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Physicochemical Characteristic of Ultrafine *Sparassis crispa*(cauliflower mushroom) Powder

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Abstract : In this study, *Sparassis crispa*(cauliflower mushroom), which is rich in beta-glucan, was pulverized using ultrafine grinding technology for its potential utilization as a diverse food ingredient. The physical and antioxidant properties of cauliflower mushroom powder were evaluated at various grinding times. The results showed that as the grinding time of cauliflower mushroom increased, the average particle size significantly decreased ($p<0.05$). Additionally, the water-holding capacity, swelling capacity, and water solubility index of cauliflower mushroom increased significantly($p<0.05$). Based on the analysis mentioned above, cauliflower mushroom prepared as a superfine powder for 5 minutes exhibited superior physical and chemical properties as well as antioxidant characteristics and is expected to be widely used in various foods.

Keywords : *Sparassis crispa, ultrafine grinding, physical properties, antioxidant properties, superfine powder*

1. Introduction

In recent years, there has been an increasing interest among modern individuals in health-related matters, leading to a growing body of research on functional natural substances with potential benefits such as anticancer properties, antioxidant effects, and anti-aging capabilities[1, 2]. Among various natural substances, mushrooms are known to possess a unique flavor and taste, along with a wide array of nutrients. They are also renowned for their diverse beneficial effects, including antioxidative, anti-inflammatory, anticancer, and immune-boosting properties[3, 4]. Among these mushrooms, *Sparassis crispa*, also known as cauliflower mushroom, stands out. It constitutes 70% of the total nutritional content, primarily consisting of carbohydrates, with more than half of the carbohydrates being β-glucan. In addition to β-glucan, it contains various other components such as glycogen and chitosan. According to the Japan Food Analysis Center, the β-glucan content in cauliflower mushroom was approximately 43.6%, which is more than two to three times higher compared to other mushrooms like Agaricus (11.6%), Shiitake (8-15%), Maitake (18.1%), Pleurotus (15-20%), and Enoki (7-12%)[5-7].

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The technology of ultrafine grinding and nano-technology has gained significant attention in the recent food industry as an advanced technology[8]. It is considered the next-generation core technology with various applications aimed at improving the solubility and absorption of bioactive compounds. Ultrafine grinding is being utilized to maximize the surface area of food ingredients, thereby enhancing the solubility of various substances[9. 10]. Additionally, it increases the extraction yield of beneficial compounds, such as phenolic compounds. Ultrafine grinding not only increases the surface area but also induces capillary effects. The increase in surface area can influence catalytic reactions, leading to changes in the expression of physiological activities. Additionally, the capillary effects are reported to introduce new physical phenomena by altering fundamental properties. This ultrafine grinding technology goes beyond simple primary processing techniques that increase the extraction yield of beneficial compounds. It can be utilized as a processing method for high-value materials such as functional food ingredients, pharmaceutical ingredients, and functional cosmetic ingredients by enhancing the solubility of physiologically active substances found in herbal medicines, thereby increasing their absorption within the body[11].

In this study, the effects of different ultrafine grinding time on the physicochemical properties of cauliflower mushrooms were investigated. The results of this study are expected to provide a theoretical basis for the further processing and redevelopment of cauliflower mushroom.

2. Experiment

2.1. Materials

The cauliflower mushrooms used in this study were supplied by Beta-Glucan Company located in Pyeongtaek, Gyeonggi Province, South Korea, and were cultivated in 2022. The provided cauliflower mushrooms were dried in an infrared dryer at 80° C until their moisture content reached 12%. All chemicals and reagents were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

2.2. The preparation of ultrafine grinding samples

Ultrafine cauliflower mushroom powder was produced using a planetary ball mill (Plverisette 6, Fritsch, Idar-Oberstein, Germany) in the grinding process. The operational conditions of the grinding device involved placing 100 g of cauliflower mushrooms and 250 g of 2.0 mm zirconia balls into a 500 mL grinding bowl, followed by grinding at a speed of 300 rpm for 5, 10, and 20 minutes, respectively. To control temperature rise during the grinding process, the lid of the grinder was left open. After pulverization, the resulting ultrafine powder was sieved through a 200mesh sieve. Three different cauliflower mushroom powders with varying particle sizes were obtained, sealed in bags, and stored in a refrigerator maintained at −30℃.

