Optimization of watermelon peel yogurt fermentation process and study on antioxidant capacity

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Abstract. This study aims to explore the variation patterns of total flavonoid and other antioxidant activities in watermelon peel yogurt, providing a basis for the production of a functional yogurt. Using watermelon peel as the raw material and total flavonoid content as the indicator, the optimal process of watermelon peel yogurt was optimized through single-factor and orthogonal experiments. The results indicate that yogurt fermented with a mass ratio of watermelon juice to milk at 1:3, fermentation time of 10 hours, and fermentation agent dosage of 0.10% exhibits a refreshing watermelon aroma, excellent texture, and almost no whey separation. After process optimization, the polysaccharide content of watermelon peel yogurt increased by 19%, and water-holding capacity rose to 63.3%. Texture analysis revealed a hardness of 81.50 g, viscosity of 20.00 g, adhesiveness of 1.20 mJ, elasticity of 8.83 mm, and a draw length of 6.76 mm. The physicochemical and microbiological properties of watermelon peel yogurt meet national standards, and it possesses a good free radical scavenging ability.

Keywords: Watermelon Peel, Total Flavonoid Content, Yogurt, Processing Technology

1. Introduction

Watermelon Peel, also known as “Xi Gua Cui” or “Xi Gua Qing”, referred to as “Xi Gua Cui Yi” in Chinese medicine [1-3]. Recognized as a dual-purpose medicinal and edible food [4], it is characterized by a refreshing fragrance, rich flavor, sweet taste, cool nature, and non-toxic properties. It is known for its functions of generating fluids, relieving thirst, clearing summer heat, and promoting diuresis [2-3]. Comprising one-third of the total watermelon volume, watermelon peel is often used as fertilizer or urban waste during the hot summer season. Due to its high fiber, low protein, and high-water content, improper disposal can lead to the release of ammonia, hydrogen sulfide, methane, and other hydrocarbons, causing serious impacts on crops and the environment [1, 5]. In recent years, research on the nutritional value of watermelon peel has been increasing. Utilizing watermelon peel as a raw material, various products like fermented beverages [6], watermelon peel and kudzu root wine [7], and watermelon peel hot sauce [8] have been developed, aiming to increase the utilization of beneficial components while reducing pollution. Yogurt, fermented by combining milk with lactic acid bacteria under specific conditions, is rich in active probiotics and has various functions such as anti-pathogenic bacteria, promoting intestinal peristalsis, and enhancing immunity [9]. The lactic acid produced during lactic acid bacteria fermentation can stimulate the stomach and intestines, increase intestinal peristalsis, and prevent constipation [10-12]. In recent years, the development of compound fermented milk has

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become a hot topic, but its main drawbacks include a singular taste, flavor, and functionality [13]. In this study, we utilize the traditional process of yogurt fermentation, using watermelon peel as a raw material. The impact of watermelon peel juice and milk mass ratio, fermentation time, and fermentation agent dosage on the total flavonoid content in watermelon peel yogurt is investigated as a single-factor experiment. Orthogonal optimization is then employed to optimize the fermentation conditions, aiming to develop a functional yogurt. This product is convenient to source, promoting the reuse of watermelon peel to reduce pollution. Simultaneously, we explore the variation patterns of total flavonoids, polysaccharides, and other antioxidant activities in watermelon peel yogurt. This study provides fundamental data for further exploration of other nutritional components in watermelon peel and the development of watermelon peel products.

2. 2. Materials and Methods

2.1. Materials and Equipment

2.1.1. Materials and Reagents

Watermelon peel: Purchased from the fruit market in Nanning (Kirin watermelon).

Defatted milk: Inner Mongolia Yili Industrial Group Co., Ltd.

Sucrose: Guangxi Yangpu Nanhua Sugar Industry Group Co., Ltd.

Lactic acid bacteria: Angel Yeast Co., Ltd.

Food-grade anhydrous ethanol: Wuhan Canosi Technology Co., Ltd.

Sodium nitrite: Dezhou Runxin Experimental Instrument Co., Ltd.

Aluminum nitrate: Guangzhou Xinhong Trading Co., Ltd.

Sodium hydroxide: Dezhou Runxin Experimental Instrument Co., Ltd.

