Preparation of Rose Vinegar Rich in Gamma-Linolenic Acid and Analysis of its Nutritional Composition and Antioxidant Activity

Yao Zhang, Qing Liu, Zhuo Liu, Chao Zhang and Yuanda Song

Food Bioengineering and Technology Laboratory, Department of Food Science and Nutrition, College of Culture and Tourism, University of Jinan, 13 Shungeng Road, Jinan 250022, China
Colin Ratledge Center for Microbial Lipids, School of Agricultural Engineering and Food Science, Shandong University of Technology, 266 Xincun West Road, Zibo 255000, China
Shandong Baofeng Food Company, Zibo 255000, China

Abstract: Despite the rapid development of various types of fruit vinegar or nutrient-fortified vinegar, the weakened functions or high cost is still the main limiting factor for healthy vinegar. In the present study, a new type of rose vinegar was obtained from raw grain supplemented with natural extract of roses through the cooperative fermentation of multiple microorganisms by adding Mucor circinelloides and lactic acid bacteria. The chemical composition analysis showed that this rose vinegar was rich in various organic acids and amino acids, which endowed the rose vinegar with a special taste and nutritional value. In particular, fatty acid content results revealed that the rose vinegar contained several long-chain unsaturated fatty acids, especially the valuable Gamma-Linolenic Acid (GLA), which was mainly due to the addition of M. circinelloides that could synthesize GLA during the fermentation of vinegar. The antioxidant activity of the rose vinegar indicated that the contents of gallic acid and vanillic acid were relatively high and thus it had a certain scavenging ability on DPPH free radicals, superoxide anion free radicals, and hydroxyl free radicals. This is the first study to develop a healthy vinegar containing GLA with antioxidant activity by combining the rose extract with a variety of beneficial microbial fermentation.

Keywords: Rose Vinegar, Gamma-Linolenic Acid, Mucor circinelloides, Lactic Acid Bacteria, Antioxidant Activity

Introduction

With the continuous progress of society and the improvement of people's living standards, people's health awareness and the demand for healthy food are increasing. Vinegar, as a traditional indispensable condiment in people's diets, contains abundant organic substances such as carbohydrates, organic acids, acetic acid, alcohol, amino acids, and volatile compounds (Budak et al., 2014; Zhao et al., 2020). As a good carrier of health food, vinegar has many health benefits such as appetizing and invigorating the spleen, waking up and warming the blood, eliminating fatigue, and so on (Budak et al., 2014; Chen et al., 2023). Due to the destruction of the ecological environment, the accelerated pace of life, excessive mental pressure, and other factors, people are easily in a state of sub-health, and people's understanding of healthy food and the demand for healthy vinegar is further stimulated. The development of vinegar with more health functions, in line with people's eating habits and consumption habits, has broad market prospects.

Traditional brewing vinegar is mostly made from starchy grain raw materials through microbial fermentation by a three-stage process. Firstly, starch is degraded to fermentable sugars by mold. And then, fermentable sugars are converted to ethanol by yeast. Finally, ethanol is oxidized to acetic acid by bacteria (Ye et al., 2023; Zhao et al., 2020). In recent years, various kinds of vinegar drinks with fruits and vegetables as raw materials have developed rapidly, such as apple cider vinegar, hawthorn vinegar, grape

© 2023 Yao Zhang, Qing Liu, Zhuo Liu, Chao Zhang and Yuanda Song. This open-access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.
vinegar, mulberry vinegar, onion vinegar, and seaweed vinegar, etc. (Bang et al., 2022; Cantadori et al., 2022; Li et al., 2023; Ousaaid et al., 2021; Özdemir et al., 2022; Singh et al., 2023). There are also many types of research on nutrient-fortified vinegar drinks with adding various nutritional factors, such as vitamins, mineral elements, pearl powder, oligosaccharides, etc., (Budak et al., 2014; Chen et al., 2019). Ousaaid et al. (2023). However, this kind of vinegar produced from fruit or fruit processing waste rather than raw grains, may undermine the health advantage of vinegar itself. And the addition of various nutrients increases the cost of vinegar.

