Analysis of Physicochemical, Antioxidant Properties and Sensory Characteristic of Shiitake Mushroom Pickles

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Abstract Mushrooms are perishable foods; they required processing technologies that preserves chemical and nutritional characteristics of fresh forms. The principal objective of this research was to analyze the physicochemical changes and sensory properties of Shiitake pickles with and without mustard oil in order to contribute to the diversification of food. The antioxidant activity of fresh shiitake mushroom pickles with and without mustard oil demonstrated a significant result in Scavenging free radicals except for DDPH. The pH of MOVS and SWVS was reduced to 3.92±010 and 2.80±0.26 over time during 20 days of storage and the acidity was gradually increased for up to storage. However HPLC analysis of both pickles revealed the presence of four acids were present in the MOVS pickle (malic acid at 4.354 with the retention time of 4.2 minutes). The second peak indicates lactic acid at 5.069 with the retention time of 4.3 min. The third peak indicates citric acid at 5.489 with the retention time of 4.5 min. The fourth peak indicates succinic acid at 7.301 with the retention time of 6.8 min. Whereas the SWVS pickle showed the peak for three acids present in the pickle sample, where the first peak indicates malic acid at 4.303 with the retention time of 4.2 min, the second peak indicates acetic acid acid at 6.573 with the retention time of 6.2 min, and the final peak was found of succinic acid at 7.304 with the retention time of 6.8 min. The pickle formulated with (MOVS) showed 5.70 for overall acceptability of pickle.

Keywords: shiitake mushroom, antioxidant activity, pickle, organic acid


1. Introduction

Mushrooms have a long tradition of use in many Asian countries and have been used as a food and as a medicine. Specifically, polysaccharides have been demonstrated to play an important role as a dietary free radical scavenger in the prevention of oxidative damage in living organisms [1,2]. Mushrooms are acknowledged as functional foods because of their bioactive compounds. Mushrooms that contain antioxidant polysaccharides may be useful in treating cancer and other devastating diseases.

The mushrooms are acknowledged as functional foods owing to their bioactive compounds. These mushrooms that contain the antioxidant polysaccharides may be useful in treating cancer and other devastating diseases. Long-term storage of the mushrooms generally, canning and drying processes along with some value addition technology are used since a long time. The value of the preserved products is seldom comparable with that of the fresh mushrooms, and these methods are not always appropriate for all types of the mushrooms. The mushrooms are highly perishable by nature with short shelf-life in the ambient environment, owing to their high moisture contents, other essential nutrients, and lack of physical protection to avoid water loss or microbial attack [3]. Therefore, the mushrooms are generally used in the processed form [4]. Owing to their unique organoleptic characteristics, taste, texture, flavor and nutritional value, the edible mushrooms are popular all over the world as well as valuable component of the diet [5-12]. The approximate world production of the mushroom consists of about 5 million tones of fresh weight per annum [13]. The increase in the mushrooms demand is due to their nutritional benefits and other important aspects of processing of the freshly harvested mushrooms for manufacturing pickles, soups, ketchup, and sauce.

The fermentation process of pickles is very simple and there is no need for specific equipment. Salt, sugar, vinegar and water are the essential or basic ingredients in making pickles. Pickles also contain acetic acid which acts as preservative in order to keep the product quality for a longer time. With living developments, fast food and
instant food have become more popular than ever before; thus, there is need for mushroom processing to convert them into delicious products. The research was focused on the development of a pickle product; pickles are favoured as a side dish with a main meal or appetizer in China and other parts of the world. Pickles are a type of mildly salted and lactic acid fermented vegetable. Lactobacillus bacteria have been utilized for many centuries in food fermentation processes for producing flavour and a sour taste. They also have natural beneficial probiotic properties and organoleptic characteristics. Therefore, current research has been focused on the development and formulation of a pickle production technology with and without mustard oil processes. This study investigated the physicochemical, antioxidant properties and sensory characteristic of shiitake mushroom pickles. Pickle was prepared according to the methods reported by [14].

2. Materials and Methods

Shiitake was purchased from Hubei Province, China. Potassium meta bisulphates (KMS) and all other chemicals used were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Folin-Ciocalteu’s phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferrous sulfate (FeSO4), ethylene diamine tetra-acetic acid (EDTA), Tris–HCl buffer, H2O2, ascorbic acid, trifluoroacetic acid (TFA), pyridine, methanol, and acetic acid, ethanol, acetic anhydride and all other chemicals and reagents were obtained from the Shanghai Reagent Co. (Shanghai, China).

