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Optimization Design for Enhancing the Jelly Formation Rate of *Mesona Chinensis Benth.*

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Abstract: This study aimed to optimize the auxiliary materials required for enhancing the jelly formation rate of *Mesona chinensis Benth.* First, different thickeners were selected, and a single-factor analysis was conducted to screen potential formulations of composite thickeners that could improve the jelly formation rate of *Mesona chinensis Benth.* Subsequently, with sensory evaluation score as the key index, an orthogonal experimental design was employed to determine the optimal thickener formulation—one that not only enhances the jelly formation rate but also achieves the optimal sensory quality of the final product. When the jelly formation rate of *Mesona chinensis Benth.* was set at 1:100, the sensory evaluation results indicated that the optimal thickener formulation for preparing *Mesona chinensis Benth.* jelly from 25 g of dried *Mesona chinensis Benth.* was as follows: the mass ratio of coix seed powder to dried *Mesona chinensis Benth.* was 1:1.5; the addition amount of gelatin with a gel strength of 180 (B) was 5 g; and the addition amount of lotus root starch (C) was 4 g.

Keywords: *Mesona Chinensis Benth.* Jelly; Jelly Formation Rate; Sensory Evaluation Score; Orthogonal Experimental Design

1. Introduction

Mesona chinensis Benth., also known as Immortal Grass), Cool Jelly Grass, and Black Tofu Grass, is a perennial herb belonging to the genus *Mesona Bl.* of the *Lamiaceae* family [1,2,3]. Globally, there are 8 to 10 recognized species of the *Mesona* genus, with 3 species distributed in China. In China, *Mesona chinensis Benth.* is primarily cultivated in provinces and autonomous regions including Fujian, Guangdong, Guangxi, Zhejiang, Jiangxi, Taiwan, and Yunnan, among which Fujian and Guangdong provinces boast the largest cultivation areas. Wuping County, located in Longyan City, Fujian Province, is highly conducive to the growth of *Mesona chinensis Benth.*, owing to its slightly acidic soil and high fertility. These favorable environmental conditions contribute to both a high jelly formation rate and superior taste of the *Mesona chinensis Benth.* produced in this region. Notably, the *Mesona chinensis Benth.* from Wuping County meets approximately 80% of the national demand for this herb [3] (see Figure 1 for dried *Mesona chinensis Benth.* obtained through local planting and harvesting practices). Since ancient times, the Hakka people have had the custom of consuming *Mesona chinensis Benth.*, primarily in summer, as it serves to clear heat and remove toxins. According to the Dictionary of Chinese Materia *Medica*, *Mesona chinensis Benth.* has astringent, sweet, and cold properties, and possesses the effects of relieving summer heat and quench thirst, cooling blood to alleviate summer heat-related disorders, and promoting diuresis. In traditional Chinese medicine (TCM), it is also commonly used to treat conditions such as heatstroke, infantile miliaria, erysipelas involving the abdomen, common cold, hypertension, jaundice, kidney diseases, diabetes mellitus, and joint and muscle pain [4]. Modern research has also indicated that *Mesona chinensis Benth.* contains a variety of compounds, including mesona gum (polysaccharides), flavonoids, triterpenoids, and phenolic compounds. Additionally, *Mesona chinensis Benth.* exhibits biological activities such as antioxidation, anti-hypoxia, hypotension, hypoglycemia, and hypolipidemia [3]. Yang Min and Xu Zhengping from Zhejiang University also confirmed through pharmacological experiments that the aqueous extract of *Mesona chinensis Benth.* exerts a certain protective effect against renal damage in diabetic patients [5]. At present, in addition to traditional products such as *Mesona chinensis Benth.* jelly, *yogurt-Mesona chinensis Benth.* jelly, and *konjac-Mesona chinensis Benth.* jelly, a series of *Mesona chinensis Benth.*-related products have been developed, including health-care tea, lipid-lowering tea, and buccal tablets [6]. With the growing emphasis on health among modern people, *Mesona chinensis Benth.* can be further developed into more healthy foods with specific health-care functions tailored to different populations [3,7] or Special medical products[8,9].