2.3. The particle characteristics of cauliflower mushroom powder

2.3.1. Particle size analysis

Particle size distribution ratio, mean particle diameter, and specific surface area were measured using a particle size analyzer (10064, CILAS, Orleans, France).

2.3.2. Molecular weight distribution

The sample, weighing 20 mg, was dissolved in 1 mL of 1N NaOH solution, followed by neutralization using 1N HCl solution. Subsequently, a 50 mM $NaNO_3$ solution was added to reach a total volume of 20 mL. The mixture was heated at 121℃ for 20 minutes and then passed through a 5 μm membrane filter before being used for analysis. Under these measurement conditions, the column used

was Toyppearl 65F, the mobile phase consisted of 50 mM $NaNO_3$, and the flow rate was set at 1.5 mL/min.

2.4. The antioxidant activity of cauliflower mushroom powder

2.4.1. Determination of Total phenol contents

The analysis of total phenols was conducted based on the ultrafine grinding time. 1 g sample was quantified and mixed with 20 mL of distilled water, followed by extraction in a water bath at 95℃ for 3 hours. The extraction was then filtered using Toyo No. 2 filter paper and subjected to vacuum concentration using a rotary evaporator. The quantification of total phenols for each grinding time was performed using the Folin-Ciocalteu method[12]. Specifically, 0.1 mL of the sample was mixed with 2.8 mL of distilled water, 2 mL of 2% sodium carbonate (Na_2CO_3), and 0.1 mL of 50% Folin-Ciocalteu reagent. This mixture was allowed to react at room temperature for 30 minutes, and the absorbance was measured at 750 nm using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan). Caffeic acid was used as a standard substance, and the content was measured based on the caffeic acid standard curve

2.4.2. DPPH assay

The remaining concentration of radicals was measured at 517 nm using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan) after dissolving 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma, USA) in 95% ethanol (800 μL) and adding 200 μL of each experimental group to an E.P tube. The mixture was vortexed for 3 minutes and then allowed to react in a chamber for 30 minutes. Gallic acid (Sigma, USA) was used as the standard substance[13].

2.4.3. ABTS assay

In this experiment, a modified version of the method by Zhu *et al.*, [14] was used. A mixture of 7 mM ABTS and 2.45 mM potassium persulfate dissolved in distilled water was diluted 1:1 with PBS (pH 7.4) to achieve an absorbance value of 0.70±0.02. The radical stock solution was left to stand for 12-16 hours, and then, for each concentration, 200 μL of the prepared sample was added to 800 μL of the diluted solution. After 15 minutes of incubation, the absorbance was measured. Gallic acid (Sigma, USA) was used as the standard substance.

2.4.4. Hydroxyl radical scavenging assay

The sample of 0.5 mL was mixed with 1 mL of 9 mM $FeSO_4$, 1 mL of 9 mM salicylic acid, and 0.5 mL of 0.3% H_2O_2. The mixture was allowed to react at room temperature for 30 minutes, and then the absorbance was measured at 510 nm using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan) [15].

2.5. Physical properties of cauliflower mushroom powder

2.5.1. Water-holding capacity

The water-holding capacity (WHC) was measured with some modifications based on the method by Zhang *et al.*,[16]. Each cauliflower mushroom powder sample, obtained through different grinding times, was weighed (M_1), and the weight of a centrifuge tube (M_2) was also measured. To the centrifuge tube, 25 mL of distilled water was added for dispersing the cauliflower mushroom powder. The sample tubes were left at room temperature(25℃±3℃) for 4 hours and then centrifuged at 2,500 rpm for 20 minutes. After removing the supernatant, the weight of the centrifuge tube and the remaining powder (M_3) were measured. WHC (g/g) was calculated using the following equation.

$$\text{WHC(g/g)} = \frac{M_1(g)}{M_3(g) - M_2(g)}$$

2.5.2. Swelling capacity

The swelling capacity (SC) was measured following the method of Jafari *et al.*,[17]. 1g of sample was hydrated in a brown falcon tube containing 10 mL of distilled water. The sample tube was then shaken to obtain a homogeneous dispersion and allowed to stand at room temperature(25℃±3℃) for 12 hours, Afterward, the sediment volume of the sample was measured. Swelling capacity (mL/g) was calculated using the following equation.

$$SW(mL/g) = \frac{\text{The sediment volume of sample(mL)}}{\text{Weight of dry sample(g)}}$$

