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Research Article

Variations in Oxidative Stability of Walnut Oil with Rosmarinic Acid Added under Different Ultraviolet Radiations

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Rosmarinic acid (RA) is a natural phenolic compound extracted from the Labiatae family and is a natural antioxidant. In this study, walnut oil added with RA was treated with different ultraviolet radiations, and stabilization effects in terms of the same conditions (0.2 mg/g) were compared with synthetic antioxidant (BHT). In order to compare the effectiveness of three UV treatments, different lab tests were conducted, namely, the acid value, peroxide value, iodine value, anisidine value, DPPH free radical scavenging rate, and malondialdehyde content. The enhanced UV-A, UV-B, and UV-C radiation intensities have increased the oxidation stability of RA-added walnut oil, of which UV-B has the greatest influence on the oxidative stability of walnut oil. When both RA and BHT were added at 0.2 mg/g, the antioxidant effect of RA is superior to the general antioxidant BHT.

1. Introduction

Walnut oil is a nutrient-rich source, containing essential unsaturated fatty acids like linoleic acid; linolenic acid, which mitigates cardiovascular disease risk; and oleic acid, known for its blood pressure-lowering effects [1, 2]. Numerous studies have affirmed walnut oil's substantial role in preventing cardiovascular and cerebrovascular diseases, diabetes, cancer, multiple sclerosis, and cognitive enhancement [3–8]. Consequently, walnut oil holds considerable appeal for consumers and boasts high economic value. Walnuts serve as the primary raw material for walnut oil production. They are widely sourced and distributed, featuring high oil content in their kernels with oil yields ranging from 52% to 70% [9]. Nonetheless, due to the high content of unsaturated fatty acids in walnut oil, it is susceptible to oxidation when exposed to the environment for an extended period, raising significant concerns about its quality. Oxidation of fats and oils poses considerable health risks, leading to the formation of hydroperoxides. Over time, these oils undergo further oxidation, resulting in secondary decomposition products like aldehydes, ketones, acids, and alcohols. These byproducts not only alter the oil's taste but also have a detrimental impact on its nutritional value and product quality [10]. Peroxides in oils and fats are recognized as catalysts for hypertension, liver damage, and atherosclerosis and significant contributors to the aging process. Extensive peroxidation of oils and fats can influence DNA production directly or indirectly in the body, ultimately impacting gene expression and potentially leading to the development of tumors [11]. Furthermore, the ingestion of oxidized fats exerts a more pronounced toxic effect on the human digestive system and other organs. Thus, the pivotal factor in maintaining the quality of walnut oil lies in preventing its oxidation.

Oil oxidation primarily falls into three categories: enzymatic oxidation, auto-oxidation, and photo-oxidation. Enzymatic oxidation, among these, necessitates specific conditions for its occurrence and can be mitigated. Auto-oxidation can

be delayed by incorporating antioxidants. Managing the impact of photo-oxidation, particularly from ultraviolet radiation, on grease oxidation proves challenging. The mechanism underlying photo-oxidation involves the conversion of ground state oxygen to excited oxygen by the light-sensitive substance (chlorophyll) in the oil upon ultraviolet light absorption. Excited oxygen then proceeds to attack the double bonds in the unsaturated fatty acids present in the oil, leading to the shifting of these double bonds and ultimately culminating in the formation of hydroperoxides [12].

Despite containing inherent antioxidants like polyphenols, vitamin E, and sterols, walnut oil still faces a significant challenge in preventing oxidation. To retard the oxidation of walnut oil, the addition of antioxidants has become a vital strategy. Currently, two primary categories of antioxidants are employed in oils and oil-containing products. One category includes synthetic antioxidants like butylhydroxyanisole (BHA), dibutylhydroxytoluene (BHT), and tert-butylhydroquinone (TB-HQ). The other category comprises natural antioxidants, including rosmarinic acid, tea polyphenols, tea polyphenol palmitate, licorice antioxidant, vitamin C, and phytic acid. Synthetic antioxidants offer robust antioxidant potential, stability, and cost-effectiveness. However, they carry potential toxic effects on humans and animals. Several developed nations, including Japan and Canada, have prohibited the use of synthetic antioxidants like TB-HQ and BHA due to their carcinogenic properties [13]. Natural antioxidants offer the benefits of safety, low toxicity, and harmlessness. Hence, the inevitable trend is the substitution of synthetic antioxidants with natural alternatives [10]. Rosmarinic acid (RA), a natural antioxidant, is a phenolic compound extracted from plants within the Lamiaceae family and is abundant in various plant species like Lamiaceae, Comfrey, Cucurbitaceae, Tiliaceae, and Umbelliferae [14]. Research indicates that rosmarinic acid possesses pharmacological properties for treating diabetes, hypertension, inflammation, and cancer [15–17]. Simultaneously, RA exhibits superior antioxidant activity attributed to its ability to inhibit nitroso formation, solubility, stability, and free radical scavenging effect. Rosmarinic acid finds utility in the preservation of meat products and soybean oil, with demonstrated excellent antioxidant properties [18, 19]. Hence, investigating the utilization of rosmarinic acid as an antioxidant in walnut oil holds substantial importance. While rosmarinic acid remains stable under various conditions, light, Ca^{2+}, and Mg^{2+} have a more pronounced impact on its stability [14]. Light not only affects the stability of rosmarinic acid to some extent but also significantly impacts the quality of the oil. The primary source of light-induced effects on oils and fats is ultraviolet radiation, making it crucial to explore the impact of ultraviolet radiation on both oils and rosmarinic acid. Previous experiments have investigated the impact of visible light on oil oxidation [20]. Wang et al. [21] examined the influence of various storage conditions (distinct ultraviolet radiation scenarios) on the oxidation level of pine nut oil-containing carnosic acid. Currently, there is a lack of literature regarding the oxidation of fragrant acids and oils. In this study, rosmarinic acid served as an antioxidant. The experiment was involved exposing walnut oil fortified with RA to various forms of ultraviolet radiation. Parameters were measured including acid value, peroxide value, iodine value, anisidine value, DPPH clearance rate, and MDA content. Scientific analysis was conducted to assess the impact of RA as an antioxidant on the degree of oil and fat oxidation, with a focus on the influence of ultraviolet radiation wavelength on fats and oils enriched with RA.