In order to give vinegar more healthy properties, it is an effective way to add natural ingredients or microorganisms during the fermentation process. Rose water extract is rich in protein, vitamins, unsaturated fatty acids, anthocyanins, polysaccharides, flavonoids, and other active substances. It has free radical scavenging, antioxidant, anti-tumor, prevention and treatment of cardiovascular diseases, and other effects (Mileva et al., 2021; Song et al., 2020). The high edible and medicinal value of rose made it an ideal raw material for developing healthy food. The oleaginous mold Mucor circinelloides can synthesize a valuable omega-6 polyunsaturated fatty acid, Gamma-Linolenic Acid (GLA), which possesses important biological effects such as anti-atherosclerosis, anti-inflammation, anti-cancer, etc., (Zhang et al., 2022). Probiotics, which mainly refers to lactic acid bacteria, is a general term for microorganisms that are beneficial to human health. Lactic acid bacteria can not only regulate the intestinal flora of the body but also have anti-cancer, enhance immunity and reduce serum cholesterol (Xia et al., 2022; Yassunaka Hata et al., 2023; Ye et al., 2023). In our previous study, a high lipid-yielding fungus M. circinelloides, and two lactic acid bacteria with antioxidant activity were screened from our lab, which could be used in vinegar brewing (Wang et al., 2022; Zhang et al., 2023).

The present study was aimed at developing a new type of rose vinegar rich in GLA with bright color, strong aroma, and high nutritional value, which caters to people's demand for natural food, nutrition, and health preservation. Firstly, the rose vinegar was prepared by using the natural extract of roses as brewing material combined with M. circinelloides and lactic acid bacteria fermentation. And then, the appearance characteristics, nutrient composition, and antioxidant capacity of the rose vinegar were also analyzed. Our study not only diversifies the varieties of healthy vinegar but also provides a theoretical basis for further development of healthy vinegar products.

Materials and Methods

Strains, Chemicals, and Reagents

M. circinelloides WJ11, Lactobacillus casei (CGMCC No. 16750) and Lactobacillus plantarum (CGMCC No. 16751) were screened by our laboratory and preserved in China Microbiological Species Preservation Center. Saccharomyces rouxii Boutroux and Acetobacterium were purchased from Kangyuan Biological Co., Ltd (Zibo, China). Roses are food grade purchased from the Laizhou planting area in Shandong, China. All reagents used for analysis were analytical grade standard products purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All kinds of organic acid standard products are chromatography grade purchased from Beina Chuanglian Biotechnology Research Institute (Beijing, China).

Preparation Method of Rose Vinegar

Fresh rose petals were washed, dried, and weighed. Then the petals were homogenized and added to distilled water with a solid-liquid ratio of 1:3. 0.6% citric acid (w/w) was added to the rose liquid for color protection. The mixture was soaked in a water bath at 50°C for 2 h, centrifuged for 20 min and the supernatant was taken. The extraction operation was repeated three times. The three times of extraction solution was mixed and stored at 4°C.

Starchy raw materials such as rice or glutinous rice were soaked, steamed, and cooled to 30-40°C. Then, 25% rose extract, 5% amylase and 10% M. circinelloides spore were added and stirred evenly and liquefied and saccharified at 30°C for 6-8 h. Then, 15% S. rouxii was added with the proper supplement of water to make the moisture of fermented grains 50-60% and alcohol fermentation was carried out at 28-30°C for 6-7 d. After alcohol fermentation, 10% Acetobacterium, 5% L. plantarum, and 5% L. casei were added to the fermented grains. The fermentation temperature was controlled at 30-32°C for 12-15 d with the supplement of oxygen by stirring thoroughly. After acetic acid fermentation, the fermentation solution was squeezed and filtered, and then 15% sugar, honey, and other accessories were added. After sterilization and aging, rose probiotic vinegar with a total acid content of ≥4% was obtained. The processing diagram is shown in Fig. 1.