2.1. Preparation of Shiitake Mushroom Pickle

Fermented mushrooms were prepared using a Chinese traditional anaerobic method. Pickle preparation was carried out according to the methods of [14]. Both types of pickle were termed as follow:
1. Mustard oil, plus vinegar and salt (MOVS)
2. Soft water, plus vinegar and salt (SWVS).

2.2. Proximate Composition

Proximate analyses were carried out from the pickle products of Shiitake mushroom. All of the analyses were conducted according to the methods of the American Association of Cereal Chemists.

2.3. Organic Acids Analysis

Organic acids obtained from the pickled mushroom were analyzed by using HPLC, equipped with photodiode array detector, along with Li Chrospher 100 RP-18 column (4.6×250 mm, 5mm, Merck, Darmstadt, Germany) using the methods described by [15] with slight modification. Two milliliters of fermented medium were diluted in 8 ml of phosphoric acid solution (pH=2.65) and left for a period of 25 min at 75°C. After centrifugation for 20 min at 10,000 g at 4°C, the supernatant was filtered using syringe filter (Waters, Milford, MA, USA) by HPLC analysis. The mobile phase was used phosphoric solution 0.01mol/L-3.0% methanol, pH 2.8, detection at 214-nm of Ultraviolet (UV) absorbance, 0.8mL/min flow rate and 10μL injection volume. The column temperature was kept ambient.

2.4. Antioxidant Activity of AAP in vitro

2.4.1. Scavenging Ability with the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay

In vitro DPPH scavenging activity of the AAPF and AAPP was determined spectrophotometrically according to the scientific literature [16]. Vitamin C was used as the standard antioxidant. DPPH Solution (0.1 mM, in 95% ethanol) was prepared fresh for each analysis. Two millilitres of this solution was mixed with the purified polysaccharide sample with different concentrations (0.5, 0.1, 1.5, and 2.0 mg/ml). The mixture was shaken vigorously and then incubated in a dark place at room temperature for 30 min. A control sample was prepared with DPPH solution and ethanol. Absorbance was measured at 517 nm using a UV Spectrophotometer (Shanghai, China ZW0310072703). The following equation was used to determine the DPPH scavenging effect:

DPPH scavenging effect (%) = \frac{A_0 - (A - A_b)}{A_0} \times 100%

where A0:A517 of DPPH without the sample, A:A517 of sample and DPPH, and A_b:A517 of sample without DPPH.

2.4.2. ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of (MO) and (WO) was measured by the decolourisation assay as described by [16], with some modifications. The ABTS radical cation solution was produced by the reaction of ABTS stock solution (7 mM) with potassium persulfate (2.45 mM). The mixture was kept in the dark at room temperature (26°C) for 16 h prior to use. Various concentrations of polysaccharides (0.5, 0.1, 1.5, and 2.0 mg/ml) and VC as a standard antioxidant were added to the dilute ABTS solution and mixed vigorously; the mixture was allowed to react at 26°C for 6 min. The absorbance was immediately measured at 734 nm. The ABTS scavenging effect was calculated as a percentage according to the following equation:

ABTS scavenging effect (%) = \frac{A_0 - (A - A_b)}{A_0} \times 100%

where A0:A734 of ABTS without sample, A:A734 of sample and ABTS, and A_b:A734 of sample without ABTS.

2.2.3. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of AAPF and AAPP was recorded according to the method of [17] with some modifications. Different concentrations (0.5, 0.1, 1.5, and 2.0 mg/ml) of polysaccharide extract were incubated with 2.0 mM EDTA (0.5 ml), 3% H2O2 (1.0 ml) and 360 μg/ml crocus in 4.5 ml sodium phosphate buffer (150 mM,
pH 7.4) for 30 min at 37 °C. The hydroxyl radical absorbance was measured at 520 nm. The hydroxyl radical scavenging effect was calculated as follows:

\[
\text{Hydroxyl radical scavenging effect (\%)} = \frac{A_c - (A_{cs} - A_c)}{A_c} \times 100
\]

where \(A_c\): \(A_{520}\) of the sample and \(A_{cs}\): \(A_{520}\) of the control.

In the control, the sample was substituted with distilled water and sodium phosphate buffer replaced \(\text{H}_2\text{O}_2\).