2. Materials and Methods

2.1. Experimental Materials. Walnut was purchased from the supermarket. Dibutylhydroxytoluene (BHT), rosmarinic acid (RA), ethanol, ether, phenolphthalein reagent, potassium hydroxide, potassium iodide, sodium thiosulfate, isooctane, glacial acetic acid, carbon tetrachloride, phenobarbital acid, methanol, and trichloroacetic acid were provided by Shanghai Sinopharm Chemical Reagent Co. Ltd. UV-A, UV-B, and UV-C irradiators, as well as ultraviolet and other radiation intensity testers, were all acquired from Shanghai Sigma Company.

2.2. Extraction of Walnut Oil. The walnut shells were manually removed, and any moldy or black walnut kernels were discarded. Walnut kernels were processed using a KOMET oil press (CA59G type, Germany) with the following settings: a rotation speed of 20 rpm, a pipe aperture of 6 mm, and an oil temperature of 60°C. Walnut oil was subjected to centrifugation at 10,000 rpm and 4°C for 10 minutes to separate impurities and isolate the pressed walnut oil. The gathered walnut oil was transferred to a beaker, sealed, protected from light, and refrigerated for further analysis.

2.3. Sample Preparation. Initially, a small amount of walnut oil was taken, to which the premeasured amounts of RA and BHT were added. Subsequently, the mixture was heated to ensure complete dissolution, following which the remaining walnut oil was incorporated. Four solutions were prepared, the blank solution, a 0.1 mg/g RA walnut oil solution, a 0.2 mg/g RA walnut oil solution, and a 0.2 mg/g BHT walnut oil solution. UV-A light ranged from 320 to 400 nm, UV-B light spanned 280 to 320 nm, and UV-C light covered 100 to 280 nm. Equal portions of the samples were distributed into Petri dishes measuring 9 cm in diameter and 1 mm in thickness. These dishes were then exposed to UV-A, UV-B, and UV-C light sources at intensities of 150 μW/cm^2 and 300 μW/cm^2, with no other concurrent light sources, while maintaining ventilation and a constant temperature of 25°C.

2.4. Walnut Oil Oxidation Stability Test

2.4.1. Determination of Acid Value. The acid value of walnut oil was determined with reference to the method of measuring the acid value of Ogata et al. [22]. Precisely, 1 gram of the samples was weighed and dissolved in a 1 : 1 (v/v) mixed solution of 100 mL ethanol and ether. Subsequently, phenolphthalein was introduced as an indicator into the solution. The solution was titrated with a 0.1 mol/L KOH standard solution, and the acid value (AV) was determined based on the amount of consumed potassium hydroxide solution. The acid value was then measured using

$$\mathrm{AV} = \frac{56.11V}{10M}, \tag{1}$$

where 56.11 (g/mol) represents the molar mass of potassium hydroxide, V represents the volume (mL) of 0.1 N/L potassium hydroxide consumed, and M represents the sample mass (g).

2.4.2. Determination of Peroxide Value. Peroxide value (PV) was determined using the acetic acid-chloroform method as described in reference [23]. Samples, ranged 0.5 g to 5 g, were accurately weighed precisely into a 250 mL iodine measuring flask. These samples were thoroughly mixed with a 30 mL solution of acetic acid-chloroform (3 : 2, *v*/*v*) and then supplemented with 0.5 mL of saturated potassium iodide solution The solution was left to stand in darkness for 1 min, and 30 mL of distilled water was promptly added. The mixture was thoroughly agitated to ensure uniformity. During titration, continue to stir while titrating until the yellow color has nearly vanished. Subsequently, 2.0 mL of starch indicator (0.05%) was added, and titration with sodium thiosulfate was continued until the blue color disappeared. A blank control was prepared using an equivalent amount of acetic acid-chloroform solution, potassium iodide solution, and water. The peroxide value of fats and oils was calculated based on the volume of sodium thiosulfate used and expressed in milligrams of active oxygen per kilogram of fats and oils. The peroxide value was then measured using

$$X = \frac{[(V - V_0) \times N \times 126.9]}{1000M}, \tag{2}$$

where V is the volume of sodium thiosulfate solution consumed by the sample (mL), V_0 is the volume of blank sodium thiosulfate solution consumed (mL), N is the concentration of sodium thiosulfate standard solution (mol/L), 126.9 is 1N sodium thiosulfate 1mL equivalent to the number of grams of iodine, and M represents the mass of the sample kg.

2.4.3. Determination of Anisidine Value. The anisidine value of walnut oil was determined following the method described in reference [23]. Samples weighing between 0.5 g and 4 g were placed in separate 25 mL volumetric flasks and diluted to the mark with isooctane. Isooctane was employed as a blank control, and the absorbance was measured at a wavelength of 350 nm. Five milliliters of the isooctane oil and fat mixture and pure isooctane solvent was taken, and 1 mL of p-anisamine glacial acetic acid solution (0.25 g/100 mL) was added to each. After a 10-minute reaction period, the pure solvent group was employed as the blank solution, and the absorbance was measured again at a wavelength of 350 nm. The anisidine value was then measured using

$$P - \text{Anv} = \frac{25 \times 1.2(\text{As} - \text{Ab})}{m}, \tag{3}$$

where As represents the absorbance of fatty isooctane solution after reaction with the p-anisidine reagent, Ab represents the absorbance of fat isooctane solution, and m represents the mass of the test sample (g).

2.4.4. Determination of Iodine Value. The iodine value of walnut oil was determined using the method described by Otabor et al. [24]. Transfer 1 g of the oil sample into a 500 mL volumetric flask, and add carbon tetrachloride (15 mL) to the sample, and then stir until uniform. Subsequently, 25 mL of the Wijs solution was then added to the volumetric flask containing the sample. Keep the sample in the dark at room temperature for 30 minutes. Following the reaction, add 20 mL of a 10% potassium iodide (KI) solution and 150 mL of distilled water. Titrate the mixture with a 0.1 N thiosulfate ($Na_2S_2O_3$) solution until the yellow color completely vanishes. Add 1.5 mL of starch indicator solution to the sample and continue titration until the blue color vanishes. A blank measurement was conducted using the same procedure. The iodine value (IV) was expressed as grams of iodine absorbed per 100 grams of sample. The iodine value was then measured using

$$\text{Iodine value} = \frac{12.69 \times (V1 - V2) \times N}{W}, \tag{4}$$

where W is the weight of the sample (g), $V1$ is the volume of $Na_2S_2O_3$ used in the test (mL), $V2$ is the volume of $Na_2S_2O_3$ used in the blank (mL), and N is the normality of HCl.