Phenol: Guangzhou Xinhong Trading Co., Ltd.

D-sodium isoascorbate (food grade) and L-ascorbic acid: Henan Zhongchen Biological Technology Co., Ltd.

DPPH free radical and hydroxyl free radical scavenging capability test kits: Ke Yizhe (Shanghai) Mechanical and Electrical Engineering Co., Ltd.

2.1.2. Instruments and Equipment

LRH-250A biochemical incubator: Shaoguan Taihong Medical Equipment Co., Ltd.

TDZ5-WS medical centrifuge: Hunan Xiangyi Laboratory Instrument Development Co., Ltd.

BSA224S electronic analytical balance: Sartorius Scientific Instruments (Beijing) Co., Ltd.

HH-4 digital constant temperature water bath: Changzhou Guohua Electric Appliance Co., Ltd.

CT3-10K texture analyzer: Brookfield Ball Fly Company.

2.2. Methods

2.2.1. Process Flow. Refer to Figure 1 for the process flow.

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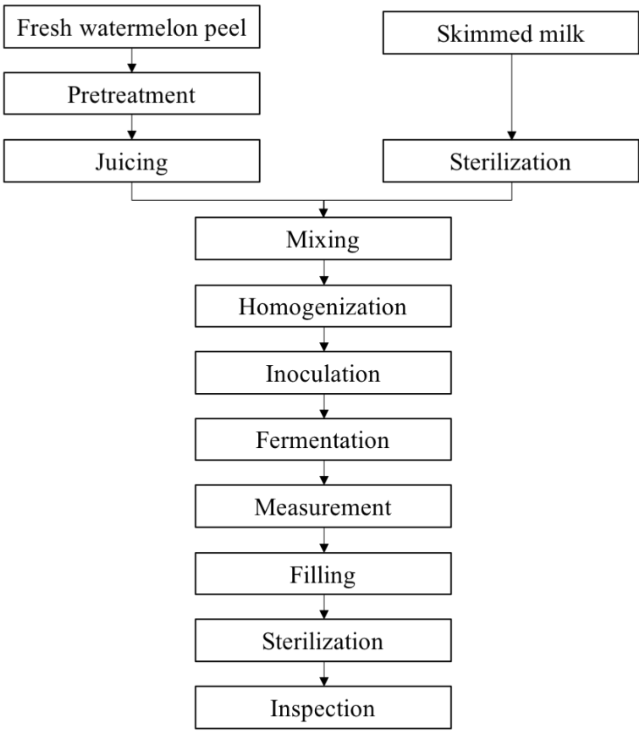

Figure 1. Process Flow

2.2.2. Operational Points

(1) Peeling: Remove the outer green hard peel of watermelon peel and wash it thoroughly.

(2) Juicing: Extract juice according to the ratio of watermelon peel to water, remove residue, and obtain watermelon peel juice.

(3) Sterilization: Take defatted milk and watermelon peel juice in a certain ratio. The milk undergoes high-temperature instant sterilization at 121 ℃ for 10 minutes, while the watermelon juice undergoes pasteurization at 60 °C for 30 minutes.

(4) Mixing and Inoculation: After sterilization, mix the milk and watermelon juice, add commercially available lactic acid bacteria at 0.10%, and ferment in a constant temperature incubator at 44 ℃.

(5) Measurement: Determine the total flavonoid content in watermelon peel juice before fermentation and compare it with the total flavonoid content in watermelon peel yogurt after fermentation to determine the optimal process.

(6) Filling: After cleaning yogurt bottles, sterilize them with steam at 100 ℃ for 10 minutes, and boil the bottle caps for 5 minutes. Quickly fill the yogurt within 30 minutes.

(7) Sterilization and Cooling: Bacterial sterilization is performed using the Pasteurization method, and after extraction, the sample is placed in an environment with a temperature ranging from 4 to 6 ℃.

2.2.3. Production of Watermelon Peel Yogurt: Single-Factor and Orthogonal Experimental Design. In this experiment, the total flavonoid content was used as an indicator to investigate the influence of the mass ratio of watermelon peel juice to milk (A), fermentation time (B), and fermentation agent dosage (C) on the total flavonoid content during the production of watermelon peel yogurt. The goal was to determine the optimal conditions for the fermentation of watermelon peel yogurt. The levels of the single-factor experimental design were as follows: watermelon peel juice to milk mass ratios of 1:2, 1:3, 1:4, 1:5, and 1:6; fermentation times of 7, 8, 9, 10, and 11 hours; and fermentation agent dosages of 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%.

