Composition and antioxidant analysis of jiaosu made from three common fruits: watermelon, cantaloupe and orange

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ABSTRACT
Jiaosu, also called as fermented fruit and vegetable juices, is becoming an increasingly popular health food, especially in East Asian markets. Watermelon, cantaloupe and orange were used as the raw materials to produce five kinds Jiaosu with natural fermentation, including single source Jiaosu and mixed jiaosu. Then the composition and antioxidant capacity were determined. All Jiaosu appear brown and different Jiaosu vary greatly. The pH of five kinds Jiaosu range from 3.06 to 4.55. Between the five kinds Jiaosu, orange Jiaosu is the highest in total protein (9.46 ± 0.41 mg/ml), total phenol (0.32 ± 0.01 µg/ml) and alcohol (56.51 ± 0.03 µg/ml). All Jiaosu have strong antioxidant capacity and watermelon Jiaosu shows the strongest antioxidant capacity. The antioxidant capacity of five kinds Jiaosu determined by FRAP assay is significantly correlated with the total protein content and total phenol content, respectively. The results indicated the raw materials had a decisive effect on the components and antioxidant capacity of Jiaosu.

1. Introduction
Jiaosu is a functional microbial fermentation product made by fermenting one or more different vegetables, fruits, cereals, seaweeds and mushrooms through natural or artificial accession of bacteria. Jiaosu have a variety of functions, including: promoting metabolism (Dong et al., 2009), promoting blood circulation (G. Y. G. Y. Zhao et al., 2015), eliminating toxins, regulating the body’s acid-base balance, enhancing the digestive and absorption functions of the gastrointestinal tract (Parvez et al., 2006), and also have antioxidant and anti-fatigue functions. It can be used as a functional food (M. M. M. M. Zhao et al., 2017), providing a new nutritional pathway for humans. In recent years, many health foods with added Jiaosu have been produced. According to the “Guideline for Jiaosu products classification” published by Ministry of Industry and Information Technology of the People’s Republic of China, Jiaosu products can be divided into the following six categories according to the application field: (1) Edible Jiaosu; (2) Environmental Jiaosu; (3) Household Jiaosu; (4) Feed Jiaosu; (5) Agricultural Jiaosu; (6) Other Jiaosu (China, 2018).

The current researches about Jiaosu mainly focus on the functional characteristics (Bai et al., 2017; Han et al., 2019), antioxidant capacity (Jiang et al., 2013; Luo et al., 2019; Shu et al., 2019), fermentation processes (Liu et al., 2019; Qin et al., 2019), and practical applications (Jing et al., 2019; Pu et al., 2019; Tong, 2017). Qin et al. (2019) study on antioxidant activity of Rubus idaeus-Punica granatum compound juice Jiaosu prepared by different fermentation technologies showing that the mixed fermentation method was superior to the single fermentation and natural fermentation method. Mei et al. (2020) study on the effect of different fermentation processes on the equality of Stropharia rugosoannulata Jiaosu and showed that the S. rugosoannulata Jiaosu products had...
strong anti-oxidation ability. The *S. rugosoannulata* Jiaosu produced by the artificial inoculation fermentation process is superior to natural fermentation in following aspects: the ability to scavenge free radicals, lipase, amylase, and organic acids.

At present, the comparative studies on the types and properties of Jiaosu mostly focus on the different fermentation processes, such as the comparison of natural fermentation and fermentation methods of different strains. However, there are limited comparative studies on the properties of Jiaosu made from different raw materials. Watermelon, cantaloupe and orange are all very common fruit in our daily life. Although some jiaosu may add the above fruits as one of the ingredients. There is no Jiaosu product based on above fruit and no comparing between them. Therefore, in this study, the above three common fruits were used as raw materials, and five kinds of Jiaosu were prepared by the same fermentation method (natural fermentation). Then, the content of each component and antioxidant capacity of the five Jiaosu were compared and analyzed. In addition to the fermentation process, we should pay more attention to the impact of raw materials on Jiaosu quality.