2.4.5. Determination of Total Oxidation Value. Because the peroxide value can only evaluate the initial rancidity of the oil, the acid value and anisidine value measure the degree of oxidation in the middle and late stages of oil oxidation. In order to comprehensively assess the degree of oil oxidation, the concept of total oxidation value is introduced here. The total oxidation value was then measured using

$$\text{Totox value} = 2 * \text{PV} + P - \text{AV}. \tag{5}$$

2.4.6. Determination of DPPH Free Radical Scavenging. The antioxidant activity of walnut oil was determined following the procedure outlined in the study by Pandini et al. [20]. Take 1 mL of the sample and combine it with 3 mL 0.2 mmol/L DPPH-methanol solution. Allow the reactions to proceed in the dark at room temperature for 30 min. Using methanol as the reference solution, measure the absorbance of the sample, a wavelength of 517 nm. DPPH free radical scavenging rate was then measured using

$$\text{DPPH free radical scavenging rate\%} = \frac{A0 - (A1 - A2)}{A0} \times 100, \tag{6}$$

where $A0$ was the absorbance of the DPPH solution when no sample was added, $A1$ is the absorbance after adding DPPH solution for 30 minutes, and $A2$ is the absorbance of the sample without the DPPH solution.

2.4.7. Determination of Malondialdehyde Content. The content of malondialdehyde (MDA) was determined following the method outlined by Leong [25]. Accurately measure 0.5 mL of walnut oil and combine it with 2 mL of a

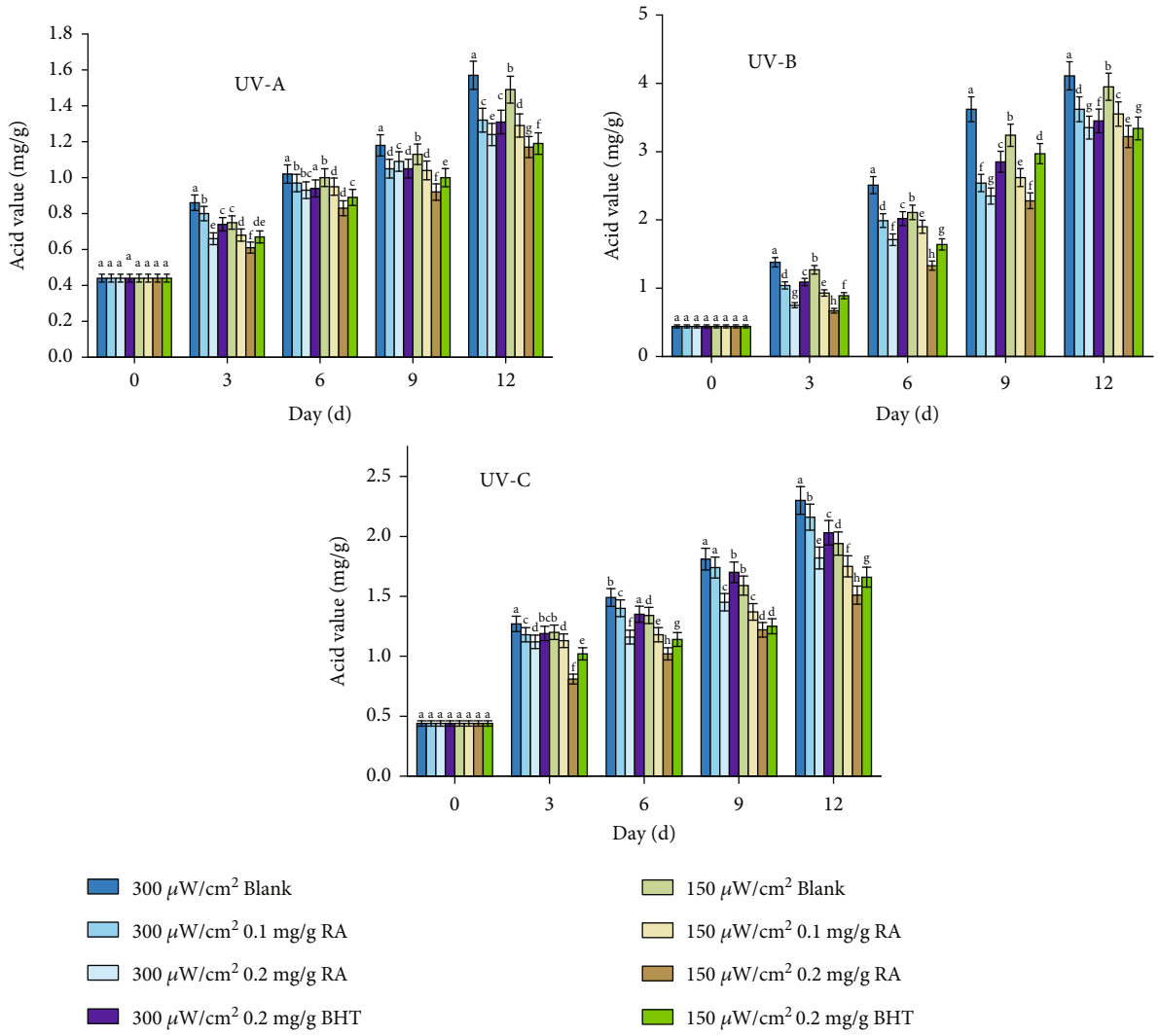

Figure 1: The acid value of each group of samples under UV-A, UV-B, and UV-C conditions.

thiobarbituric acid- (TBA-) trichloroacetic acid (TCA) solution. The TBA-TCA solution consists of 15% (*w*/*v*) TCA and 20 mM TBA in an aqueous solution. Combine 0.5 mL of the oil with 2 mL of the TBA-TCA solution in a centrifuge tube. Mix the contents, place the tube in a water bath at 95°C for 30 min until the solution becomes pale pink, allow it to cool for 10 minutes, and then centrifuge (centrifuge corporation) at 3900 rpm at room temperature for 25 minutes. As a blank control, prepare 0.5 mL of distilled water using the same procedure. Measure the absorbance of the supernatant containing the pink compound at 532 nm with an ultraviolet-visible spectrophotometer. The results are expressed in milligrams of malondialdehyde (MDA) per kilogram of walnut oil.