Building upon the single-factor experiments, Design-Expert 10.0.4 was utilized for an L_9 (3^3) orthogonal experimental design, with sensory scores as the orthogonal experimental indicators. The factors and levels are presented in Table 1.

Table 1. Orthogonal Experimental Factor Level Coding Table

Level	Factor		
	Watermelon Peel Juice to Milk Mass Ratio (A)	Fermentation Time (hours) (B)	Fermentation Agent Dosage (%) (C)
1	1:2	8	0.10
2	1:3	9	0.15
3	1:4	10	0.20

2.2.4. Measurement Methods

2.2.4.1. Determination of Total Flavonoids. In both single-factor and orthogonal experiments, experiments were conducted with the total flavonoid content as the indicator at different levels. The determination of total flavonoid content followed the method outlined in DB84T 476-2009 for the determination of total flavonoids in plant-derived foods [14].

2.2.4.2. Sensory Evaluation Experiment. During the production of the product, considering the influence of sucrose content on the taste of yogurt, five levels of sucrose addition were considered: 7%, 8%, 9%, 10%, and 11%. Simultaneously, 50 students (half male and half female) were invited to conduct sensory evaluations based on three indicators: texture, taste, and coagulation status. The total score was 100 points, with the average score of the 50 individuals considered as the sensory indicator score. The scores were summarized, analyzed, and the optimal sugar quantity was determined. The evaluation criteria are presented in Table 2 [15].

Table 2. Sensory Comprehensive Evaluation Criteria

Item	Evaluation Standards	Score Range
Texture (30 points)	Smooth surface, no whey separation or minimal separation	20~30
	Relatively smooth surface, with a small amount of whey separation	10~19
	Rough surface, significant whey separation	0~9
Taste (40 points)	Pleasantly sour and sweet, fine and smooth taste, pure flavor	26~40
	Improper balance of sourness and sweetness, not a smooth taste, relatively rough texture	10~25
	Overly sour or sweet, rough taste	0~9
Coagulation Status (30 points)	Good coagulation, thick texture, no bubbles	20~30
	Relatively good coagulation, some particles, lower viscosity, few bubbles	10~19
	Poor coagulation, thin texture, appearance of milk fat separation, numerous bubbles	0~9

2.2.4.3. Physicochemical and Microbiological Indicator Determination. Microbiological indicators of the final product were tested according to the national standard GB 19302-2010 “National Standard for Food Safety - Fermented Milk” [16]. This includes testing for fecal coliforms [17], Staphylococcus aureus [18], and lactic acid bacteria count [19]. The acidity of the yogurt was determined according to the national standard GB5009.239-2016 using the phenolphthalein indicator method [20].

2.2.4.4. Antioxidant Activity Determination. After process optimization through single-factor and orthogonal experiments, watermelon peel yogurt and a control yogurt from other studies [13, 21] were subjected to a positive control for ascorbic acid, and their antioxidant capacity was measured. The DPPH free radical scavenging ability and ·OH free radical scavenging ability were determined according to the method of Zhao Zhongxia et al. [22].

2.2.4.5. Polysaccharide Determination. Polysaccharide determination of the finished product followed the method outlined by Li Jingjing et al. [23] with slight modifications. A 1 g sample was mixed with 1 mL of 6% phenol solution, then 5 mL of concentrated sulfuric acid was added. After standing for 10 minutes, the mixture was heated in a boiling water bath for 15 minutes. After cooling to room temperature, the absorbance at 490 nm was measured using a UV spectrophotometer.

2.2.4.6. Water-Holding Capacity Determination. To evaluate the coagulation status of the finished product, water-holding capacity was determined, following the method of Cheng Shaoning et al. [24] using the weight method.