General Components and Chemical Analysis

The pH was measured using a pH meter (Mettler Toledo, PE28-Standard, USA), and the content of reducing sugar was determined by the 3,5-Dinitrosalicylic acid (DNS) colorimetric method (Wang et al., 2019). Determination of total phenolic content was performed by the Folin-Ciocalteu assay using gallic acid as a standard phenolic compound (Song et al., 2016). The content of total flavonoids was determined by the colorimetric method as described before with minor modifications using tannic acid as the standard (Song et al., 2016).
The content of total acids was determined according to the national standard GB/T12456-2008 "Determination of total acid in food". General components such as moisture (GB/T5009.3-2016: Determination of moisture in food), ash (GB/T5009.4-2016: Determination of ash in food), protein (GB/T5009.5-2016: Determination of protein in food), fat (GB/T5009.6-2016: Determination of fat in food), energy (GB/T28050-2011: National standards for food safety pre-packaged food nutrition label general rules), sodium (GB/T5009.91-2017: Determination of potassium and sodium in food), dietary fiber (GB/T5009.88-2014: Determination of dietary fiber in food) were carried out according to the national standard prescribed method.

**Determination of Organic Acids and Phenolic Substances**

The standard analysis and sample determination were detected by HPLC with InfinityLab Porshell.120 EC-C18 (150 mm × 4.6 mm, Agilent Technologies, Inc., USA). The analysis of organic acids used 0.05 mol/L Na2HPO4 (pH 2.8) as effluent with a flow rate of 0.5 mL/min for 20 min and the sample size was 10 μL. The detection wavelength of the UV detector was set at 210 nm. The temperature of the column oven was set at 30°C and the detection wavelength of the UV detector was set at 280 nm.

**Determination of Free Amino Acids**

Rose vinegar samples were hydrolyzed with 6 mol/L HCl at 150°C for 1 h and T-Butyldimethylsilyl (TBDMS) was used as a pre-column derivatization reagent for sample analysis. GC-MS measurement was performed with the method described in our previous work (Wang et al., 2019). The fused-silica capillary column (30.0 m × 250 nm × 0.25 μm) by Shimadzu 2010 GC was used. The GC process was set as follows: The initial temperature was 60°C for 2 min, then it rose to 180°C at 5°C/min and 260°C at 10°C/min, respectively, and then continued for 5 min. The MS detecting conditions were set as follows: The ion source and vaporization temperatures were respectively set at 230°C and 250°C and the detector voltage and power voltage were respectively fitted at 350 V and 70 eV.

**Determination of Fatty Acid Composition and Content**

After liquefaction and saccharification, alcohol fermentation, and acetic acid fermentation, the
fermentation mixture was repeatedly squeezed and filtered out to obtain the vinegar sample. Total lipids were extracted from 1 mL of vinegar sample by chloroform/methanol (2:1, v/v) and then methylated at 60°C for 3 h with 10% HCl/methanol (v/v). Pentadecanoic acid (C15: 0, Sigma) was used as the internal standard. The obtained fatty acid methyl esters were separated by n-hexane and analyzed by Gas Chromatography (GC) fitted on a 30 mm × 0.32 mm DB-Waxetr column with a film thickness of 0.25 µm. The GC program was set with our previous method as follows: 120°C for 3 min, rising to 200°C at 5°C/min, then rising to 220°C at 4°C/min and keeping for 2 min (Zhang et al., 2023).

Determination of Antioxidant Activity

The 1,1-Diphenyl-2-trinitrophenylhydrazine (DPPH) radical scavenging activity, superoxide radical scavenging assay, and hydroxyl radical scavenging ability of the rose vinegar were assessed according to the method as described by Liu et al. (2022).

Statistical Analysis

SPSS statistics 22 software was used for statistical analysis. All data are expressed as mean ± standard deviation. The mean values and the standard errors of the mean were calculated by three independent experiments. Student’s t-test was used for differences between test means and p<0.05 was considered statistically significant.

