecules.

3.2 Analysis of potential

Potential is an important indicator for judging the stability of the emulsion. The smaller the dispersed particles, the higher the absolute value of the potential, the greater the electrostatic

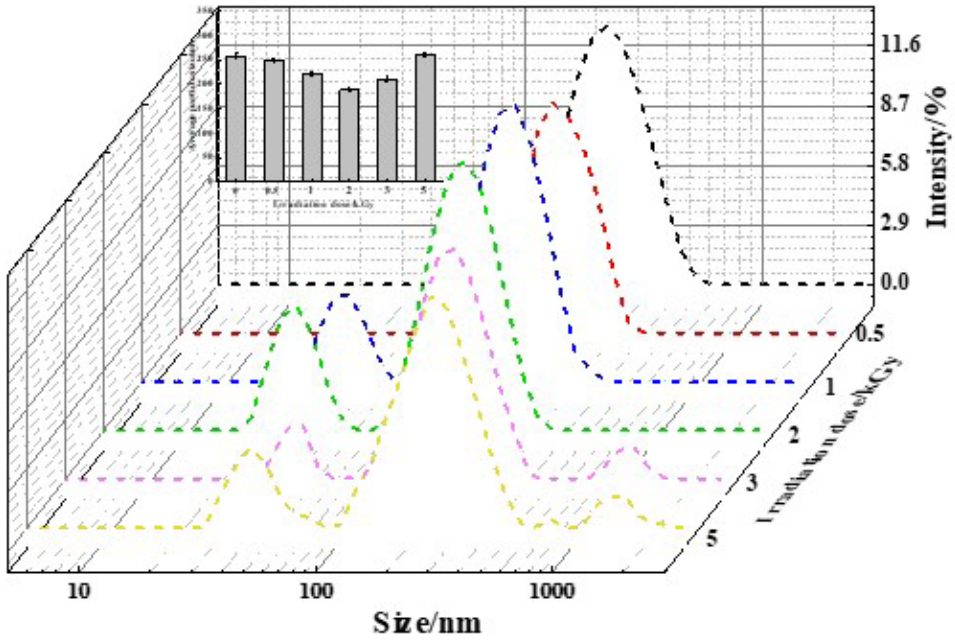

Figure 1. Effect of γ-irradiation on particle size distribution and potential of rice protein. Note: the illustration shows the effect of γ-irradiation on the average particle size of rice protein.

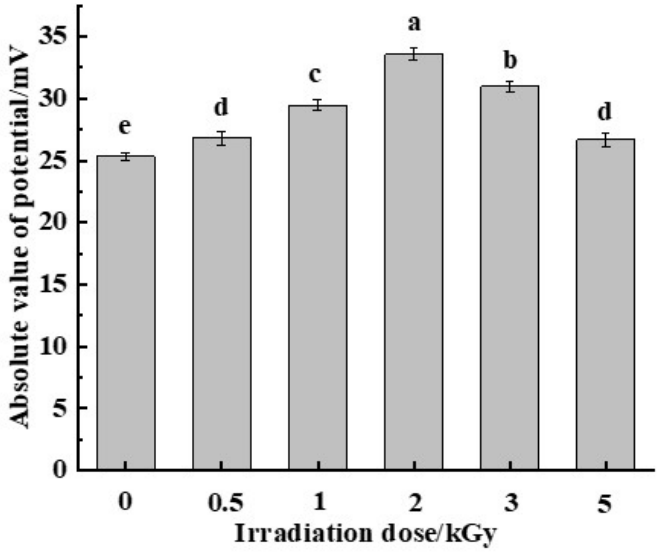

Figure 2. Effect of γ-irradiation on potential of rice protein. Note: Different letters represents significant difference.