2.4.8. Data Analysis. Each sample analysis is repeated at least three times to ensure that the overall accuracy is at least 5% of the CV (coefficient of variation), and data were subjected to analysis of variance (ANOVA). ANOVA analyses were performed according to SPSS software. Significant differences between means were determined by Duncan's multiple range tests; P values less than 0.05 were considered statistically significant.

3. Result and Discussion

3.1. Comparison of Acid Value of Walnut Oil under Different UV Conditions. Comparison of the acid value of each experimental sample group under different UV conditions is shown in Figure 1.

Determining the acid value of fats and oils is a method for assessing rancidity or the extent of rancidification. As shown in Figure 1, the acid value exhibits an increasing trend, albeit not very pronounced, primarily due to the initial formation of hydroperoxides during the oil's oxidation. With prolonged storage, hydroperoxides further transform into aldehydes, ketones, acids, and other compounds [26]. This is when the acid value experiences a significant upswing. During the 12-day observation period, the most rapid acid value increase was observed in the 300 μW/cm^2 blank group. The acid values under the three radiation conditions were 1.57 ± 0.08 mg/g, 4.11 ± 0.21 mg/g, and

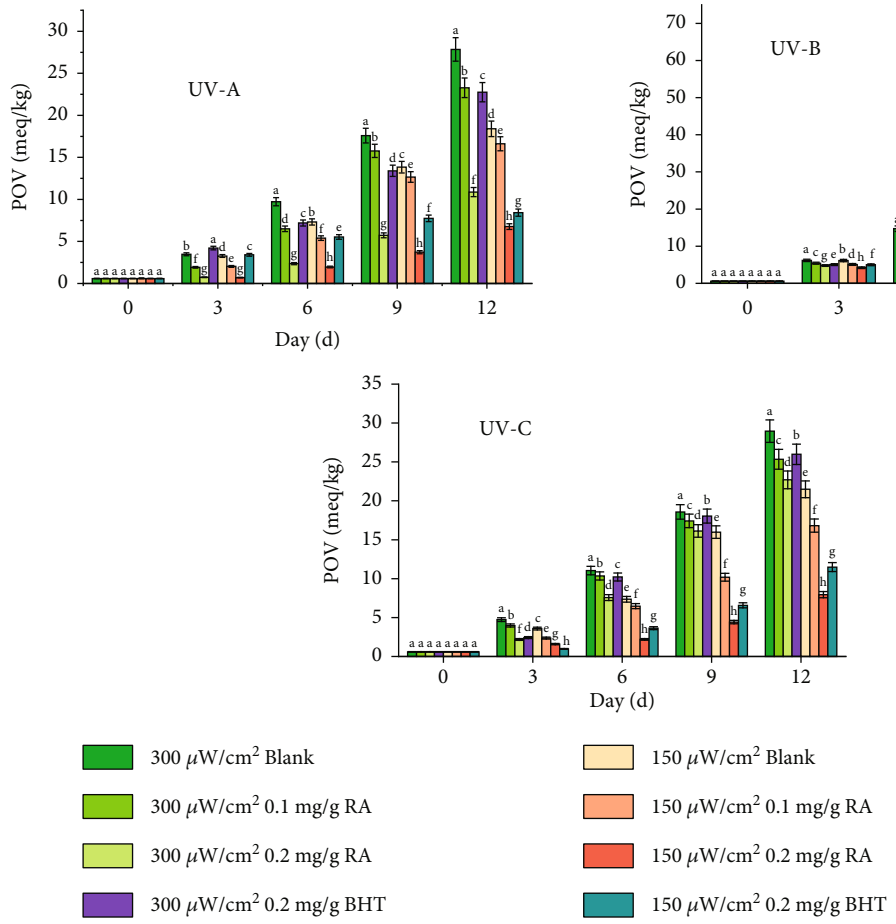

Figure 2: Peroxide value of each group of samples under UV-A, UV-B, and UV-C conditions.

2.30 ± 0.12 mg/g. Among the UV radiation conditions, UV-B exhibited the most rapid increase, with a growth rate of 833.41% compared to day 0, followed by UV-C at 422.73%. The mildest impact on oil and fat was observed under UV-A conditions, with an increase of 168.94%. In the case of the three distinct radiation types, identical samples were exposed to varying intensities of ultraviolet radiation, leading to significant differences in the samples ($P < 0.05$). This observation highlights the substantial impact of ultraviolet radiation intensity on the acid value of oil. A comparison of the acid value between the blank group and the antioxidant group leads to the conclusion that antioxidants exhibit antioxidative effects under ultraviolet conditions, and the extent of oil and fat rancidity is linked to the concentration of RA. Within a specific concentration range, a higher RA concentration corresponds to a lower degree of sample rancidity. Under the UV-A, UV-B, and UV-C radiations, the RA group containing 0.2 mg/g and the equivalent BHT group, whether exposed to 300 μW/cm^2 or 150 μW/cm^2, exhibited lower acid values in the RA group. This suggests that an identical concentration of RA possesses a more potent antioxidant capacity.

3.2. Comparison of Peroxide Value of Walnut Oil under Different UV Conditions. Comparison of the peroxide value of each experimental sample group under different UV conditions is shown in Figure 2.