2.5.3. Water solubility index

The water solubility index (WSI) was measured with some modifications based on the method by Wang *et al.,* [18]. About 1 g of the sample (W_1) was dispersed in 25 mL of distilled water and placed in a 50 mL falcon tube. The sample tubes were then left at room temperature(25℃±3℃ for 30 minutes before being centrifuged at 2500 rpm for 20 minutes. The supernatant liquid was collected in an aluminum weighing dish (W_2) and dried at 105° C for 5 hours. The weight of the dried residue (W_3) was measured. The formula used to calculate the water solubility index (WSI) is as follows

$$WSI(\%) = \frac{W3(g)-W2(g)}{W1(g)} \times 100$$

2.6. Statistical analysis of data

All measurements and analyses were performed in three replicates. Data were analyzed using the ANOVA procedure of statistical analysis system(version 9.0, SAS Institute Inc. Cary, NC, USA). Results were expressed as mean ± standard deviation. The differences in mean were determined by Duncan's multi-range test and was considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Particle size analysis of cauliflower mushroom powder

The particle size distribution, average particle diameter, and specific surface area of *Sparassis crispa* mushroom powder according to ultrafine grinding time were presented in Table 1. The D50 values for grinding times of 5, 10, and 20 minutes were 403.37, 72.76, and 18.56, respectively. It indicated that as the particle size decreased, the specific surface area increased. This reduction in particle diameter was observed in the 10%, 50%, and 90% cumulative volume distributions. The specific surface area of the particles also showed a significant increase with longer grinding times. In other words, as the particle size decreased, the specific surface area of *Sparassis crispa* mushroom powder increased rapidly from 42.18 to 385.55 m^2 /kg, showing a 9.14-fold increase. This increase in specific surface area can expose active groups, potentially enhancing the physiological activity of *Sparassis crispa* mushrooms [19]. Additionally, this increase in specific surface area is expected to significantly improve the taste of *Sparassis crispa* mushroom powder. Therefore, superfine grinding can drastically reduce the particle size of powder in food.

3.2. Molecular weight distribution

The results of measuring the molecular weight distribution of cauliflower mushroom powder at different ultrafine grinding times are presented in Table 2 and Fig. 1. As seen in Fig. 1, when the grinding time is 20 minutes, the intensity of the first peak with a large molecular weight decreases, and with increasing grinding time, the intensity of the peak increases. The peak area values for 5 minutes of grinding were 32.87%, 10 minutes

Table 1. Mean particle size and specific surface area of cauliflower mushroom powder

Grinding time(min)	D10(μm)	D50(μm)	D90(μm)	Specific surface area (m^2/kg)
5	271.77±2.88[a1)]	403.37±15.33[a]	752.15±27.87[a]	42.18±2.76[c]
10	57.03±0.24[b]	72.76±0.10[b]	101.33±10.78[b]	177.78±4.27[b]
20	7.88±0.25[c]	18.56±0.21[c]	36.01±0.67[c]	385.55±5.13[a]

[1)] Values represent means±deviation. Values with different letter within a same row (a–c) are significantly different ($p<.05$) by Duncan's test.

Table 2. Average molecular weight on each peak, particle size distribution, and peak area at different pulverization times

Grinding time(min)	Peak		Area of peak(%)
	Mw[1)](10^8g/mol)	Rg[2)](nm)	
5	12.69±2.76[a3)]	243.28±25.17[a]	32.87±2.65[a]
10	8.97±0.23[a]	73.16±8.26[b]	73.11±6.5[b]
20	1.03±0.40[b]	39.33±1.27[c]	101.13±6.85[c]

[1)] Molecular weight
[2)] Radius of gyration
[3)] Values represent means±deviation. Values with different letter within a same row (a–c) are significantly different ($p<0.05$) by Duncan's test.

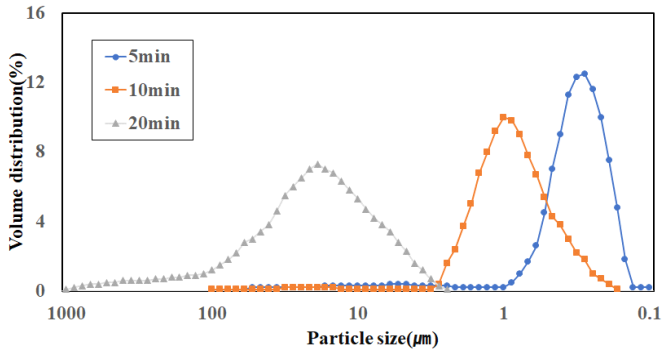

Fig. 1. Molecular weight distribution diagram of cauliflower mushrooms at different ultrafine grinding times.

were 73.11%, and 20 minutes were 101.13%, indicating an increase in the relative peak area value as the grinding time increased. The results from this study indicate that the ultrafine grinder can effectively reduce the particle size of the mushroom powder to a superfine scale of 5 μm or less. These findings are consistent with experiments conducted on mushroom stipe and cap powders[20].