repulsion between the particles, and the more stable the emulsion system (Markiewicz et al., 2013; Vallar et al., 1999). It can be seen from Figure 2 that the absolute value of the rice protein potential increased significantly after γ-irradiation treatment compared with the untreated sample. With the increase of γ-irradiation dose, the absolute value of rice protein potential increased first and then decreased. When the γ-irradiation dose is 2 kGy, the maximum absolute value of the rice protein potential is 33.58 mV, and the solution system is the most stable at this time. This may be because the γ-irradiation treatment opens the protein structure and exposes the charged groups, which increases the electrostatic repulsion and the absolute value of the potential, thereby making the solution more stable (Markiewicz et al., 2013) . When the dose of γ-irradiation is too large, the charged groups in the protein are destroyed, resulting in a decrease in the polarity or quantity of the charge of the rice protein, which reduces the electrostatic attraction, thereby reducing the potential value of the rice protein (Xu & Liu, 2016). The above results indicate that the γ-irradiation treatment changes the exposure of the charged groups of the protein, thereby changing its potential.

3.3 Analysis of secondary structure

Studies have shown that the protein secondary structure measured by infrared spectroscopy can reflect the stability of protein molecular conformation (Wang et al., 2020). It can be seen from Table 1 that the highest β-sheet content of untreated rice protein samples is 33.62%, and the lowest irregular curl content is 12.45%. With the increase of γ-irradiation dose, the content of α-helix and random coils first increased and then decreased, the content of β-turn angle first decreased and then increased, and the content of random coils increased. When the γ-irradiation dose is 2 kGy, the content of β-sheets and β-turns decreases, and the content of α-helix and random coils increases; when the γ-irradiation dose is greater than 2 kGy, the contents of β-sheets and β-turns As it increases, the content of α-helix and random coils decreases again. This may be because the γ-ray irradiation treatment opens the protein structure and breaks the intermolecular bonds of the protein, thereby reducing the ordered structure of the protein (Moon & Song, 2001). When the γ-irradiation dose is too high, a cross-linking reaction occurs between protein molecules, and the intermolecular interaction increases. Studies by Lv et al. (2018) and others have shown that

the secondary structure of a protein is positively correlated with the interaction between different parts of the molecule. The above results indicate that the γ-irradiation treatment changes the interaction between the protein molecules by destroying the intermolecular bonds of the rice protein, resulting in changes in its secondary structure.

3.4 Analysis of scanning electron microscope

Note: (1) is the control, (2) ~ (6) the radiation dose is 0.5, 1, 2, 3, 5 kGy.

The surface microstructure of rice protein was observed by scanning electron microscope. It can be seen from Figure 3 that the surface of the untreated sample is smooth and flat. After γ-irradiation treatment, there are pores and more fragmented protein particles on the protein surface, and the degree of pore depression first deepens and then decreases as the irradiation dose increases. When the γ-irradiation dose is 2 kGy, the surface of rice protein is rough and the degree of pore depression is the deepest. This may be due to the high-intensity rays generated by γ-irradiation that damage the surface structure of protein molecules, which makes the surface rough and the pores are deeply recessed (Yang et al., 2017; Gani et al., 2014). When the γ-irradiation dose is too high, the cross-linking reaction or aggregation between the protein and the protein will reduce the degree of pore depression on the surface of the protein molecule (Malik et al., 2017). The above results show that the γ-irradiation treatment destroys the flat and dense structure of the protein, and the high-energy rays of the irradiation cause the protein to produce more fragmented particles.

3.5 Analysis of surface hydrophobicity (H_0)

Surface hydrophobicity (H_0) refers to the ability of the hydrophobic groups in the protein to contact the surface aqueous environment, reflecting the stability of the protein conformation and affecting the functional properties and interactions of the protein (Uruakpa & Arntfield, 2006). It can be seen from Figure 4 that the H_0 of the untreated sample is the smallest. As the irradiation dose increases, the H_0 of the rice protein first increases and then decreases. When the irradiation dose is 2 kGy, the H_0 of rice protein is the highest 160.45 ± 2.98.

Table 1. Effect of γ-irradiation on the secondary structure of rice protein.