There are two varieties of *Mesona chinensis Benth.* in Wuping County: one with good gum secretion and high jelly formation rate, and the other with low gum secretion and low jelly formation rate. Using the variety with good gum secretion as the raw material, the optimal ratio of dried *Mesona chinensis Benth.*, food-grade alkali, and starch for *Mesona chinensis Benth.* jelly preparation was screened out through preliminary orthogonal experiments. The jelly formation rate of this variety is approximately 1:60, meaning 0.5 kg of dried *Mesona chinensis Benth.* can produce about 30 kg of *Mesona chinensis Benth.* jelly [3]. In response to the needs of the Wuping *Mesona chinensis Benth.* Industry Association, a service unit of science and technology commissioners, efforts were made to further improve the jelly formation rate of *Mesona chinensis Benth.* and enhance the taste of the jelly. *The Mesona chinensis Benth.* of Wuping area is shown in Figure 1, where a is *the Mesona chinensis Benth.* planting base of Wuping and b is the dried *Mesona chinensis Benth.* of 2023 that has been picked and sun-dried. The dried *Mesona chinensis Benth.* used in this study was the 2023 harvest from Wuping County, which is known for its good jelly-forming performance. First, based on preliminary experiments, the ratio of dried *Mesona chinensis Benth.* to coix seed powder and the jelly formation rate under this ratio were evaluated. On this basis, different jelly-forming agents (mainly natural gelatin or other starches) were added to further screen and evaluate the sensory quality of the formed *Mesona chinensis Benth.* jelly, aiming to improve the jelly formation rate while ensuring a high sensory score. This study is expected to promote the development of the *Mesona chinensis Benth.* industry and provide a further foundation for the development of functional foods derived from *Mesona chinensis Benth.*.

a. Mesona Chinensis Benth. Planting Base in Wuping County

b. Harvested Dried Mesona Chinensis Benth.

Figure 1. Mesona Chinensis Benth. in Wuping County

2. Equipment and Reagents

2.1 Instruments

Induction Cooker (Midea Group Co., Ltd., Model: C22-RT22E0102)
Pressure Cooker (Midea Group Co., Ltd., Model: YL50Q3-451)
Electronic Balance (Shanghai Jinghai Instrument Co., Ltd., Model: YP601N)

2.2 Consumables and Reagents

Dried *Mesona chinensis Benth.* (Supplied by Longyan Wuping Lulu *Mesona chinensis Benth.* Professional Cooperative, harvested in 2024 with excellent jelly-forming capacity); Medical Gauze (Commercially available); Food-Grade Alkali (Jinjiang Guliangdao Food Co., Ltd., Batch Number: 20240103); Coix Seed Powder (Supplied by Longyan Wuping Lvlu *Mesona chinensis Benth.* Professional Cooperative); Edible Gelatin (Gel Strength: 180, Xin'errui Biotechnology Co., Ltd., Contact Number: 19903853686); Edible Gelatin (Gel Strength: 200, Shanghai Xintai Food Chemicals, Contact Number: 18637053132); Pure Lotus Root Starch

(Yangzhou Qinlianxin Food Co., Ltd., Batch Number: 20240920)

3. Methods and Results

3.1 Preparation Process of *Mesona Chinensis Benth.* Jelly [3]

In the preparation of *Mesona chinensis Benth.* jelly, since both the enhancement of jelly formation rate and the improvement of taste need to be taken into comprehensive consideration, the approach adopted in previous experiments, using coix seed powder as the sole thickener, was deemed insufficient to meet the dual objectives. Furthermore, as any modification to the type of thickener requires systematic experimental validation, the preparation process was revised as Figure 2:

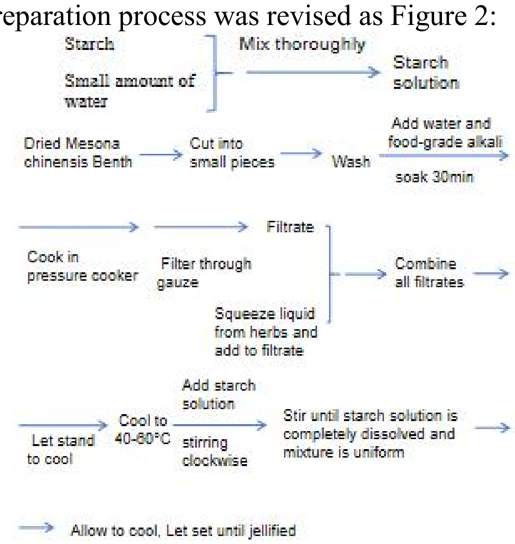


Figure 2. Preparation of Starch Solution

3.2 Single-Factor Analysis and Results

For each boiling trial, 25 g of dried *Mesona chinensis Benth.* was mixed with 2.5 kg of drinking water. With sensory evaluation score serving as the core index, and based on the findings of preliminary experiments, coix seed powder produced locally in Wuping was selected as the starch source. The ratio of dried *Mesona chinensis Benth.* to coix seed powder was adjusted to screen for the optimal jelly-forming ratio applicable to dried *Mesona chinensis Benth.* from different harvest years.