### 2.4.4. Measurement of the Reducing Power

The reducing power of AAPF and AAPP was determined as described by [18] with minor modifications. Different concentrations (0.5, 0.1, 1.5, and 2.0 mg/ml) of sample extracts were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide \([K_3\text{Fe(CN)}_6]\). The mixture was allowed to incubate at 50 °C for 20 min; afterwards, 2.5 ml of trichloroacetic acid solution (10 %, w/v) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with (2.5 ml) distilled water and \(\text{FeCl}_3\) (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. The greater the absorbance value, the stronger the reducing power. VC was used for comparison.

### 2.5. Sensory Evaluation

For analysis of sensory qualities, all samples were evaluated according to the 7 points hedonic scaling method summarized by [19]. The sensory qualities were estimated by 10 members of students and staff, college of Food Sciences and Technology of Huazhong Agricultural University Wuhan China. Appropriate questionnaire description presented to the panelist to evaluate the differences in color, flavor, taste, texture and overall acceptability. Samples were evaluated in triplicate the sensory parameters investigated according to descriptions of each score were as follows: 7–9 like very much, 5–6 like slightly, 3–4 neither like nor dislike 2– dislike slightly and 1– dislike very much. Sensory tests were replicated thrice and water was present for taste cleansing amongst samples.

### 2.6. Statistical Analysis

The results of chemical and sensory evaluation were statistically analyzed for analysis of variance (ANOVA) \(P<0.05\) using the SPSS (IBM Statistics version 20).

### 3. Result and Discussion

#### 3.1. Proximate Composition

Figure 1A shows the mean values obtained for proximate compositions of MOVS and SWVS pickled. It was observed that MOVS contained higher pH (4.61±0.10%) compared to SWVS (3.21±0.26%) the pH continuously decreases during 20 days of storage. Decrease in pH was observed in both pickles during storage may be due to the activity of certain types of the bacteria, reported by several authors [20-25]. The titratable acidity results showed that MOVS contained the lowest percentage, 0.93±0.39%, in contrast to SWVS, 0.70±0.31% (Figure 1C). At the initial stage, the processing technique seemed to influence titratable acidity retention to different extents. The loss of titratable acidity According to Jones [26], severe loss of nutrients occurred during brining due to leaching of the material into the brine. The mean values revealed no significant differences \((p>0.05)\) between both pickles. Whereas, the polysaccharide contents of both types of pickles are presented in (Figure 1B). In the present study, there were no significant changes recorded. Mean values were recorded for pickle formulated with SWVS were ranged from 7.72% to 6.67% after 5 and 20 days, respectively. Whereas, the pickle formulated with MOVS mean values were ranging from the 8.43% to 7.23% after 5 and 20 days, respectively. The mean values revealed no significant differences \((p>0.05)\) between both pickles.

#### 3.2. Organic Acid

The organic acids contents were analyzed by using the HPLC technique and the chromatograms of pickle are shown in Figure 2. Organic acids of the fermented mushrooms were analyzed using a modified method of [15]. Five standard acids i.e. malic acid, lactic acid, citric acid, acetic acid, and succinic acid were used. The standard volume of sample was 10µL as compare to the pickle sample. The standard acid was standard mix 100 mM. The organic acids detected in the mushroom pickles during the fermentation were varied. Four acids were present in the MOVS pickle (malic acid at 4.354 with the retention time of 4.2 minutes). The second peak indicates lactic acid at 5.069 with the retention time of 4.3 min. The third peak indicates citric acid at 5.489 with the retention time of 4.5 min. The fourth peak indicates succinic acid at 7.301 with the retention time of 6.8min. Whereas the SWVS pickle showed the peak for three acids present in the pickle sample, where the first peak indicates malic acid at 4.303 with the retention time of 4.2 min, the second peak indicates acetic acid at 6.573 with the retention time of 6.2 min, and the final peak was found of succinic acid at 7.304 with the retention time of 6.8 min. Further study of pickles products during storage conditions is required.

#### 3.3. Antioxidant Activity

##### 3.3.1. Effect of Scavenging on DPPH Radicals

DPPH is an accurate, convenient and quick method used to analyse the free radical scavenging ability of natural compounds in contrast to some other methods [27]. DPPH is a stable radical measured at 517 nm, and the results of the (MOVS and SWVS) and Vitamin C are revealed in (Figure 3a). The results revealed that SWVS was found to be highest at all concentrations of DPPH radical scavenging activities compared to that of MOVS. (Figure 3a) demonstrated that the pickled DPPH significantly increased very significantly with increasing concentrations \((0.5 \text{ mg/ml to } 2 \text{ mg/ml})\); DPPH radical scavenging activity reached 5.28 to 7.40 % of the MOVS and was higher than the SWVS sample at each concentration point, while the result obtained from SWVS.
also showed an increase in DPPH range from (1.5 mg/ml to 2 mg/ml) concentration changed its range from 4.48 to 6.23%. The results of AAPP showed activity near to that of VC. The reaction among antioxidant molecules and the radical caused the decrease in absorbance of DPPH radical. The AAPP extract can be measured as a product with highly potent antioxidant activity.