Irradiation dose/kGy	α-helix	β-sheet	β-turn	Random coil
0	12.52 ± 0.07^{c}	33.62 ± 0.13^{a}	41.41 ± 0.09^{b}	12.45 ± 0.07^{e}
0.5	11.57 ± 0.10^{e}	33.34 ± 0.15^{b}	42.54 ± 0.05^{a}	12.55 ± 0.05^{e}
1	12.27 ± 0.08^{d}	33.01 ± 0.20^{bc}	41.28 ± 0.04^{c}	13.44 ± 0.05^{d}
2	15.72 ± 0.05^{a}	31.16 ± 0.16^{f}	38.56 ± 0.09^{f}	14.56 ± 0.06^{a}
3	14.25 ± 0.10^{b}	31.60 ± 0.15^{e}	40.59 ± 0.05^{d}	13.56 ± 0.05^{c}
5	12.68 ± 0.09^{c}	32.71 ± 0.20^{d}	40.29 ± 0.05^{e}	14.32 ± 0.07^{b}

Note: different lowercase letters in the same column indicate significant differences.

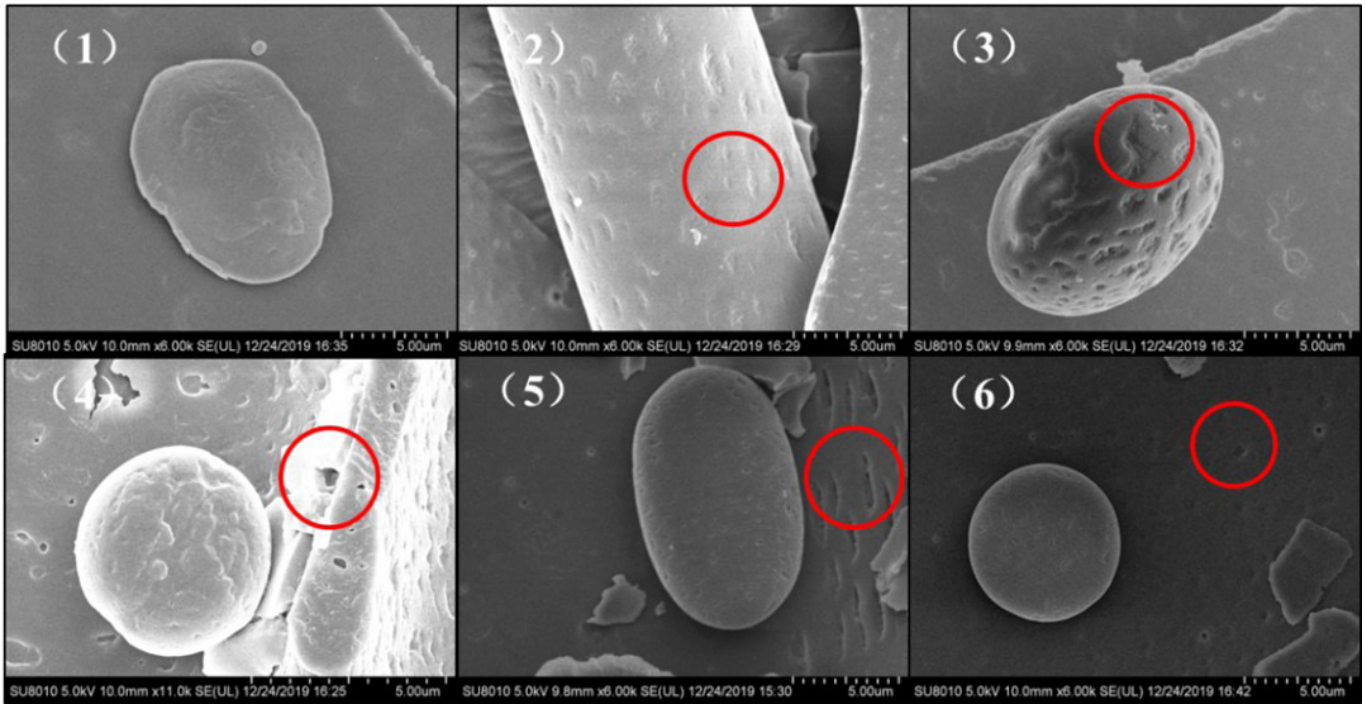

Figure 3. Effect of γ-irradiation on the microstructure of rice protein. Note: (1) is the control, (2) ~ (6) the radiation dose is 0.5, 1, 2, 3, 5 kGy.