This suggests that an identical concentration of RA possesses a more potent antioxidant capacity. A comparison of the peroxide values of the samples under the three conditions reveals that UV-B has the most pronounced impact on the oxidation stability of walnut oil. The three samples are all 300 μW/cm^2. All three samples were exposed to 300 μW/cm^2. The blank sample exhibited the highest peroxide value. On the 12th day, under UV-B conditions, the peroxide value of the 300 μW/cm^2 blank group reached (62.09 ± 0.44 meq/kg), with a growth rate of 10423.21%. The oxidation process of oils and fats initially generates hydroperoxides, making the peroxide value of oils and fats an early indicator of oil oxidation and rancidity [27]. Figure 2 illustrates that the peroxide value is on the rise, and its growth rate is notably rapid compared to the growth rate of the acid value, in alignment with the theory that the peroxide value measures the initial oxidation product of oil. When comparing the peroxide values of the samples under the three radiation conditions, the order of oxidation severity is UV-B > UV-C > UV-A, corresponding with the experimental findings for the acid value. Compare the peroxide values of the blank group and the sample group with added antioxidants (best 0.2 mg/g RA) under the same radiation condition (300 μW/cm^2). From this, it is deduced that the antioxidant effect is most pronounced under a UV-B conditions, with an inhibition rate of 66.56%, followed by a

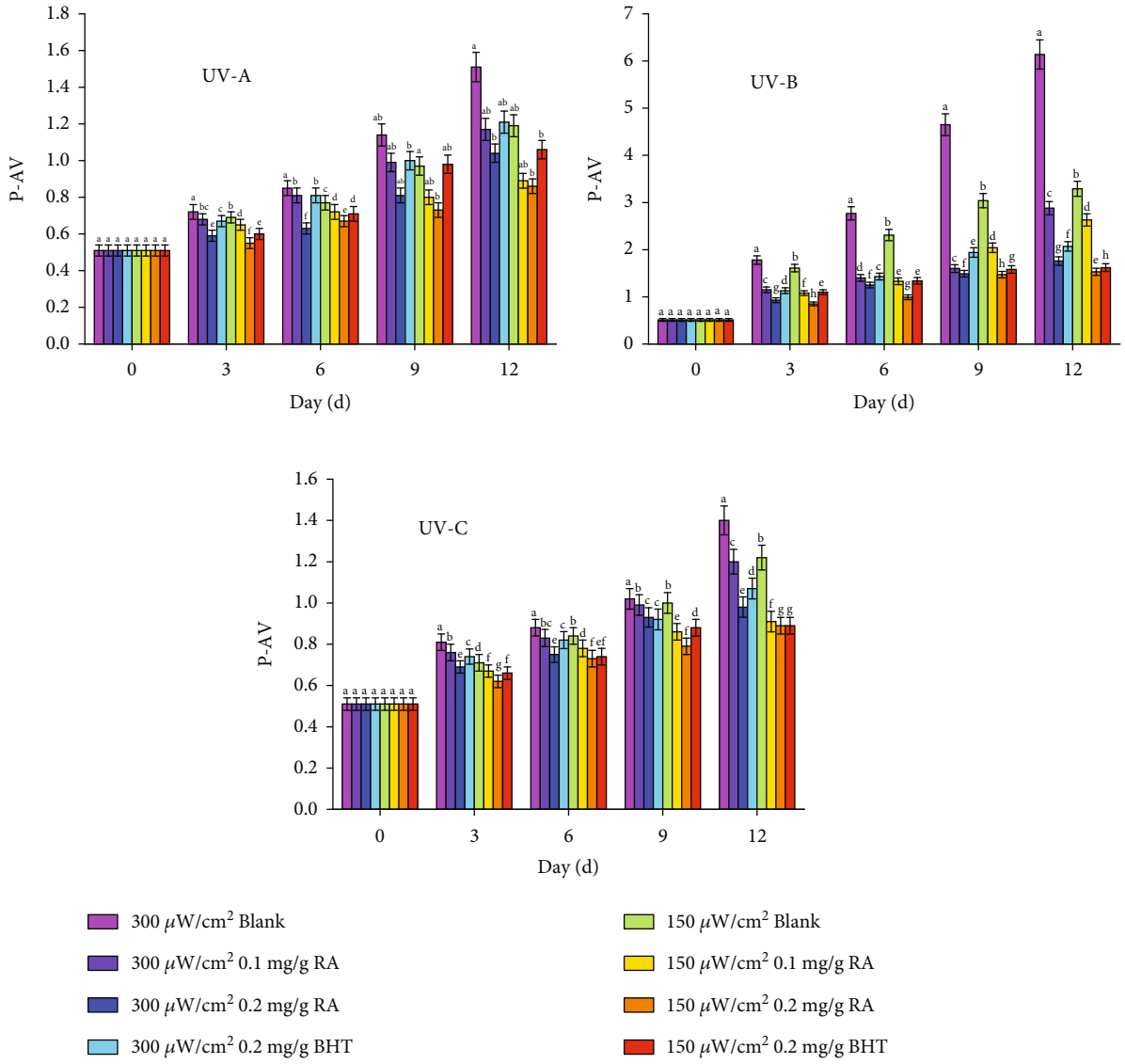

Figure 3: Anisidine value of each group of samples under UV-A, UV-B, and UV-C conditions.

UC-A inhibition rate of 60.96%, and a minimum inhibition rate of 20.62% under a UV-C conditions. Among the three types of ultraviolet radiation, namely, UV-A, UV-B, and UV-C, UV-C possesses the shortest wavelength and the highest energy, followed by UV-B, and lastly UV-A. However, UV-C has limited penetration ability. Consequently, UV-B exerts the most significant influence on oils and fats among the three types of radiation. Subjecting the same sample to varying UV radiation intensities demonstrated a notable disparity ($P < 0.05$), suggesting a certain correlation between UV radiation intensity and the peroxide value of oil. Higher radiation intensity corresponds to a higher peroxide value of the fat. Within the three conditions, the concentration of antioxidants also exerts an influence on the peroxide value of fats and oils. Greater antioxidant concentration results in a lower peroxide value of the sample ($P < 0.05$). When comparing 0.2 mg/g RA with the equivalent concentration of BHT, the peroxide value of the RA group is lower than that of the BHT group, indicating that RA possesses a more robust antioxidant capacity.

3.3. The Change Trend of p-Anisidine Value of Walnut Oil under Different UV Conditions. Comparison of p-anisidine values of different experimental sample groups under different UV conditions is shown in Figure 3.

Anisidine value detection measures the presence of secondary oxidation products in oils and fats, particularly aldehyde compounds. Aldehydes are byproducts of advanced oil and fat spoilage and are responsible for unpleasant odors in oils. It can be stated that the measurement of the p-anisidine value provides a numerical assessment of the sensory attributes of oils and fats [28]. Figure 3 presents a bar chart depicting the anisidine value of each sample group under various conditions. The figure illustrates an increasing trend in anisidine values, with each sample group displaying the most rapid rise in anisidine values under UV-B conditions.