Results and Discussion

Appearance Characteristics of Rose Vinegar

The rose extract and its fermented vinegar were shown in Fig. 2. The extract of roses was bright color, rich flavor, and uniform texture without precipitation. The rose vinegar was prepared from rice as raw grain with the supplement of rose extract and M. circinelloides and lactic acid bacteria were added to participate in biological fermentation. This vinegar was golden yellow, clear and transparent, and evenly distributed (Fig. 2).

Chemical Composition and Properties of Rose Vinegar

The determination results of protein, lipid, carbohydrate, sodium, and other essential nutrients and energy in the rose vinegar were summarized in Table 1. The main component of the rose vinegar was water. The contents of energy, carbohydrate, and sodium were 70.8 kJ/100 mL, 4.9 g/100 mL, and 17.8 mg/100 mL, respectively. Compared with other vinegar drinks, this rose vinegar was low in carbohydrates and energy, making it suitable for daily drinking.

Table 1, a high value of acidity was observed in the rose vinegar and the pH of the vinegar was 3.01. Organic acids in the vinegar might be generated by various fermentation bacteria. The rose vinegar was rich in acetic acid, malic acid, succinic acid, citric acid, and so on, among which acetic acid accounts for more than 90% of the total acid content since acetic acid was thought to be the main cause of the large changes in pH and acidity. The contents of various organic acids such as acetic acid, succinic acid, malic acid, and citric acid, meet the requirements of the main organic acids in the national standards. Acetic acid has a pungent sour taste, succinic acid has an umami taste, malic acid is refreshing, and citric acid sour taste and is rounded (Shi et al., 2022). These organic acids vary in acidity and their proportions are in harmony, which together constitute the unique sour taste of rose vinegar.

Amino acid is an important substance to maintain human life activities. It not only has various physiological functions but also has a cushioning effect on the sour taste of the vinegar, making it soft, delicious, and mellow and providing energy to body tissues (Kong et al., 2017; Liu et al., 2023). The free amino acids in the rose vinegar were determined and the results were shown in Table 1. This vinegar contains 16 kinds of free amino acids. The total amount of amino acids was 387.94 µg/mL, in which the contents of isoleucine, proline, aspartic acid, and serine were high, reaching 156.42, 72.13, 56.47, and 32.94 µg/mL, respectively. Isoleucine could cooperate with leucine and valine to repair muscles and control blood sugar (Ullrich et al., 2016).
Table 1: Chemical and antioxidant analysis of rose prebiotic vinegar