This may be because the radiation treatment opens the protein structure, exposes the hydrophobic residues inside the protein, increases the number of hydrophobic sites, and enhances the ability to contact the external environment, thereby increasing H_0 (Wang et al., 2017b). When the γ-irradiation dose is too large, due to the increase of β-sheets in the secondary structure of the protein, the hydrophobic sites of the protein are reduced, thereby reducing H_0 (Yuan et al., 2009). The above results indicate that the γ-irradiation treatment changes the structure of the protein and exposes its internal groups, thereby changing the H_0 of the protein.

3.6 Analysis of Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to determine the effect of γ-irradiation on the denaturation temperature (T_d) and denaturation enthalpy (△H) of rice protein. T_d is a measure of the thermal stability of the protein, and △H is related to the content of the ordered secondary structure of the protein, indicating the proportion of protein in the isolate that is not denatured during processing (Arntfield & Murray, 1981; Koshiyama et al., 1981). It can be seen from Table 2 that all samples have a single endothermic peak (denaturation temperature T_d) and the T_d and △H of the untreated sample are the largest. With the increase of the irradiation dose, the T_d and △H of rice protein showed a trend of first decreasing and then increasing. When the γ-irradiation dose was 2 kGy, the T_d and △H of rice protein were the lowest. Studies have shown that the denaturation temperature and enthalpy of protein are related to its spatial structure (Yang et al., 2019), because γ-irradiation treatment reduces β-sheets and increases random coils in the secondary structure of the protein, the ordered structure of the protein is reduced, and the disordered structure is increased, so as to reduce the T_d and △H of the irradiated protein during heating. When the γ-irradiation treatment dose is too high, the protein molecules will be cross-linked, and the denaturation of the cross-linked protein molecules will require more energy (Benbettaïeb et al., 2016), which will increase T_d and △H. This is consistent with the results of Abu et al. (2006) and others studying the thermostability of cowpea protein. The above results show that the γ-irradiation treatment changes the spatial structure of the protein and the intermolecular forces, thereby changing the T_d and △H during the heating process.

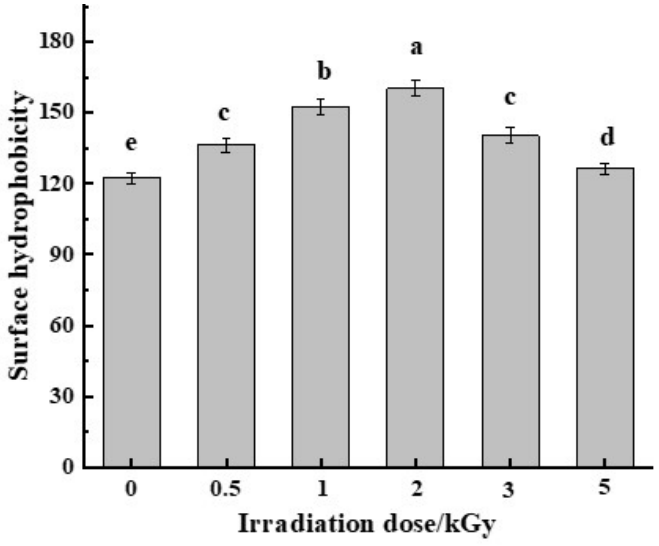

Figure 4. Effects of γ-irradiation on the surface hydrophobicity of rice protein. Note: Different letters represents significant difference.