2.2.4.7. Yogurt Texture Determination. In this study, the texture characteristics such as viscosity, hardness, and other properties were analyzed with reference to the research method of Yang Beibei et al. [25], with slight modifications. A cylindrical extrusion test probe (TA4/1000:D) was used under the following conditions: target measurement of 10.0 mm, pre-test speed of 2.00 mm/s, test speed of 5.00 mm/s, trigger point load of 3g, and two cycles. Each sample was measured in triplicate, and the average value was taken.

2.3. Data Processing

Data processing and analysis, as well as graphical representation, were performed using Desgin-Expert 10.0.4 and Origin2018 software.

3. Results and Analysis

3.1. Single-Factor Experiment Results and Analysis for Watermelon Peel Yogurt

3.1.1. Influence of Different Mass Ratios on Total Flavonoid Content in Watermelon Peel Yogurt.

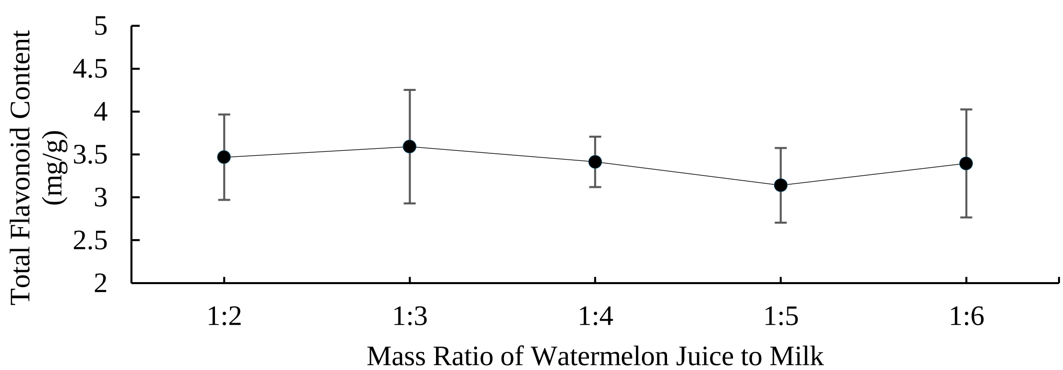

Figure 2. Impact of Different Mass Ratios on Total Flavonoids

As shown in Figure 2, the maximum utilization of substrates and the highest flavonoid content were achieved when the mass ratio was 1:3. While there is no literature directly describing the impact of mass ratio on the total flavonoid content during lactic acid bacteria fermentation, research has indicated that too high a sample extract concentration can inhibit the growth of lactic acid bacteria [26] when studying the growth and antioxidant effects of three medicinal and edible raw materials on lactic acid bacteria.

Therefore, it can be inferred that an increase in sample concentration may influence the action of lactic acid bacteria, and too low a concentration may result in insufficient reaction substrate, affecting the total flavonoid extraction rate.

3.1.2. Influence of Different Fermentation Times on the Total Flavonoid Content in Yogurt.

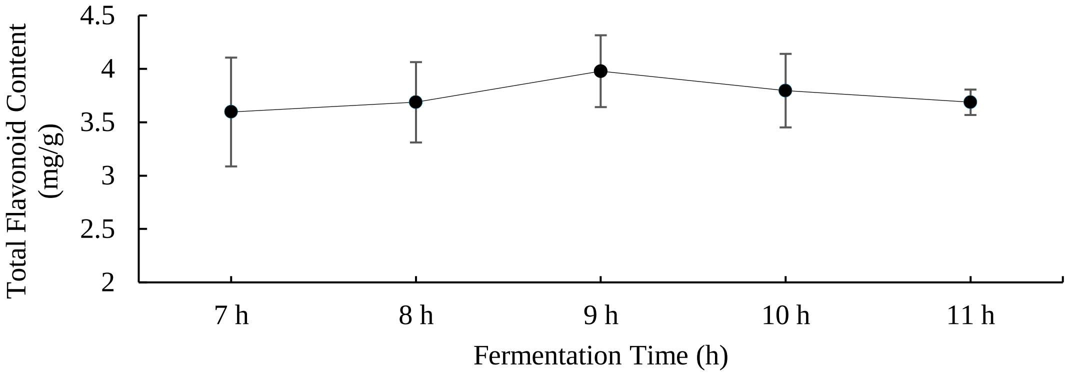

Figure 3. Impact of Different Fermentation Times on Total Flavonoids

As shown in Figure 3, the total flavonoid content is positively correlated with the extension of fermentation time. This is attributed to the influence of proteases and pancreatin during the fermentation process, which affects the generation and breakage of covalent bonds, resulting in an increase in the content of flavonoids [27]. Beyond 9 hours, an extended fermentation time leads to insufficient fermentation substrates to sustain the growth and metabolism of lactic acid bacteria, causing the total flavonoid content to plateau and even incur some losses in supporting bacterial growth [28].