3.3. Antioxidant activity of cauliflower mushroom powder

The excessive accumulation of free radicals poses a threat to human health by damaging molecules within living cells[21]. Therefore, effectively removing these free radicals is crucial for the body's antioxidant defense. This is because antioxidants can protect the body from the harmful effects of free radicals. These facts have been confirmed through extensive research[22]. The antioxidant activity of the super-fine powdered extract of cauliflower mushroom was analyzed, and the results are presented in Table 3. As shown in Table 3, ultrafine-ground cauliflower mushroom exhibited antioxidant properties, with the highest $ABTS^+$ scavenging rate. With increasing grinding time, the TP content and the scavenging rates of DPPH, $ABTS^+$, and Hydroxyl radical decreased, and significant differences were observed among groups ($p<0.05$). At a 5-minute grinding time, cauliflower mushroom reached its maximum TP content (537.34 mg/100g), and the scavenging rates of DPPH, $ABTS^+$, and Hydroxyl radical also reached their maximum values(65.39%, 85.39%, 48.87%, respectively). These results suggest that the antioxidant activity of cauliflower mushroom powder can be enhanced by superfine grinding. These findings are consistent with previous studies on the antioxidant activity of ginger[23] and quercus blume leaf[24] subjected to superfine grinding. Furthermore, the changes in antioxidant activity corresponded to the alterations in functional components. Superfine grinding led to reduced particle size, uniform distribution, increased specific surface area, and a completely disrupted structure. Consequently, antioxidant components were effectively dissolved. In cauliflower mushroom, β-glucan and total phenols were identified as the main antioxidant components[25].

3.4. Water holding capacity, swelling capacity and water solubility index

The water holding capacity, swelling capacity and water solubility index of cauliflower mushroom powder as a function of ultrafine grinding time is presented in Fig. 2, 3, and 4, respectively. In general, it is known that ultrafine grinding technology significantly influences moisture-related properties such as water holding capacity, swelling capacity, and water solubility index. As seen in Fig. 2, it can be observed that as particle size decreases, water holding capacity significantly increases. Water holding capacity typically represents a material's ability to retain water when subjected to external centrifugal gravity force.

Table 3. Effect of superfine grinding on the antioxidant activity of cauliflower mushroom powder based on ultrafine grinding time

Grinding time (min)	TP[1)](mg/100g)	DPPH-scavenging activity (%)	$ABTS^+$ radical scavenging activity (%)	Hydroxyl radical scavenging activity (%)
5	$37.34\pm10.78^{a2)}$	65.39 ± 0.51^{a}	85.39 ± 1.17^{a}	48.87 ± 0.92^{a}
10	501.40 ± 11.46^{b}	65.27 ± 0.14^{a}	83.87 ± 0.46^{b}	43.98 ± 1.17^{b}
20	448.56 ± 10.67^{c}	53.29 ± 0.59^{b}	77.08 ± 0.55^{c}	38.40 ± 0.46^{c}

[1)] TP: Total polyphenol

[2)] Values represent means ± standard deviations. Means followed by different letters (a-c) in the same column represent significant differences ($p<0.05$).

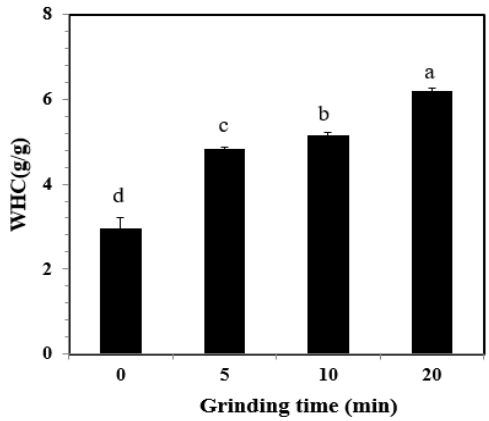

Fig. 2. Effect of utrafine grinding time on the water holding capacity of cauliflower mushroom powder.
Means ± SD (n = 3) with different letters (a–d) are significantly different (p<0.05).