Table 2. Effects of γ-irradiation on denaturation temperature (T_d) and enthalpy (△H) of rice protein.

Irradiation dose/kGy	T_d (°C)	△H (J/g)
0	77.59 ± 0.06^a	2.04 ± 0.01^a
0.5	75.96 ± 0.07^b	1.71 ± 0.01^b
1	72.35 ± 0.06^e	1.47 ± 0.02^d
2	70.49 ± 0.05^f	1.30 ± 0.01^e
3	72.93 ± 0.06^d	1.45 ± 0.02^d
5	73.73 ± 0.07^c	1.62 ± 0.01^c

Note: different lowercase letters in the same column indicate significant differences.

3.7 Analysis of sensory evaluation

According to Table 3, when the irradiation dose of γ- was less than 5 kGy, the color of rice protein did not change, and when the irradiation dose was 5 kG, the color of rice protein was slightly yellow. After irradiation, the protein samples had peculiar smell, but the peculiar smell disappeared immediately after being placed, and all samples of rice protein had no peculiar taste. Therefore, when the γ- irradiation dose was less than 5 kGy, the sensory quality of rice protein was not significantly affected.

3.8 Analysis of solubility

Solubility is used to measure protein aggregation and is an important indicator of protein functional properties (Hu et al., 2013). It can be seen from Figure 5 that compared with the untreated sample, the solubility of rice protein after γ-irradiation treatment increased significantly; with the increase of the irradiation dose, the solubility of rice protein first increased and then decreased. When the γ-irradiation dose is 2 kGy, the solubility of rice protein is 69.18 ± 1.07%. This may be because γ-irradiation treatment reduces β-sheets in the secondary structure of the protein, opens the protein structure, and exposes the internal hydrophilic groups (Nazari et al., 2018), making it easier for water molecules to bind, thereby increasing the

Table 3. Effect of γ-irradiation on sensory evaluation of rice protein.

Sensory evaluation	0	0.5 kGy	1 kGy	2 kGy	3 kGy	5 kGy
Color	Milk white	Milk white	Milk white	Milk white	Milk white	Slightly yellow
Smell	No special flavor	No special flavor	No special flavor	No special flavor	No special flavor	No special flavor
Taste	No special flavor	No special flavor	No special flavor	No special flavor	No special flavor	No special flavor

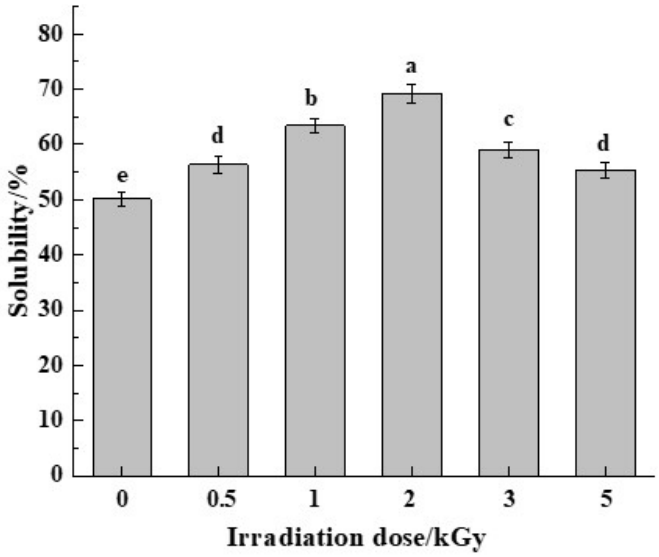

Figure 5. Effect of γ-irradiation on solubility of rice protein. Note: Different letters represents significant difference.