3.1.3. Effect of Starter Addition on the Total Flavonoid Content in Yogurt

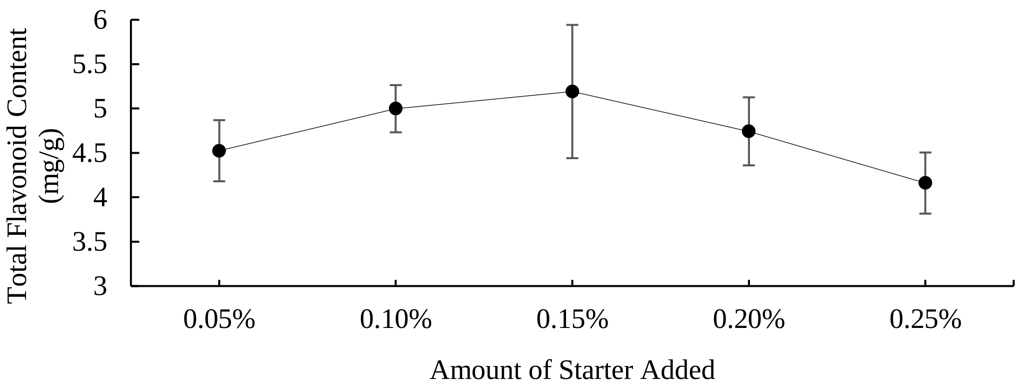

Figure 4. Effect of Different Starter Addition Levels on Total Flavonoids

As depicted in Figure 4, the total flavonoid content in yogurt is positively correlated with the amount of starter added. However, after reaching an addition level of 0.15%, the decrease in total flavonoid content may be attributed to a reduction in metabolic activity and the utilization of nutrients and active components by the strains during fermentation [29].

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3.2. Results and Analysis of Orthogonal Experiments

Table 3. Orthogonal Experiment Results

Level	Factor				
	A	B	C	D (Blank Column)	Experimental Results
1	1	1	1	1	3.91
2	1	2	2	2	3.32
3	1	3	3	3	5.07
4	2	1	2	3	5.29
5	2	2	3	1	4.20
6	2	3	1	2	5.36
7	3	1	3	2	4.56
8	3	2	1	3	4.78
9	3	3	2	1	4.78
k_1	4.100	4.587	4.683	4.297	
k_2	4.950	4.100	4.463	4.413	
k_3	4.707	5.070	4.610	5.047	
R	0.850	0.970	0.220	0.750	

Table 4. Analysis of Variance of Orthogonal Test Results

Source of Variance	Deviation	Degrees of Freedom	Mean Square Deviation	F-Value	Significance
A	2.300	2	1.150	5.328	*
B	2.831	2	1.416	6.557	**
C	0.149	2	0.074	0.344	
Error (QE)	2.375	11	0.216		

Note: * denotes significance, and more * indicates a higher degree.

From Tables 3 and 4, it is evident that the factors affecting the total flavonoid content in watermelon peel yogurt are in the order of B > A > C (fermentation time > ratio of watermelon peel juice to milk > fermentation agent addition). The optimal combination is a ratio of watermelon peel juice to milk of 1:3, a fermentation time of 10 hours, and a fermentation agent addition of 0.10%, with the fermentation time having a highly significant impact. Fermenting under conditions of 44 ℃ yields watermelon peel yogurt with a high total flavonoid content. The sensory evaluation indicates almost no whey separation, no bubbles, good coagulation status, a distinct watermelon peel fragrance, and a delicate taste.