2. Materials and methods

2.1. Preparation of jiaosu

Watermelon, cantaloupe, orange and brown sugar were used and all were purchased in the regular market. The fruits were all ripe and were washed with clean water. The watermelons and cantaloupes were cut into pieces with a width and height of about 5 cm, and each orange was divided into four pieces. We put the treated raw materials into a 50 L plastic barrel according to the weight ratio of brown sugar, fruit and water of 1:3:10, and then stirred them for ten minutes. Finally, we sealed and marked them. With different raw materials, five kinds Jiaosu were produced (Table 1). The fermentation temperature was controlled between 20°C to 30°C and fermentation process lasted six months. After the fermentation completed, the Jiaosu solution was filtered out for determination.

2.2. Determination of pH and components

All five kinds Jiaosu were filtered again with a circular fast qualitative filter paper of 11 cm and poured the filtrate into a clean tube. The color and taste of the five kinds Jiaosu were compared and recorded. Then the pH values of the five kinds Jiaosu were measured using the Leici PHS-3 C PH meter (INESA Scientific Instrument Co., Ltd, Shanghai).

Total protein, total phenol, total sugar, vitamin E and alcohol content of five kinds Jiaosu were determined. The total protein quantification kit (Coomassie Brilliant Blue), total phenol kit and Vitamin E assay kit (Colorimetric method) were purchased from Nanjing Jiancheng Bioengineering Institute (http://www.njjcbio.com) and all specific methods referred to the instructions. The total sugar kit was purchased Shanghai yuanye Bio-Technology Co., Ltd (http://www.shyuanye.com/), following method of Glucose Determination With 3, 5-Dinitrosalicylic Acid. The colorimetric method was used to determine the alcohol content (Wei et al., 2001). We took 5 mL of diluted five-fold sample solution into a test tube and added 10 mL of 2% potassium dichromate solution, 5 mL of concentrated sulfuric acid, then shaking for 10 minutes. The absorbance was determined at the wavelength of 610 nm. A standard curve was prepared basing different concentrations of alcohol. According to the regression equation and the absorbance of the sample solution, the alcohol content of each sample solution was calculated.

2.3. Determination of antioxidant capacity

The antioxidant capacity of the five kinds Jiaosu were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, 2,2-Azino-Bis (3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS) assay and ferric reducing antioxidant power (FRAP) assay respectively. The determination of DPPH free radical scavenging ability referring to the method of He et al. (2020). First, we took 2 mL of 0.1 mmol/L DPPH solution in a test tube, gradually added Jiaosu filtrate with shaking. When the color of the solution has basically disappeared, recorded the volume of the added Jiaosu filtrate. Basing the above experiment, 5 arithmetic series volume were set for gradient test. Then the five different volumes (less than 1 mL) of the Jiaosu samples were taken into the test tube, supplemented with absolute ethanol to 1 mL. 2 mL of 0.1 mmol/L DPPH solution was added to each test tube. Placed the test tube in the dark at room temperature for 30 minutes. The absorbance was measured at a wavelength of 517 nm. Simultaneously, the absorbance at 517 nm of the mixture of 1 mL sample solution and 2 mL absolute ethanol were measured as a blank. The absorbance at 517 nm of a mixture of 1 mL of absolute ethanol and 2 mL of DPPH solution were measured as a control. The free radical scavenging activity of each concentration of the sample against DPPH free radical was calculated by the following formula (1). A regression equation has been obtained and the IC50 was calculated. The IC50 value indicated the volume to scavenge 50% of free radicals, so the smaller the IC50 value, the stronger the antioxidant capacity. Total antioxidant capacity assay kit (ABTS method) and total antioxidant capacity assay kit (FRAP method) were purchased from Nanjing Jiancheng Bioengineering Institute (http://www.njjcbio.com) and all specific methods referred to the instructions.