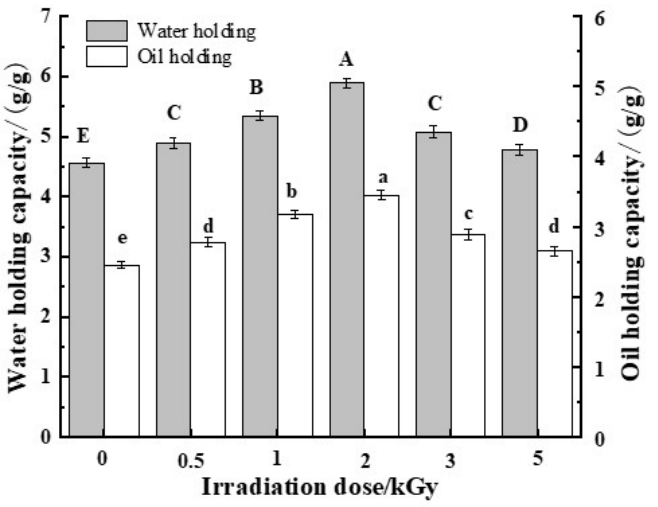

Figure 6. Effects of γ-irradiation on the water holding and oil holding capacity of rice protein. Note: Different capital letters represents significant difference in water holding. Different lowercase letters represents significant difference in oil holding.

solubility of the protein. When the γ-irradiation dose is too high, the protein molecules will aggregate or the protein residues will be oxidized to produce disulfide bonds (Amagliani et al., 2017), which reduces the hydration of the protein and reduces the solubility of rice protein. The above results indicate that γ-irradiation treatment changes the degree of exposure of the hydrophilic groups in the rice protein to change the interaction between the protein and water molecules, thereby changing the solubility of the rice protein.

3.9 Analysis of water and oil retention

The water-holding and oil-holding properties of protein can improve product texture and maintain product flavor, so it is an important functional property of protein (Kinsella & Melachouris, 2009). It can be seen from Figure 6 that compared with the untreated sample, the water and oil holding capacity of rice protein is significantly improved after γ-irradiation treatment; with the increase of the irradiation dose, the water holding and oil holding capacity of rice protein shows a tendency to increase first and then decrease. When the γ-irradiation dose is 2 kGy, the highest water and oil holding capacity of rice protein are 5.89 ± 0.08 g/g and 3.45 ± 0.04 g/g, respectively. This may be because γ-irradiation treatment opens the protein structure and exposes internal groups. The water-holding capacity of the protein is closely related to the exposure of hydrophilic groups, which increases the chance of contact with water molecules, thereby enhancing the water-holding capacity (Hettiarachchy & Kalapathy, 1998). The increase in oil holding capacity may be due to the increase in H_0 of the protein, which promotes more effective adsorption and diffusion between the protein and the oil droplets (Li et al., 2019a). When the γ-irradiation dose is too high, the protein secondary structure β-sheet increases, the structure becomes tighter and orderly, and the proteins are more prone to cross-linking, which reduces the contact between the protein and water and oil droplets, resulting in the decline of water and oil holding capacity (Wang et al., 2017a). The above results indicate that the γ-irradiation treatment further changes the contact ability of the protein with water and oil by changing the structure of the protein, thereby changing its water and oil holding capacity.

3.10 Analysis of emulsifying activity and emulsifying stability

Emulsification activity refers to the ability of protein to quickly adsorb on the oil-water interface (Pearce & Kinsella, 1978), and emulsification stability is to evaluate the stability of the protein staying at the water-oil interface for a period of time (Mohanty et al., 1988). As can be seen from Figure 7, compared with untreated samples, γ- Irradiation treatment significantly improved the emulsifying activity and emulsifying stability of rice protein. With the increase of irradiation dose, the emulsifying activity and emulsifying stability of rice protein increased first and then decreased. When the γ-irradiation dose is 2 kGy, the emulsification activity and emulsification stability of rice protein are highest at 45.65 ± 1.26 m^2/g and 208.33 ± 4.79 min, respectively. This may be because the γ-irradiation treatment makes the ordered structure of the protein tend to be disordered, exposing its internal hydrophilic groups and hydrophobic side chains, thereby making the hydrophilic groups more conducive to binding to water molecules and hydrophobic The sexual side chain interacts with the oil and stabilizes the droplets through steric effects, which improves the emulsification activity and emulsification stability of the protein (Wang et al., 2017b; Li et al., 2016). The high dose of γ-irradiation treatment causes cross-linking between proteins, formation of disulfide bonds in the molecule, reduction of hydrophobic sites, and reduction of H_0, which reduces the ability of proteins to adsorb to the water-oil interface, thereby reducing the emulsification ability (Shi et al., 2015; Bandyopadhyay et al., 2008). The above results show that the γ-irradiation treatment changes the H_0 of the rice protein