3.3. Sensory Evaluation Results and Analysis

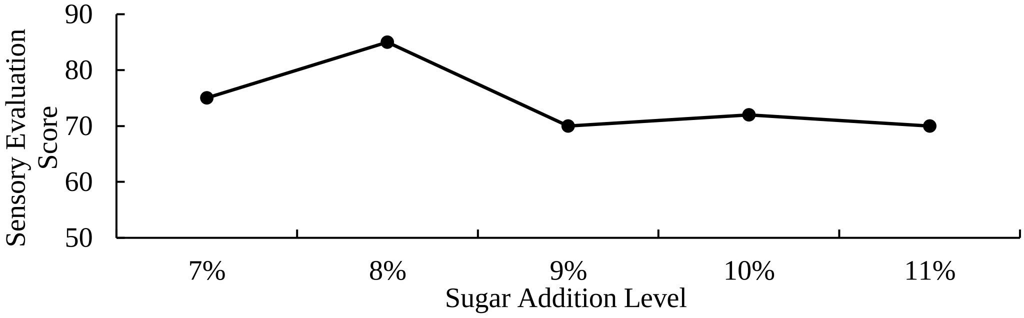

Figure 5. Sensory Evaluation Results

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The sensory evaluation scores were highest when the sugar content was 8%. At this level, the yogurt exhibited a balanced sweetness and acidity, had a pronounced watermelon flavor, and possessed a delicate texture. Therefore, in this study, a sugar addition of 8% was determined to be the most suitable.

3.4. Physicochemical and Microbiological Test Results and Analysis

The microbiological test results of the final product were conducted in accordance with the national standard GB 19302-2010 "National Food Safety Standard Fermented Milk" [16]. The results are presented in Table 5.

Table 5. Microbiological Test Standards

Item	Limit [CFU/g(mL)]	Test Result
Coliform Count	1	ND
Staphylococcus aureus Count	0 /25	0 CFU/mL
Lactic Acid Bacteria Count ([a]≥)	1×10^6	6.5×10^6 CFU/mL

Note: "a" indicates that the product, after fermentation, does not have specific requirements for the count of lactic acid bacteria; "ND" stands for "Not Detected."

According to national standards, fermented milk should have an acidity of ≥70 °T [16]. Therefore, acidity testing was conducted for watermelon peel yogurt, resulting in an acidity of 77.1 °T, which complies with national standards. In comparison, commercially available yogurts, such as AITHENON yogurt, have an acidity of 86.05 °T. Watermelon peel yogurt exhibits a lower acidity. According to the research by Cheng Shaoning and others, flavonoid content can inhibit yogurt fermentation, leading to a decrease in acidity [24].