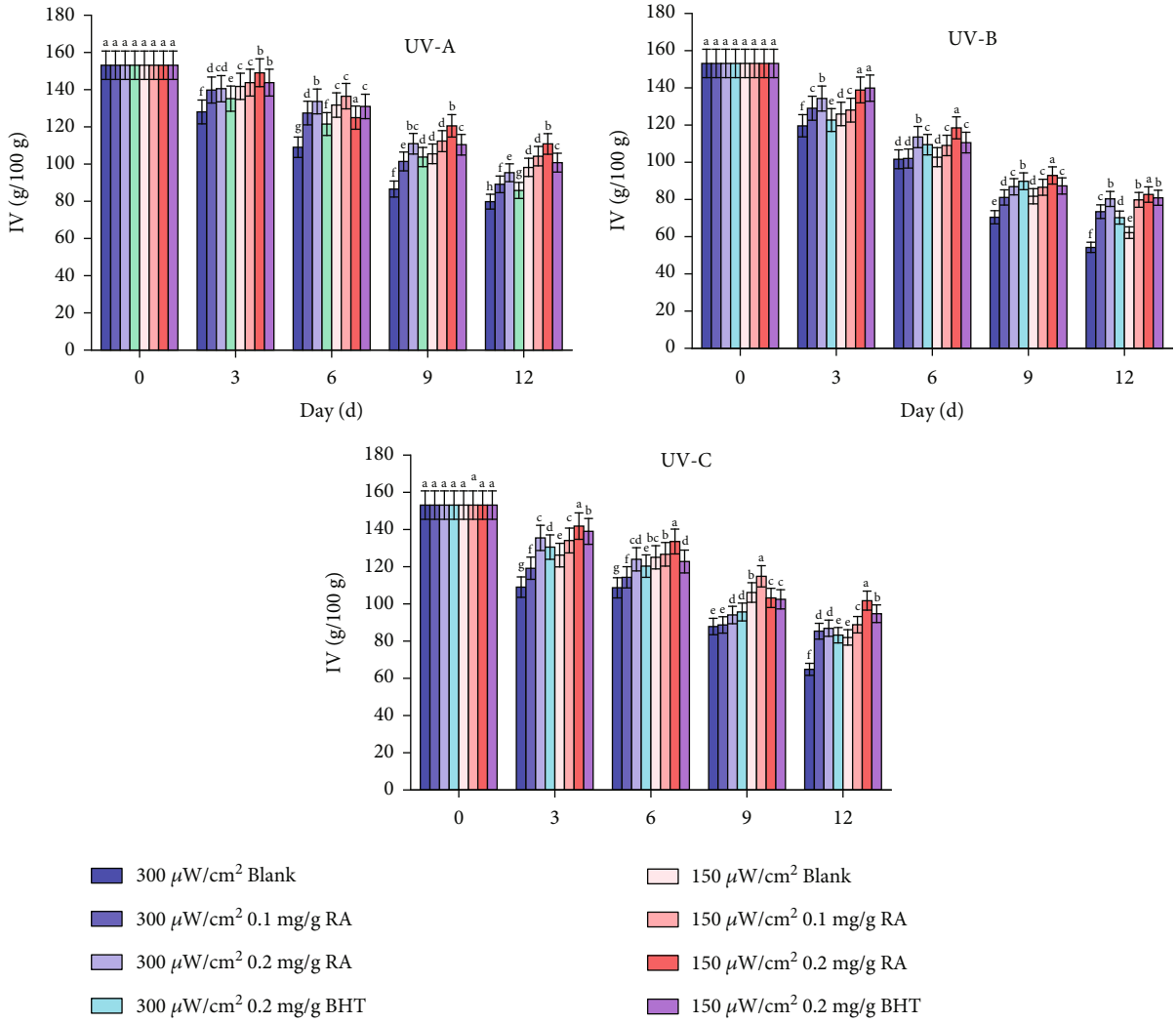

Figure 4: Iodine value of each group of samples under UV-A, UV-B, and UV-C conditions.

Upon comparing varying radiation intensities under different conditions, it can be concluded that higher ultraviolet radiation intensity corresponds to an increased anisidine value in the oil, signifying a greater degree of oil oxidation. These conclusions align with the earlier findings regarding acid value and peroxide value.

3.4. Comparison of Iodine Value of Walnut Oil under Different UV Conditions. Comparison of iodine value of each experimental sample group under different UV conditions is shown in Figure 4.

Determining the iodine value of oils and fats assists in assessing the level of unsaturation in them, providing an indirect measure of their oxidation degree [29]. Examining Figure 4 reveals a decreasing trend in the iodine value. As the storage duration increases, the concentration of unsaturated fatty acids in walnut oil gradually diminishes, aligning with the prolonged oxidation of the oil. In UV-A, UV-B, and UV-C conditions, the 300 μW/cm^2 blank group displayed the most rapid decline in iodine value. The observed sequence UV-B > UV-C > UV-A aligns with the previous patterns observed in acid value, peroxide value, and other experiments. Figure 5 reveals that the iodine value does not exhibit the same regularity as the acid value and peroxide value. This variation can be attributed to the consumption of unsaturated bonds in unsaturated fatty acids during the oxidation of fats and oils, alongside the production of other compounds containing unsaturated bonds. Notably, the formation of unsaturated aldehydes during oil and fat oxidation exerts a specific influence on the iodine value. Nevertheless, it is evident that the same conclusion as the earlier test remains valid: stronger ultraviolet radiation leads to a higher degree of oil oxidation.

3.5. Total Oxidation Value. The standardization trend of the total oxidation value of the samples under UV-A, UV-B, and UV-C conditions is shown in Figure 5.