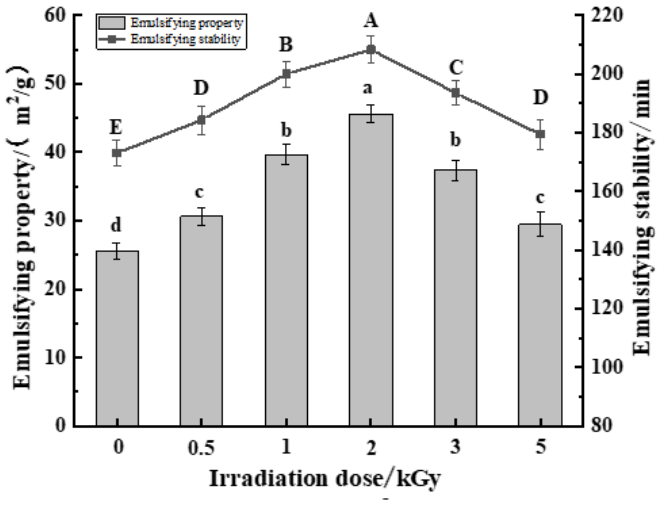

Figure 7. Effect of γ-irradiation on the emulsifying activity and emulsifying stability of rice protein. Note: Different capital letters represents significant difference in emulsifying stability. Different lowercase letters represents significant difference in emulsifying property.

by changing the exposure of the internal groups of the protein, and then changes the interaction between the protein molecule and the water-oil interface, thereby changing the emulsifying ability of the rice protein.

4 Conclusion

Using γ-irradiation technology to irradiate rice protein with different doses, explore the effect of radiation dose on the physicochemical and functional properties of rice protein. The results of the physicochemical properties of rice protein show that when the γ-irradiation dose is 2 kGy, the average particle size of rice protein is the smallest, and the absolute value of the potential is the highest 33.58 mV, and the β-sheet content in the secondary structure is at least 31.16 ± 0.16. The content of random curl is at most 14.56 ± 0.06, the surface of the microstructure is rough and the degree of pore depression is the deepest, the highest surface hydrophobicity (H_0) is 160.45 ± 2.98, and the lowest T_d and △H are 70.49 ± 0.05 °C and 1.30 ± 0.01 J/g. This indicates that the γ-irradiation treatment reduces the particle size of rice protein, improves the stability of the solution, changes the secondary structure, and the structure of the protein tends to become disordered, exposing its internal hydrophobic groups, thereby increasing H_0. The high, disordered structure makes the intermolecular bonds of the protein easier to break during the heating process, which leads to the decrease of T_d and △H. The results of the functional properties of rice protein show that when the γ-irradiation dose is 2 kGy, the solubility of rice protein is the best 69.18 ± 1.07%, and the water-holding and oil-holding capacity are the strongest at 5.89 ± 0.08 g/g and 3.45 ± 0.04 g/g, the highest emulsification activity and emulsion stability are 45.65 ± 1.26 m^2/g and 208.33 ± 4.79 min. This indicates that γ-irradiation treatment can also improve the functional properties of the protein by changing the structure of the protein. When the irradiation dose was less than 5 kGy, the sensory quality of rice protein was not significantly affected. In this study, γ-irradiation technology was used as pretreatment to provide a theoretical basis for the deep processing and value-added of rice protein.

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