| Watermelon | Cantaloupe | Orange | Brown sugar | Water |
|------------|------------|--------|-------------|-------|
| Watermelon Jiaosu | 8.57 | - | - | 2.86 | 28.57 |
| Cantaloupe Jiaosu | 8.57 | - | - | 2.86 | 28.57 |
| Orange Jiaosu | 8.57 | - | - | 2.86 | 28.57 |
| Watermelon-Cantaloupe Jiaosu | 4.28 | 4.28 | - | 2.86 | 28.57 |
| Orange-Cantaloupe Jiaosu | - | 4.28 | 4.28 | 2.86 | 28.57 |
DPPH free radical scavenging rate

\[
(\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%
\]

(A1: the absorbance of the sample; A2: the absorbance of the blank; A0: the absorbance of the control).

2.4. Statistical analyses

Each experiment was performed in triplicate to avoid errors. The statistical analysis was performed using IBM SPSS Statistics 26.0. Data was expressed as mean ± standard deviation (SD) indicating sample variability. The Least Significant Difference (LSD) was employed to determine whether the differences between the Jiaosu were significant \((p <.05)\). Pearson correlation coefficient method was used to explore the correlation relationship between factors.

3. Results and analysis

3.1. Color and taste characteristics of Jiaosu

The Jiaosu is sanitary after the filter. All Jiaosu appear brown and different kinds Jiaosu vary greatly in color (Figure 1). The color of watermelon Jiaosu is the darkest and orange Jiaosu appears lighter than other. The pH of the five kinds Jiaosu range from 3.06 to 4.55 (Table 2). Each Jiaosu smell different and all Jiaosu have a wine flavor. Orange Jiaosu smell the most comfortable.

3.2. Comparative analysis of Jiaosu component

The total protein content of the five kinds Jiaosu ranges from \(5.98 \pm 0.47 \text{mg/ml (orange Jiaosu)}\) to \(9.46 \pm 0.41 \text{mg/ml (cantaloupe Jiaosu)}\). There is no significant different of the total protein content between the orange Jiaosu and watermelon Jiaosu \((8.90 \pm 0.63 \text{mg/ml})\), while they are higher than others \((p < .05)\). The total phenol content of the five kinds Jiaosu is low, ranging from \(0.32 \pm 0.01 \mu\text{g/ml (cantaloupe Jiaosu)}\) to \(0.54 \pm 0.03 \mu\text{g/ml (orange Jiaosu)}\). The total phenol content of watermelon Jiaosu \((0.40 \pm 0.01)\) is between cantaloupe Jiaosu and orange Jiaosu and the different is significant \((p < .05)\). There is no significant different between two mixed Jiaosu, watermelon-cantaloupe Jiaosu and orange-cantaloupe Jiaosu \((p > .05)\). The content of vitamin E of the five kinds Jiaosu ranges from \(1.92 \pm 0.26 \mu\text{g/ml (cantaloupe Jiaosu)}\) to \(2.76 \pm 0.43 \mu\text{g/ml (watermelon-cantaloupe Jiaosu)}\). The vitamin E content of two mixed Jiaosu is higher than single source Jiaosu, while there is no significant different between all five kinds Jiaosu \((p > .05)\). The total sugar content of the five kinds Jiaosu span largely and the variance is also large, ranging from \(247.70 \pm 30.00 \mu\text{g/ml (watermelon-cantaloupe Jiaosu)}\) to \(1070.46 \pm 68.74 \mu\text{g/ml (cantaloupe Jiaosu)}\). The total sugar content of cantaloupe Jiaosu is much higher than other Jiaosu \((p < .05)\). With wine flavor, there are alcohol in five kinds Jiaosu and the content are low. The highest alcohol content is orange Jiaosu \((56.51 \pm 0.03 \mu\text{l/ml})\), and the lowest content is watermelon Jiaosu \((15.37 \pm 0.46 \mu\text{l/ml})\) respectively (Table 3).

Except vitamin E, there are significant differences in the contents of the other four components for different Jiaosu. The total protein content, total phenol content and alcohol

![Figure 1](image)

Figure 1. The color of the five kinds Jiaosu.