The following conclusions can be derived from the three graphs depicting changes in total oxidation values: the total oxidation value serves as an index that offers a comprehensive assessment of oil oxidation by incorporating both peroxide and anisidine values [30]. With prolonged storage of

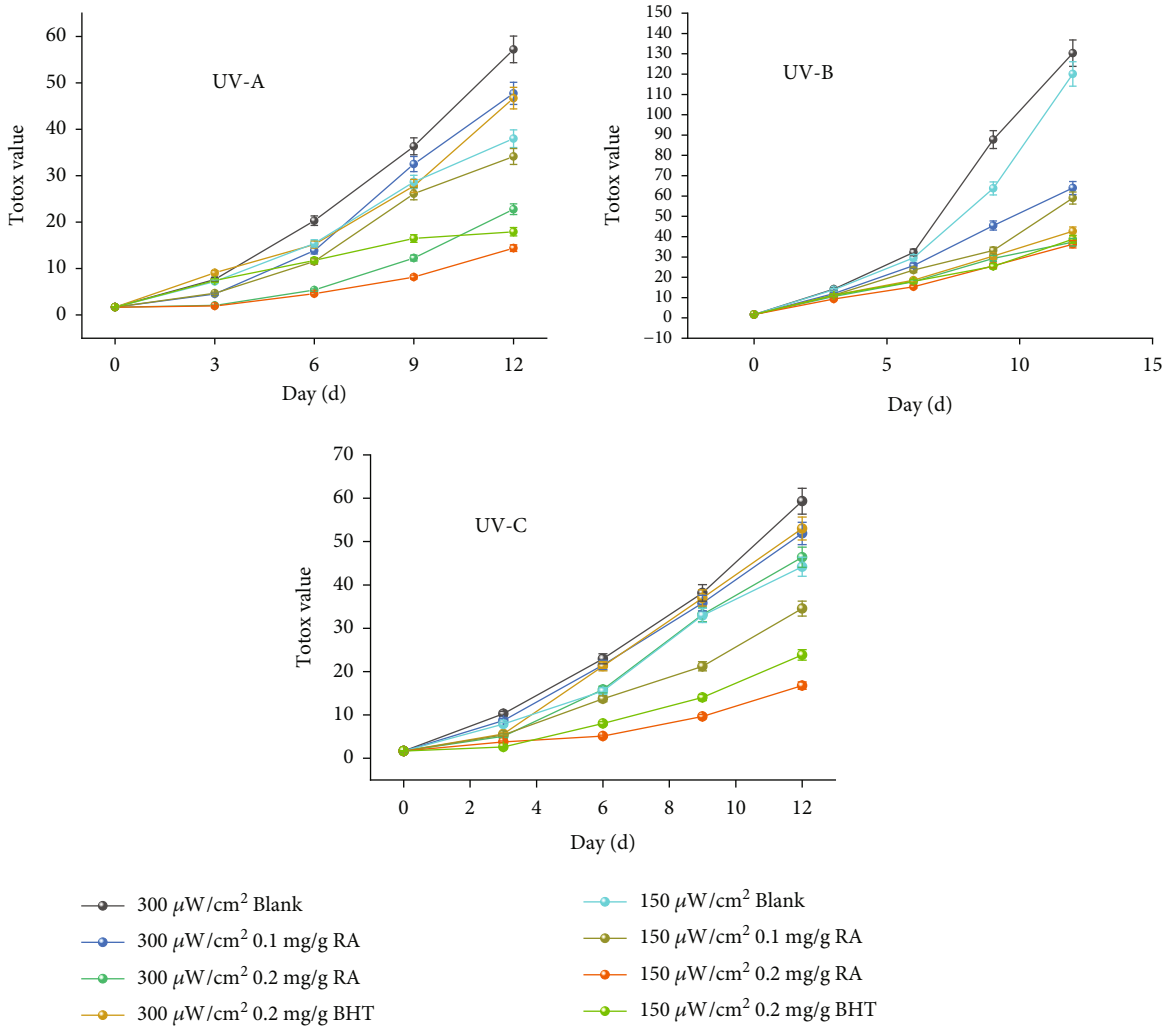

Figure 5: The changing trend of the total oxidation value of each sample under the conditions of UV-A, UV-B, and UV-C.

the oil, the total oxidation value rises. The total oxidation value trend graphs reveal that, under all three radiation conditions, the 300 μW/cm^2 blank group exhibits the highest degree of oxidation. A comparison of the three conditions allows us to determine the impact on oil oxidation strength: UV-B > UV-C > UV-A. This conclusion contradicts the notion that shorter ultraviolet wavelengths have a stronger impact on oil oxidation. Following further research, it is noted that UV-C is referred to as shortwave ultraviolet. Despite its harmful short wavelength, UV-C has limited natural penetration and struggles to pass through the atmospheric ozone layer to reach the ground, causing harm to humans. Despite its limited penetration, UV-C poses significant harm to the human body. In this experiment, the use of glass containers limits the penetration of UV-C, resulting in minimal impact on oil oxidation. Nevertheless, the impact of UV-C on oil oxidation is still more significant than that of UV-A. Among the three radiation conditions, the 0.2 mg/g RA sample group at 150 μW/cm^2 experienced the lowest level of oxidation. However, under UV-B conditions, the degree of oxidation for the 0.2 mg/g RA and 0.2 mg/g BHT samples at 150 μW/cm^2 is nearly identical. This could be attributed to the stability of rosmarinic acid under ultraviolet radiation. Furthermore, rosmarinic acid exhibits greater safety than BHT, making it advantageous for use. The analysis of the total oxidation value reaffirms the conclusion from the previous test: the concentration of antioxidants exhibits an inverse relationship with the degree of oil oxidation, and the intensity of ultraviolet radiation is directly proportional to the degree of oil oxidation. Notably, RA demonstrates a stronger antioxidant activity compared to BHT.

3.6. Determination of the Antioxidant Activity of Walnut Oil. Antioxidant activity testing includes DPPH free radical scavenging rate and lipid oxidation (MDA) testing. The measured data results are shown in Table 1: the following conclusions can be drawn from the table.

Based on the free radical scavenging rate, UV-A > UV-C > UV-B, it is observed that the impact of ultraviolet radiation on the oxidation stability of walnut oil is UV-B > UV-C > UV-A. Notably, UV-B exerts the most pronounced effect on the antioxidant activity of the oil. Rosmarinic acid (RA) demonstrates a protective effect across various conditions, capable of mitigating or retarding the oxidative rancidity of

Table 1: DPPH free radical scavenging rate and MDA content of each sample under different conditions.