<table>
<thead>
<tr>
<th>Project</th>
<th>Content</th>
<th>Project</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.01±0.050</td>
<td>Total acid (g/L)</td>
<td>40.60±1.25</td>
</tr>
<tr>
<td>Total sugar (g/L)</td>
<td>48.36±0.150</td>
<td>Total polyphenol (g/L)</td>
<td>1.88±0.09</td>
</tr>
<tr>
<td>Total flavonoid (g/L)</td>
<td>1.74±0.070</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General contents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/100 mL)</td>
<td>70.8±2.8000</td>
<td>Protein (g/100 mL)</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>Fat (g/100 mL)</td>
<td>0.40±0.0200</td>
<td>Carbohydrate (g/100 mL)</td>
<td>4.9±0.24</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.0</td>
<td>Moisture (%)</td>
<td>90.2±2.50</td>
</tr>
<tr>
<td>Na (mg/100 mL)</td>
<td>17.8±0.9000</td>
<td>Dietary fiber (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Organic acids (μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3001.37±12.62</td>
<td>Malic acid</td>
<td>426.51±5.63</td>
</tr>
<tr>
<td>Citric acid</td>
<td>284.35±3.480</td>
<td>Lactic acid</td>
<td>5.54±0.37</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>27.66±1.220</td>
<td>Oxalic acid</td>
<td>49.42±1.48</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>475.81±3.560</td>
<td>Glutaric acid</td>
<td>4.33±0.86</td>
</tr>
<tr>
<td>Free amino acids (μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp^4</td>
<td>56.47±2.39</td>
<td>Tyr^4</td>
<td>2.61±0.02</td>
</tr>
<tr>
<td>Glu^4</td>
<td>8.32±0.16</td>
<td>Val^*</td>
<td>1.96±0.07</td>
</tr>
<tr>
<td>Ser</td>
<td>32.94±1.28</td>
<td>Met^*</td>
<td>0.62±0.02</td>
</tr>
<tr>
<td>Gly^4</td>
<td>1.56±0.04</td>
<td>Cys</td>
<td>3.48±0.05</td>
</tr>
<tr>
<td>Arg</td>
<td>3.88±0.45</td>
<td>He^*</td>
<td>156.42±3.48</td>
</tr>
<tr>
<td>Thr^*</td>
<td>15.37±0.75</td>
<td>Leu^*</td>
<td>23.69±1.01</td>
</tr>
<tr>
<td>Pro</td>
<td>72.13±1.03</td>
<td>Phe^*</td>
<td>2.35±0.04</td>
</tr>
<tr>
<td>Ala^4</td>
<td>4.82±0.21</td>
<td>Lys^*</td>
<td>1.32±0.02</td>
</tr>
<tr>
<td>Essential amino acids</td>
<td></td>
<td>Total amino acids</td>
<td>387.94</td>
</tr>
<tr>
<td>Essential amino acids/Total amino acids (%)</td>
<td>52.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acid composition (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>541.89±23.57</td>
<td>C18:0</td>
<td>339.02±16.72</td>
</tr>
<tr>
<td>C18:1</td>
<td>606.32±35.44</td>
<td>C18:2</td>
<td>476.45±13.21</td>
</tr>
<tr>
<td>C18:3</td>
<td>358.41±20.08</td>
<td>Others</td>
<td>41.92±2.460</td>
</tr>
<tr>
<td>Phenolic substances (μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>184.38±1.46</td>
<td>Caffeic acid</td>
<td>3.52±0.140</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>103.09±2.58</td>
<td>Ferulic acid</td>
<td>0.88±0.020</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>1.02±0.01</td>
<td>Quercitrin</td>
<td>1.41±0.050</td>
</tr>
<tr>
<td>Catechol</td>
<td>1.29±0.03</td>
<td>Rutin</td>
<td>1.65±0.360</td>
</tr>
<tr>
<td>Antioxidant capacity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH free radical scavenging rate (%)</td>
<td>92.34±2.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide anion free radical scavenging rate (%)</td>
<td>59.16±1.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyl free radical scavenging rate (%)</td>
<td>79.66±3.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: N.D., Not Detected
^4 Flavorful amino acid; * Essential amino acid
Data are expressed as mean ± standard deviation (n=3)

Serine was a component of seramine phosphate in the brain and other tissues, which could reduce blood cholesterol concentration and prevent hypertension (Guevara and Mani, 2016). It can be seen that the rose vinegar contained a variety of amino acids with certain nutritional value and the contents of amino acids were also high. The total amount of essential amino acids was 201.73 μg/mL, accounting for 52.00% of the total amino acids. The contents of isoleucine, leucine, and threonine in essential amino acids were relatively high. The total amount of flavored amino acids in the rose vinegar was 76.13 μg/mL, accounting for 19.62% of the total amino acid content. Among them, aspartic acid is related to the formation of a sour taste, glycine, and alanine are related to sweetness, and glutamic acid is related to the formation of umami substances (Ardö, 2006). These amino acids improve the nutritional quality of rose vinegar and further enrich the taste of vinegar.

Fatty Acid Composition and GLA Content of Rose Vinegar

Although the lipid content of rose vinegar is low, the fatty acid composition of rose vinegar was detected by GC analysis in this study. The results from Table 1 showed that the main fatty acids were octadecenoic acid (C18:1, C18:2, and C18:3) and palmitic acid (C16:0), which were basically consistent with our previous research (Zhang et al., 2022; 2023). It should be noted that rose vinegar contains several long-chain unsaturated fatty acids, especially the valuable GLA, which is mainly due to the addition of *M. circinelloides* that could synthesize GLA during the fermentation.
process of vinegar. The presence of GLA was not found in other commercially available vinegar products.