Figura 1. Color de los cinco tipos de jiaosu.

| Jiaosu                  | pH   | Representation                                      |
|-------------------------|------|----------------------------------------------------|
| Watermelon Jiaosu       | 4.49-4.55 | dark brown, obvious acid smell, lighter wine flavor, pungent smell |
| Cantaloupe Jiaosu       | 4.31-4.44 | brown, obvious acid smell, wine flavor, mild smell |
| Orange Jiaosu           | 3.06-3.12 | light brown, obvious acid smell, stronger wine flavor, has the smell of oranges, good smell |
| Watermelon-Cantaloupe Jiaosu | 4.19-4.29 | partial dark brown, obvious acid smell, wine flavor, a little pungent smell |
| Orange-Cantaloupe Jiaosu | 3.46-3.55 | light brown, obvious acid smell, stronger wine flavor, lighter orange flavor, good smell |
content of orange Jiaosu are the highest among the five kinds Jiaosu, and the total sugar content is also the second lowest. Among the two mixed Jiaosu, watermelon-cantaloupe Jiaosu has the highest vitamin E content and the lowest total sugar content of the five kinds of Jiaosu (Table 3).

3.3. Comparative analysis of jiaosu antioxidant capacity

DPPH free radical scavenging ability is widely used to evaluate antioxidant activity in a short time (Sirwardhana & Shahidi, 2002). The IC50 value of watermelon Jiaosu is the smallest (40.93 μM/ml) and the largest is orange Jiaosu with 74.71 μM/ml. The IC50 value of the other three kinds of Jiaosu range from 59.38 μM/ml to 61.18 μM/ml, and there is no significant difference between them (p > 0.05, Table 4).

The ABTS assay is fast, simple and highly correlated with the biological activity of antioxidants (Berg et al., 1999). In this study, ABTS value of watermelon Jiaosu is the largest (1.20 ± 0.01 mM), significantly higher than other types (p < 0.05). The ABTS value of other four kinds Jiaosu ranges 0.80 ± 0.10 mM to 0.90 ± 0.05 mM, and there is no significantly different from each other (p > 0.05, Table 4).

FRAP method is widely used in the analysis of antioxidant capacity of food and health products (Y. X. Y. Chen et al., 2011). In this study, the five kinds Jiaosu measured by FRAP method have the largest value of 4.75 ± 0.43 mM for orange Jiaosu, and the smallest is 3.13 ± 0.12 mM of cantaloupe Jiaosu. The FRAP values of orange Jiaosu, watermelon Jiaosu and orange-cantaloupe Jiaosu were higher than other two kinds Jiaosu (p < 0.05).

3.4. Relationship between components and antioxidant capacity

In our study, correlation analysis was also carried out between the components and the antioxidant capacity (Table 5). The analysis shows that there is no significant correlation between the components and ABTS and DPPH (p > 0.05). The correlation between the FRAP and the total protein content reached a very significant level (r² = 0.753, p < 0.01). Besides, there is also significant positive correlation between the total phenol content and FRAP (r² = 0.581, p < 0.05).

4. Discussion

At present, fruits, vegetables or plants are always used as raw materials for the fermented products, Jiaosu (Kuwaki et al., 2012; Simsek et al., 2014; Zulkawi et al., 2017). At the same time, natural fermentation or fermentation by specific bacteria are proposed as the two main fermentation methods (Kwaw et al., 2018; Zhang et al., 2020; D. Zhao & Shah, 2014). The Jiaosu in this study were made from three common fruits, watermelon, cantaloupe and orange, and were treated by natural fermentation method. Using the same process, single source Jiaosu and mixed Jiaosu were made respectively. The components and antioxidant capacity indicate that the source of raw materials has a decisive effect on the above indicators. Except the total sugar content, the maximum and minimum values of all detection indicators are from the single source Jiaosu.