	DPPH (%)				MDA												
		UV-A	UV-B	UV-C		UV-A				UV-B				UV-C			
	0 d		15 d		0 d	3 d	6 d	9 d	12 d	3 d	6 d	9 d	12 d	3 d	6 d	9 d	12 d
300 μW/cm^2 blank	56.81 ± 0.68	41.57 ± 1e	32.54 ± 0.41f	37.49 ± 0.53g	4.87 ± 0.09	5.64 ± 0.07a	8.77 ± 0.25a	10.88 ± 0.26a	16.44 ± 0.26a	5.79 ± 0.30a	9.13 ± 0.30a	12.72 ± 0.34a	17.42 ± 0.54a	5.42 ± 0.33a	9.01 ± 0.08a	12.06 ± 0.21a	16.98 ± 0.38a
300 μW/cm^2 0.1 mg/g RA		45.66 ± 1.14d	43.33 ± 0.58d	43.89 ± 0.56e		4.99 ± 0.2b	5.63 ± 0.09b	7.94 ± 0.22b	9.04 ± 0.19d	5.03 ± 0.17b	6.27 ± 0.43b	7.39 ± 0.50c	10.55 ± 0.39d	5.24 ± 0.27ab	5.89 ± 0.24bc	7.54 ± 0.33bc	10.17 ± 0.42d
300 μW/cm^2 0.2 mg/g RA		53.77 ± 1.04b	48.76 ± 0.78bc	50.43 ± 0.61c		4.9 ± 0.16b	4.93 ± 0.04c	7.28 ± 0.22cd	8.92 ± 0.30d	5.01 ± 0.14b	4.98 ± 0.07c	6.53 ± 0.11d	10.02 ± 0.15de	5.11 ± 0.07ab	5.56 ± 0.43c	7.04 ± 0.17c	9.72 ± 0.24de
300 μW/cm^2 0.2 mg/g BHT		49.58 ± 0.61c	47.95 ± 1.37c	49.04 ± 0.30cd		5.17 ± 0.09b	5.74 ± 0.05b	7.14 ± 0.14de	11.26 ± 0.25c	5.22 ± 0.15b	5.93 ± 0.22b	7.44 ± 0.45c	12.22 ± 0.31c	5.05 ± 0.16ab	5.7 ± 0.23c	7.32 ± 0.48c	11.09 ± 0.15c
150 μW/cm^2 blank		43.89 ± 0.97d	35.79 ± 1.47e	39.21 ± 1.32f		5.11 ± 0.15b	5.54 ± 0.10b	7.62 ± 0.07bc	11.96 ± 0.11b	5.17 ± 0.18b	6.33 ± 0.16b	8.29 ± 0.16b	13.47 ± 0.23b	5.39 ± 0.16a	6.19 ± 0.33b	7.93 ± 0.26b	13.14 ± 0.41b
150 μW/cm^2 0.1 mg/g RA		49.07 ± 1.57c	48.99 ± 0.36bc	48.46 ± 0.99d		4.97 ± 0.33b	4.96 ± 0.18c	6.37 ± 0.16f	9.27 ± 0.27d	4.93 ± 0.11b	4.92 ± 0.06c	6.05 ± 0.66de	9.91 ± 0.29de	4.95 ± 0.10b	4.99 ± 0.23d	6.42 ± 0.15d	9.36 ± 0.49e
150 μW/cm^2 0.2 mg/g RA		54.34 ± 1.44a	52.34 ± 0.82a	53.97 ± 1.53a		4.93 ± 0.04b	4.89 ± 0.06c	6.03 ± 0.05f	9.03 ± 0.22d	4.88 ± 0.34b	4.88 ± 0.30c	5.62 ± 0.34e	8.94 ± 0.19f	4.88 ± 0.12b	4.93 ± 0.11d	5.83 ± 0.41e	8.73 ± 0.16f
150 μW/cm^2 0.2 mg/g BHT		53.88 ± 1.24a	49.5 ± 1.09b	52.33 ± 0.70b		4.98 ± 0.11b	5.02 ± 0.17c	6.84 ± 0.41e	9.22 ± 0.06d	5 ± 0.13b	5.15 ± 0.08c	6.74 ± 0.44cd	9.74 ± 0.60e	4.98 ± 0.24b	5.07 ± 0.21d	6.26 ± 0.27de	9.39 ± 0.13e

Note: using Duncan's algorithm, the same letter in the same column means no significant difference, $P < 0.05$.

walnut oil. This protective effect exhibits a positive correlation with RA concentration within a specific range. The protective effect becomes stronger with increasing RA concentration. Furthermore, when assessing the DPPH clearance rate under two different radiation intensities and across three distinct radiation types, the findings align with the previous tests involving acid value, peroxide value, and others. In these assessments, it is reaffirmed that, at the same concentration, RA exhibits superior antioxidant activity compared to BHT. The DPPH and MDA detection methods consistently indicate that the oxidation stability of walnuts is in line with a common trend. Specifically, as the storage period extends, the free radical scavenging capacity of oil and fat diminishes gradually, leading to an increase in rancidity.

4. Conclusion

Based on this experiment, it is evident that UV-A, UV-B, and UV-C exert varying effects on the oxidation stability of walnut oil. Notably, UV-B has the most pronounced impact on walnut oil oxidation. Furthermore, the degree of oil and fat oxidation is directly proportional to the radiation intensity, underscoring the importance of storing walnut oil with rosemary acid away from strong light. Additionally, at equivalent concentrations of BHT and RA, the natural antioxidant RA exhibits superior antioxidant activity. While the distinction between the RA and BHT groups is not significant under specific conditions, it is worth noting that BHT, being a synthetic antioxidant, can have certain adverse effects on human health. Consequently, the investigation into the protective effects of rosmarinic acid against the photo-oxidation of walnut oil remains of practical significance, providing a theoretical foundation for future applications of rosmarinic acid in oil. This study focused on a limited set of indicators to assess the degree of oil oxidation when adding rosmarinic acid to walnut oil under different radiation conditions. The precise mechanism underlying the impact of rosmarinic acid on antioxidant activity in various radiation conditions warrants further exploration.

Data Availability

The data that support the findings of this study are available from the corresponding author, Shuang Jin (jinshuangzy@126.com), upon reasonable request.

Conflicts of Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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