It comprises the sum of bound water, hydrodynamic water, and physically trapped water, with the latter contributing the most to this capacity[26]. The high water holding capacity of cauliflower mushroom powder resulting from ultrafine grinding is likely due to the increased surface area and enhanced ability to bind with water. These result is similar to the study of different milling methods on mung bean flour[27]. Swelling capacity represents the amount of water that cauliflower mushroom can absorb. As shown in Fig. 3, it can be observed that with increasing ultrafine grinding time, it absorbs the highest amount of water (11.24 mL/g). This is believed to be due to the altered spatial structure of fibrous substances, resulting in an increased number of fine pores, which make easier binding with water. The water solubility index is a measure of how well a sample dissolves in water under the same conditions. As seen in Fig. 4, it showed the highest water solubility index value at a grinding time of 5 minutes, but as the grinding time increased, it exhibited a slight decrease. In general, smaller particle sizes are expected to increase the water solubility index. However, the results showed contradictions. This is believed to be due to less starch damage from heat and easier gelatinization. However, solubility appears to decrease depending on the grinder and grinding time used.

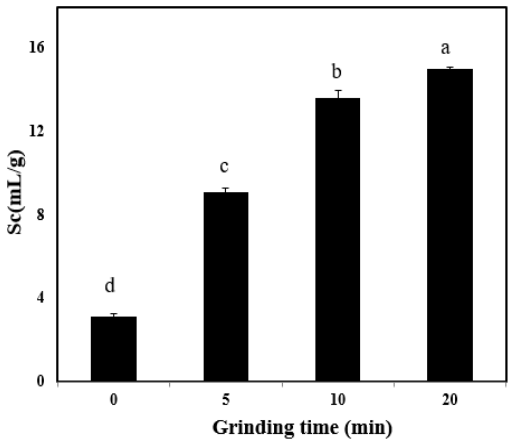

Fig. 3. Effect of utrafine grinding time on the swelling capacity of cauliflower mushroom powder.
Means ± SD (n = 3) with different letters (a–d) are significantly different (p<0.05).

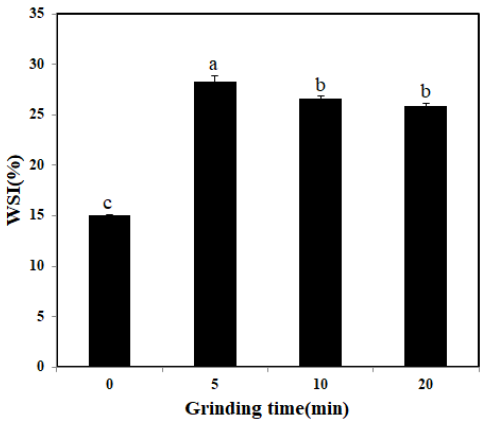

Fig. 4. Effect of utrafine grinding time on the water solubility index of cauliflower mushroom powder.
Means ± SD (n = 3) with different letters (a–c) are significantly different (p<0.05).

4. Conclusion

This study analyzed the impact of superfine grinding on the physicochemical properties and antioxidant activity of cauliflower mushroom.

1. The use of superfine grinding effectively reduced the particle size of cauliflower mushroom, and increasing grinding time indicated an enhancement in the physicochemical properties of superfine cauliflower mushroom powder.

2. The values of D50 according to ultrafine grinding times of 0, 10, and 20 minutes were 403.37, 72.76, and 18.56, respectively. As the particle size decreased, the specific surface area rapidly increased from 39.83 to 285.88 m^2 /kg.

3. The molecular weight distribution of cauliflower mushroom powder, based on ultrafine grinding time, showed values of 32.87% for 5 minutes, 73.11% for 10 minutes, and 101.13% for 20 minutes, indicating that the molecular weight of cauliflower mushroom decreased as the ultrafine grinding time increased.

4. Considering the impact of ultrafine grinding on the physical properties, adsorption properties, and antioxidant properties of cauliflower mushroom, the most suitable grinding time was found to be 5 minutes.

5. The results of this study can serve as fundamental data for the future application of superfine grinding technology in the processing and development of functional foods using cauliflower mushrooms.

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