The pH value of the five kinds Jiaosu ranges from 3.06 to 4.55, showing acidity, which is basically consistent with most fruit Jiaosu (Yan, 2019). There is alcohol in all five kinds Jiaosu, ranging from 15.37 ± 0.46 μl/ml to 56.51 ± 0.03 μl/ml. The fermented products studied by Zhang et al. (2018) and Kun et al. (2008) also had high alcohol content, and the results were generally consistent.

Total phenols play an important role in the antioxidant capacity of Jiaosu. There are many studies to explore the correlation between the total phenolic content and antioxidant capacity for most fruits or vegetables Jiaosu (Gao et al., 2020; Gu & Zhao, 2016; Jiang et al., 2013). However, the relationship between total phenol content and antioxidant properties is not completely clear. In the study, all the five kinds Jiaosu have strong antioxidant capacity, but the total phenol content is low. G. L. G. L. Chen et al. (2014) found that some fruits Jiaosu had high total phenol content, but their antioxidant capacity was normal. Even, it was found that tomatoes and carrots Jiaosu had low or almost no total phenol content, but they also had strong antioxidant capacity (Tian & Yang, 2004). On the other hand, the DPPH assay, the ABTS assay, and the FRAP assay were employed to determine the antioxidant capacity of the Jiaosu. However, the correlation between total phenols and the above three

Table 3. Comparative analysis of Jiaosu component.

| Watermelon Jiaosu | Cantaloupe Jiaosu | Orange Jiaosu | Watermelon-Cantaloupe Jiaosu | Orange-Cantaloupe Jiaosu |
|-------------------|------------------|--------------|-------------------------------|------------------------|
| Total protein(mg/ml) | 8.90 ± 0.63 | 5.98 ± 0.47 | 9.46 ± 0.41 | 6.54 ± 0.41 |
| Total phenol(μg/ml) | 0.40 ± 0.01 | 0.32 ± 0.01 | 0.54 ± 0.03 | 0.41 ± 0.02 |
| Vitamin E(μg/ml) | 2.03 ± 0.6 | 1.92 ± 0.26 | 2.59 ± 0.83 | 2.76 ± 0.43 |
| Total sugar(μg/ml) | 353.50 ± 30.00 | 1070.46 ± 68.74 | 368.94 ± 25.98 | 247.70 ± 30.00 |
| Alcohol(μl/ml) | 15.37 ± 0.46 | 29.15 ± 0.40 | 56.51 ± 0.03 | 22.44 ± 0.20 |

Mean ± SD, different letters for each sample indicate the significant differences at p < 0.05.

Table 4. Comparative analysis of Jiaosu antioxidant capacity.

| Watermelon Jiaosu | Cantaloupe Jiaosu | Orange Jiaosu | Watermelon-Cantaloupe Jiaosu | Orange-Cantaloupe Jiaosu |
|-------------------|------------------|--------------|-------------------------------|------------------------|
| ABTS (mM) | 1.20 ± 0.01 | 0.80 ± 0.10 | 0.85 ± 0.08 | 0.84 ± 0.04 |
| FRAP (mM) | 4.64 ± 0.92 | 3.13 ± 0.12 | 4.75 ± 0.43 | 3.17 ± 0.03 |
| DPPH IC50 (μl/ml) | 40.93 | 59.38 | 74.71 | 61.18 |

Mean ± SD, different letters for each sample indicate the significant differences at p < 0.05.
indicators is different. The results suggest that we should choose appropriate indicators to measure the antioxidant capacity of Jiaoos.

5. Conclusion

The raw materials play an important role for the composition and antioxidant capacity of Jiaoos. All Jiaoos appear brown and different kinds Jiaoos vary greatly in color. Between the five kinds Jiaoos, orange Jiaoos are the highest in total protein, total phenol and alcohol, while vitamin E and total sugar content are also in a better position, showing the good nutritional content. All five kinds Jiaoos have strong antioxidant capacity and watermelon Jiaoos shows the strongest antioxidant capacity. The antioxidant capacity of five kinds Jiaoos determined by FRAP assay is significantly correlated with the total protein content and total phenol content, respectively.

Disclosure statement

The authors declare that they have no conflicts of interest.

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