3.2.1 Ratio of dried *Mesona chinensis Benth.* to coix seed powder

In preliminary experiments, the optimal ratio of dried *Mesona chinensis Benth.* to coix seed powder was determined to be 1:1.3 for samples from specific harvest years. However, due to the utilization of dried *Mesona chinensis Benth.* from a different harvest year in the current study, a set of new ratios was designed for re-evaluation, with sensory evaluation score as the key assessment indicator. The ratios of dried *Mesona chinensis Benth.* to coix seed powder tested were as follows: 1:1.1, 1:1.3, 1:1.5, 1:1.7, and 1:1.9. The experimental results demonstrated that the highest sensory evaluation score was obtained when the ratio of dried *Mesona chinensis Benth.* to coix seed powder was 1:1.5 (see Figure 3a). At this ratio, the jelly formation rate reached approximately 1:70. Consequently, this ratio (1:1.5) was selected as the basis for subsequent studies focused on enhancing the jelly yield of the current batch of dried *Mesona chinensis Benth.*

In response to the requirement proposed by the Wuping *Mesona chinensis Benth.* Industry Association (a designated service unit for science and technology commissioners), the target jelly formation rate was set at approximately 1:100, with a concurrent improvement in the sensory quality of the resulting jelly. Specifically, the ratio of dried *Mesona chinensis Benth.* to filtrate was required to achieve 1:100. To fulfill this requirement, the single-thickener system was replaced with composite thickener systems, which combined coix seed powder with either gelatin or lotus root starch. The jelly-forming performance of these composite systems was then evaluated, with sensory evaluation score used as the quantitative measurement metric.

3.2.2 Selection of gelatin gel strength

In accordance with relevant literature [10,11], gelatin samples with gel strengths of 180 and 200 were selected, respectively, and dissolved in water to prepare gelatin solutions. The filtrate obtained from boiling 50 g of dried *Mesona chinensis Benth.* was mixed thoroughly with coix seed powder; after cooling, the mixture was divided into two equal portions. To each portion, 5 g of the 180-gel-strength gelatin solution or the 200-gel-strength gelatin solution was added separately. Sensory evaluation of the final *Mesona chinensis Benth.* jelly revealed that the sample supplemented with the 180-gel-strength gelatin solution achieved a higher sensory score. Therefore, gelatin with a gel strength of 180 was selected for subsequent evaluations.

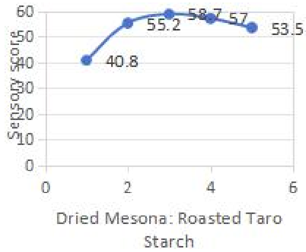
3.2.3 Evaluation of gelatin addition amount

With sensory evaluation score as the index, the experiments were conducted under the conditions of dried *Mesona chinensis Benth.* :

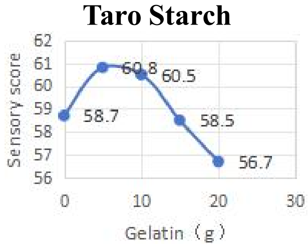
water = 1:100 and dried *Mesona chinensis Benth.* : coix seed powder = 1:1.5. After adding coix seed powder and cooling the mixture (before jelly formation), gelatin solutions prepared with 0 g, 5 g, 10 g, 15 g, and 20 g of 180-gel-strength gelatin were added to the mixture, respectively. The results indicated that the sample supplemented with 5 g of 180-gel-strength gelatin solution obtained the highest sensory score (see Figure 3b).

3.2.4 Combined use of coix seed powder and lotus root starch

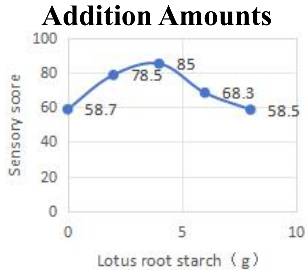
Using sensory evaluation score as the index, the experiments were performed under the conditions of dried *Mesona chinensis Benth.* : water = 1:100 and dried *Mesona chinensis Benth.* : coix seed powder = 1:1.5. While adding coix seed powder, 0 g, 2 g, 4 g, 6 g, and 8 g of lotus root starch were incorporated separately; the mixture of coix seed powder and lotus root starch was blended into a starch solution and then added to the system. The results showed that the highest sensory score was achieved when 4 g of lotus root starch was added (see Figure 3c).