Antioxidant Activity of Rose Vinegar

Phenolic substances have antioxidant, anti-free radical, and other biological activities, as well as antibacterial, antiviral, anti-tumor, and other pharmacological effects (Bodoira and Maestri, 2020; Lagana et al., 2020). The contents of monomer phenols in the rose vinegar were determined and the results were shown in Table 1. The total content of 8 monomer phenols in the rose vinegar was 297.24 μg/mL and the contents of gallic acid and vanillic acid were relatively high. Previous studies have shown that gallic acid has an obvious scavenging ability on DPPH free radicals and hydroxyl free radicals (Marino et al., 2014). The contents of gallic acid, vanillic acid, and ferulic acid in common fruit vinegar are low, while rose vinegar contains relatively high contents of phenolic acids and then it has strong antioxidant activity. This further indicated that the addition of rose extract increased the content of phenolic substances, changed the nutritional composition, and improved the antioxidant capacity of vinegar.

Antioxidant capacity is the comprehensive effect of various antioxidant health factors. The scavenging rates of DPPH free radicals, superoxide anion free radicals, and hydroxyl free radicals by the rose vinegar were detected in Table 1. The results indicated that rose vinegar had a certain scavenging ability on three kinds of free radicals and the scavenging rates of three kinds of free radicals in rose vinegar were 92.34% for DPPH free radicals, 59.16% for superoxide anion free radicals and 79.66% for hydroxyl free radicals, respectively. Adding a certain amount of rose extract as raw material in the process of vinegar fermentation could significantly affect the antioxidant capacity of the rose vinegar.

Conclusion

In this study, a new type of rose vinegar was made of raw grain supplemented with a natural extract of roses as brewing material through the cooperative fermentation of multiple beneficial strains by adding M. circinelloides and lactic acid bacteria. The rose vinegar was rich in organic acids, including acetic acid, malic acid, succinic acid, citric acid, and so on. These organic acids coordinated with each other to form the vinegar’s special sour taste. The rose vinegar contained 16 kinds of amino acids with a total content of 387.94 μg/mL. These amino acids contained 7 kinds of essential amino acids and 5 kinds of flavorful amino acids, accounting for 52.00 and 19.62% of the total amino acids, respectively. These amino acids enhanced the nutritional quality and the taste of the rose vinegar. The fatty acid composition revealed that the rose vinegar contained several long-chain unsaturated fatty acids, especially the valuable GLA, which is mainly due to the addition of M. circinelloides that could synthesize GLA during the fermentation process of vinegar. The antioxidant activity of the rose vinegar showed that the contents of gallic acid and vanillic acid were relatively high and thus the rose vinegar had a certain scavenging ability on DPPH free radicals, superoxide anion free radicals, and hydroxyl free radicals. The content of GLA and antioxidant activity in rose vinegar could be further improved through fermentation optimization. In summary, this study for the first time combined the rose extract with a variety of beneficial microbial fermentation to develop a healthy vinegar rich in GLA. It not only diversified the kinds of vinegar but also offered a theoretical foundation for further development of healthy vinegar products. It is believed that giving vinegar more new nutrients and functions will be a development trend of healthy vinegar in the future.

Acknowledgment

We would like to thank Panling Liu, a 2018 student of Food Science and Engineering at Shandong University of Technology, for her great help in this research.

Funding Information

This study was supported by the school-city integration project in Zhangdian district (2021JSCG0016), the national natural science foundation of China (32101927), and the Shandong provincial natural science foundation (ZR2020MC007).

Author’s Contributions

Yao Zhang: Involved in the study conception, experimental design, data analysis, figures and tables arrangement, result interpretation, manuscript writing, and review of the final manuscript.

Qing Liu: Carried out the experiments and collected data.

Zhuo Liu and Chao Zhang: Participated in the experimental research.

Yuanda Song: Conceived the study and reviewed the original manuscript.

Ethics

All authors read and approved the final version of this manuscript. There are not any ethical issues to declare that could arise after the publication of this manuscript.
References


200