3.5. Antioxidant Activity Test Results and Analysis

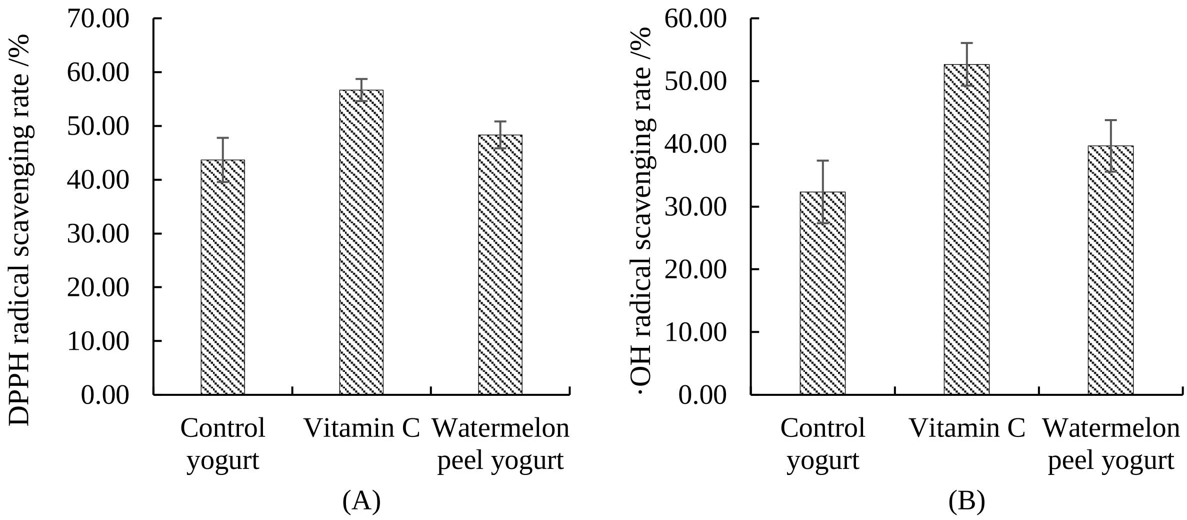

Figure 6. DPPH and·OH Radical Scavenging Rates of Different Samples

The graph illustrates the scavenging rates of DPPH (A) and ·OH radicals (B) by various samples. A study on the scavenging rates of DPPH and ·OH radicals in watermelon peel yogurt and other control yogurts reveals that yogurt possesses excellent antioxidant properties. The antioxidant activity of watermelon peel yogurt in this study is lower than that of Vitamin C at a concentration of 2 mg/mL but higher than the control yogurt, showing similar antioxidant activity to other functional yogurts [13, 21-22].

3.6. Polysaccharide Measurement Results and Analysis

Watermelon peel polysaccharides possess functions such as lowering blood sugar and exhibiting antioxidant properties [30-31]. Under optimized fermentation conditions, the polysaccharide content of watermelon peel yogurt reaches 68.11 μg/mL, which is a 19% increase compared to the polysaccharide content of watermelon peel juice (57.34 μg/mL). Extracellular polysaccharides are sugar compounds secreted from the cell to the outside during the growth and metabolism of lactic acid bacteria. During lactic acid bacteria fermentation, extracellular polysaccharides increase accordingly [32]. Additionally, the fermentation process of lactic acid bacteria can alter the microstructure of insoluble dietary fiber, making soluble dietary fiber more compact and abundant.

3.7. Water-Holding Capacity Results and Analysis

Water-holding capacity is one of the indicators for evaluating the coagulation effect of watermelon peel yogurt [33]. The water-holding capacity of watermelon peel yogurt is 63.3%, which is relatively lower compared to plain yogurt. Research indicates that an increase in the total flavonoid content inhibits yogurt fermentation [24], suggesting that the reduction in water-holding capacity in this yogurt might be attributed to the elevated total flavonoid content affecting its coagulation.

3.8. Yogurt Texture Results and Analysis

Texture is a crucial factor affecting food quality. The results show that the hardness of watermelon peel yogurt is 81.50 g, viscosity is 20.00 g, adhesiveness is 1.20 mJ, elasticity is 8.83 mm, and the draw length is 6.76 mm. This is mainly due to polysaccharides participating in the synthesis of protein microstructures during the fermentation process, enhancing the hardness and brittleness of yogurt [34]. Moreover, low concentrations of polysaccharides promote the spontaneous aggregation of casein, further explaining the increase in hardness and brittleness of yogurt [35]. Polysaccharides facilitate the production of extracellular polysaccharides in yogurt, and extracellular polysaccharides easily interact with casein, increasing stickiness and adhesiveness while influencing drawability and water-holding capacity [36]. Yogurt stickiness is obtained through the interaction between proteins and lactic acid. Polysaccharides can serve as thickeners or gelling agents, and an increase in their content can enhance the stability of proteins, thereby increasing the stickiness of yogurt [37].

4. Conclusion

Under conditions of 8% sugar content and a temperature of 44 ℃, after optimization through orthogonal experiments, the optimal mass ratio of watermelon juice to milk is 1:3, fermentation time is 10 hours, and the fermentation agent’s addition is 0.10%. The resulting yogurt has the fragrance of watermelon, excellent tissue status, and almost no whey separation. After process optimization, the polysaccharide content of watermelon peel yogurt increased by 19%, and the water-holding capacity increased to 63.3%.

Texture analysis results indicate that the hardness of watermelon peel yogurt is 81.50 g, viscosity is 20.00 g, adhesiveness is 1.20 mJ, elasticity is 8.83 mm, and the draw length is 6.76 mm. The physicochemical and microbiological properties of watermelon peel yogurt meet national standards and exhibit good DPPH and ·OH radical scavenging capabilities. The development and utilization of yogurt as a ready-to-eat food are more in line with the fast-paced lifestyle of today. Developing a new type of yogurt product using watermelon peel as raw material holds significant market potential.

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