a. Sensory Scores of Dried Mesona: Roasted Taro Starch



b. Sensory Scores at Different Gelatin Addition Amounts



c. Sensory Scores at Different Lotus Root Starch Addition Amounts

Figure 3. Single-Factor Analysis

3.3 Orthogonal Design and Results

Based on the optimized results of single-factor experiments, an orthogonal experimental design was adopted. For each experiment, 25 g of dried *Mesona chinensis Benth.* was used, with the ratio of dried *Mesona chinensis Benth.*: water set at 1:100. Factor A (Mass of Coix Seed Powder): Calculated based on 25 g of dried *Mesona chinensis Benth.*, the levels were determined as 32.5 g, 37.5 g, and 42.5 g according to the single-factor evaluation results. Factor B (Mass of Added Gelatin): Three levels were set, namely 0 g, 5 g, and 10 g. Factor C (Mass of Added Lotus Root Starch): When the addition amount of lotus root starch was 0 g, the sensory score was relatively low mainly due to poor jelly formation. To enhance the distinguishability between experimental groups, three levels of lotus root starch addition were designed: 0 g, 5 g, and 10 g.

The factor-level table is shown in Table 1.

An $L_9 (3^4)$ orthogonal array was employed, with sensory evaluation score as the key index to determine the optimal process. The factor-level design of the orthogonal experiment is presented in Table 1.

Table 1. Factor-Level Design of the Orthogonal Experiment

Factor	Level		
	1	2	3
A-(g)	32.5	37.5	42.5
B- (g)	0	5	10
C-(g)	0	2	4

Experiments were conducted in accordance with the $L_9 (3^4)$ orthogonal array design specified in Table 1. To evaluate the sensory score of the formed jelly, a *Mesona chinensis Benth.* jelly tasting activity was organized, and sensory evaluation score sheets were collected. The results are shown in Table 2 and Table 3.

Through orthogonal analysis of the sensory scores, the range analysis (R-value analysis) in Table 2 revealed that the order of influence of the three factors on the sensory score was C- > A > B-. The optimization objective was to maximize the sensory score. By analyzing the values of k_1, k_2, and k_3 (see Figure 1), the optimal conditions for achieving the highest sensory score were determined as follows Table 3.

The analysis of variance (ANOVA) results regarding the effects of each factor on the sensory score are presented in Table 3. As indicated in Table 3, A- and C- exhibited a significant influence on the sensory score ($P < 0.05$), whereas B- had no significant impact on the sensory score ($P > 0.05$). Through analysis of the F-values of the three factors, the order of their influence on the sensory score was confirmed to be C- > A- > B-. Upon comprehensive consideration of the effects of the three factors on the sensory score, the optimal combination was identified as A2B2C3. Specifically, when 25 g of dried *Mesona chinensis Benth.* was used in the experiment, the formulation of the composite thickener was determined as follows: the addition amount of coix seed powder (A) was 37.5 g (corresponding to a ratio of dried *Mesona chinensis Benth.* to coix seed powder of 1:1.5), the addition amount of gelatin with a gel strength of 180 (B) was 5 g, and the addition amount of lotus root starch (C) was 4 g. This formulation resulted in the highest sensory score.

Table 2. Orthogonal Experiment Scheme and Results

Experiment No.	A-(g)	B-(g)	C-(g)	Sensory Score
1	1 (32.5)	1 (0)	1 (0)	55.8
2	1	2 (5)	2 (2)	78.5
3	1	3 (1)	3 (4)	71.9
4	2 (37.5)	1	2	84.5
5	2	2	3	88.6
6	2	3	1	62.5
7	3 (42.5)	1	3	70.5
8	3	2	1	61.3
9	3	3	2	64.8
K_1	206.20	210.80	179.60	
K_2	235.60	228.40	227.80	
K_3	196.60	199.20	231.00	
k_1	68.73	70.27	59.87	
k_2	78.53	76.13	75.93	
k_3	65.53	66.40	77.00	
r	13.00	9.73	17.13	
Influence Order: C>A>B		Optimal Combination: $A_2B_2C_3$		

Table 3. Analysis of Variance (ANOVA) Results of Each Factor on Sensory Score

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	P-Value	Significance
A	275.28	2	137.64	21.362	0.045	*
B	144.11	2	72.05	11.183	0.082	
C	552.83	2	276.41	42.899	0.023	*
Error (e)	12.89	2	6.44			

Note: ** indicates an extremely significant effect ($P < 0.01$); * indicates a significant effect ($P < 0.05$).