3.3.2. Hydroxyl Radical Scavenging Activity

The hydroxyl radical is very reactive and has been concerned to be a very hazardous oxidant to organisms [28]. It is essential to remove hydroxyl radicals for the protection of living systems as hydroxyl radical scavengers compete with deoxyribose for the resulting hydroxyl radicals and diminish the tint formation. The study evaluated the hydroxyl radical scavenging activity of MOV and SWVS compared to those of vitamin C in the concentration range of 0.5 to 2.0 mg/ml (Figure 3b). It was observed that the scavenging capacity of MOV was found to be higher, with very significantly increased concentrations (0.5-0.2 mg/ml) in comparison with the SWVS form. At a concentration of 0.5 mg/ml, a scavenging capacity of 6.18% was observed in MOV; with the expanding concentrations (Figure 3b). At a concentration of 0.5mg/mL, a scavenging capacity of MOV 6.18% was observed, whereas the SWVS showed 2.05% at the concentrations of 0.5 mg/mL. SWVS results also showed the similar increase with increasing concentration (1.5 mg/mL and 2 mg/mL), 2.66 and 5.72%, respectively.

3.3.3. Effect of Scavenging ABTS Radicals

ABTS assay is an excellent method for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants and which frequently employed in assessing the total antioxidants [29,30]. Specific absorbance at 734-nm was used to analyze the radical scavenging effect of extracts. The results of ABTS radical scavenging of SWVS were observed to be higher than MOV, 6.83% at a concentration of 0.5 mg/mL; though, lower than that of vitamin-C (Figure 3c). The results showed that the scavenging ability of both MOV and SWVS pickles on ABTS free radicals confirmed that the activities of each sample increased with the increase in concentration.

3.3.4. Reducing Power

During this assay, the colour of the test solution changed from yellow to green or blue, depending on the reducing power of the test sample. The reducing power of various extracts might be a result of its hydrogen-donating ability as described by [30]. The reducing power of MOV and SWVS at different concentrations were demonstrated. At 2.0 mg/ml, the reducing powers were 5.05 and 5.52% for MOV and SWVS, respectively (Figure 3d). Moreover, the standard antioxidant showed a similar result of an increase in the reducing power with increasing concentration.

![Figure 1](image_url)
Figure 2. HPLC chromatogram of standard sample (B) (MOVS) (C) (SWVS)
3.4. Sensory Evaluation

Sensory analysis of both pickles was carried out for color, flavor, taste, texture, and overall acceptability scores are presented in Figure 4A and Figure 4B. The color, flavor and overall acceptability of the MOVs pickles were found to be best as compared to SWVS pickle. The pickle formulated with MOVs showed 5.7 scores for overall acceptability of pickle. Whereas, the texture of pickle was slightly ranked lower 5.06 scores than the overall acceptability, no significant difference (P>0.05) was obtained. Panelist scores demonstrated that the MOVs type of formulated pickle found to be best. Whereas, the all sensory attributes of SWVS formulated
pickle was rated lower. In terms of texture and taste, all the samples were rated quite low, indicating a poor preference for the texture and taste. This might be due to the blanching which decreases in texture firmness and brining could be related to ultra structural changes [31].

3.5. Conclusion

The present study was carried out on Physicochemical, Antioxidant Properties And Sensory Characteristic Of Shiitake Mushroom Pickles. The antioxidant activity of fresh shiitake mushroom pickles with and without mustard oil demonstrated a significant result in Scavenging free radicals. The pH of MOVs and SWVS was reduced over time during 20 days of storage and the acidity was gradually increased for up to storage. However HPLC analysis of both pickles revealed the presence of four acids i.e. pickle malic acid, lactic acid, citric acid and succinic acid, Whereas the SWVS pickle showed for three acids present in the pickle sample malic acid, acetic acid and succinic acids. The pickle product has been evaluated for colour, flavour, taste, texture and overall acceptability and is found to be excellent formulated with MOVs as compared to the SWVS. Further studies require in evaluating the Physicochemical, Antioxidant Properties of the different pickle.

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References