4. Results and Discussion

The primary objective of this study was to increase the jelly formation rate of *Mesona chinensis Benth.* jelly to 1:100 while simultaneously ensuring favorable sensory quality (i.e., taste). By integrating single-factor analysis, orthogonal experimental design, and sensory evaluation score as the core assessment index—along with the optimized protocols derived from preliminary experiments—the optimal preparation process for *Mesona chinensis Benth.* jelly was established, and the details are as follows: 1. A total of 25 g of dried *Mesona chinensis Benth.* was weighed, to which 2.5 kg of water and 7 g of food-grade alkali were added. 2. The mixture was subjected to two rounds of boiling in a pressure cooker, with each round maintaining a gentle boil for 1 hour. After each boiling cycle, the juice was squeezed from the *Mesona chinensis Benth.* residue and combined with the filtrate. 3. The combined filtrate was allowed to cool to room temperature, following which it was reheated to a gentle boil. At this stage, the composite starch solution (comprising coix seed powder and lotus root starch) was added, where the ratio of dried *Mesona chinensis Benth.* to coix seed powder was 1:1.5, and the addition amount of lotus root starch was 4 g. 4. When the temperature of the mixture decreased to approximately 50 °C, the gelatin solution (prepared with 5 g of gelatin featuring a gel strength of 180) was added to the system. The results obtained from this experiment can serve as a foundational reference for subsequent related research. Key issues encountered during the experiment are explained as follows:

(1) Preliminary evaluation experiments confirmed that coix seed powder produced locally in Longyan achieved a higher sensory score compared to corn starch and tapioca starch. Consequently, locally sourced coix seed powder from Longyan was retained as the primary thickener. However, to meet the dual requirements of improved jelly formation rate and enhanced sensory quality, gelatin and lotus root starch were incorporated into the thickener system to form a composite thickener. By integrating the assessment criteria for both jelly formation rate and sensory quality into the sensory evaluation scoring framework, the optimal formulation of jelly-forming agents required to increase the yield to 1:100 was determined: a ratio of dried *Mesona chinensis Benth.* to coix seed powder of 1:1.5, 5 g of gelatin with a gel strength of 180, and 4 g of lotus root starch.

(2) The optimized *Mesona chinensis Benth.* jelly demonstrated satisfactory gel formation at room temperature; however, it was observed to melt gradually over an extended period. Notably, when the jelly was immersed in warm water at approximately 40 °C, a more rapid melting process occurred. It is worth noting that this experimental result provides evidence for the potential of further research focused on the development of temperature-sensitive *Mesona chinensis Benth. jelly.*

(3) As reported in relevant literature, the incorporation of traditional Chinese medicine (TCM) ingredients into *Mesona chinensis Benth.* Jelly when using conventional thickening agents often results in poor gel formation[6]. In contrast, the findings of this experiment suggest that the application of composite thickeners may effectively improve the gel-forming performance of *Mesona chinensis Benth.* jelly supplemented with TCM components. Furthermore, this approach can also enhance the health-functional attributes of the jelly product, thereby expanding its application in functional food development.

(4) Variations in the required amount of thickener were observed for *Mesona chinensis Benth.* harvested in different years, which may be attributed to differences in annual precipitation, climatic conditions, and soil properties. Further evaluations can be conducted based on the findings of this experiment to address such variations.

5. Conclusion

Because of the various health benefits of the *Mesona chinensis Benth.* , it can be combined with the traditional form of *Mesona chinensis Benth.* jelly and other dual-purpose medicinal and edible materials to prepare special medical products suitable for patients with different diseases, such as those with hypertension and diabetes, which has broad development potential. This experiment evaluated the thickening agent schemes that increase the yield, improve the jelly-forming property and taste of the *Mesona chinensis Benth.*jelly. The ratio of dried *Mesona chinensis Benth.* to taro starch is 1:1.5, with 4 g of lotus root starch added and 5 g of gelatin with a gelling strength of 180. On this basis, through the further development and evaluation of the compound jelly-forming agent, different dual-purpose medicinal and edible plants can be added to the preparation of he*Mesona chinensis Benth.*jelly. to create health products suitable for different sub-healthy groups or patients with special diseases.Additionally, the further development of temperature-sensitive *Mesona chinensis Benth.* jelly can significantly reduce the risk of choking when consumed by children or individuals with swallowing difficulties. This innovation not only enhances the safety of the product but also improves its technical added value and economic potential in the functional food market.

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