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Effect of Processing Techniques on the Quality and Acceptability of *Auricularia auricula* Mushroom Pickle

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Abstract The present study aimed at the formulation of pickled product, incorporated with and without mustard oil; vinegar and salt were used as preservative. The products have been evaluated for sensory attribute i.e color, flavor, texture, taste and overall acceptability, (MOVS) formulated pickle found to be excellent and have higher score 5.7, 6.5, and 7.0 for color, flavor, and overall acceptability. It was found that the polysaccharide content and pH were decreased during processing, and significant difference (P>0.05) was found in moisture content Figure 4. The microbiological studies revealed that total viable counts (bacteria) were high in (SWVS) formulated pickle as compared to (MOVS) respectively. Storage studies demonstrated that pickles stored successfully for 90 days at ambient temperature (26±4C) without any significant change in the quality attributes of the (MOVS) pickle. Furthermore, microbiological analysis demonstrated the presence of Escherischia coli and Lactobacillus bacteria in pickled products.

Keywords: *sensory, storage, quality, pickle, microbial*

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1. Introduction

Nowadays, edible mushrooms have become an attractive, functional food, generally due to their chemical composition of polysaccharides. Owing to their unique organoleptic characteristics, taste, texture, flavour and nutritional value, edible mushrooms are popular all over the world as well valuable component of the diet [1-7]. The approximate world production of mushroom consists of about 5 million tones of fresh weight per annum [8].

Auricularia auricula, an important edible and medicinal mushroom, particularly cultivated in China and Asia followed by Europe and Africa, and fourth most important cultivated mushroom used by humans all over the world. Generally used as a healthy food in East Asian, especially in China and Korea [9]. Researchers found 100g of dried *Auricularia auricula* contained 18.3g protein, 18.9g carbohydrates and 50g dietary fiber and medicinal value in prevention of diabetes and heart attacks [10,11,12]. *Auricularia auricula* plays crucial role in China mushroom industry due to containing of high nutritional value therefore to be processed into a variety of foods [13]. As mushroom highly perishable by nature with short shelf-life in the ambient environment owing to its high moisture content and other essential nutrients and lack of physical protection to avoid water loss or microbial attack [14]. Therefore, mushrooms are generally used in the processed form [15].

While observing increased mushroom demand due to the nutritional advantages of consumption, required appropriate processing technologies in developing different new food products to preserve chemical and nutritional characteristics in fresh forms. Extending shelf-life is a very important aspect that can be enhanced for a larger period by way of processing of freshly harvested mushrooms for manufacturing of pickle, soups, ketchup, sauce and so on. Several processing industries, preserving the products via value-added technologies by different methods to extend the shelf-life and add value to the product. The pickle is a vegetable product which has a extended history and local characteristics in China and is favourite in the Chinese population, Chinese were fermenting vegetables called (Paocai) as early as the third century B.C. Chinese pickle is acknowledged to be the product of mixed fermentation processed by lactic acid bacteria (LAB), which are natural bacteria. Various groups of LAB have been isolated from fermented vegetables, such as Leuconostoc, Lactobacillus, Lactococcus, Pediococcus, etc [16]. Throughout the fermentation, LAB utilise carbohydrate substrates accessible in the fermentation method and produce organic acids, especially lactic acid, as a part of their metabolites. These

acids not simply play a significant role in the taste and aroma of the product. It has been report that numerous LAB can produce bacteriocins which are active against closely related species [17]. Salt, vinegar and spices are frequently used in consequent action in pickling and widely are, in practice within different parts of the world [18]. Pickles are good appetizers, consumed by all age of people and contain large amounts of lactobacilli bacteria which are important for the digestion of grains and vegetables, which have usual beneficial probiotic properties used by the body [19,20]. While, to our knowledge, no comprehensive study has been carried out on pickle from *Auricularia auricula*. Processed foods generally provide an acceptable nutritional component and acceptable in sensorial quality to consumers. Therefore, aim of this work was to investigate the effect of different processing methods on quality of *Auricularia auricula* pickles and to develop formulations for value added mushroom pickle products. Pickles were investigated for sensory attributes and overall storage stability; also microbiological qualities were also evaluated. *Auricularia auricula* pickle was prepared according to the literature of [21].

2. Materials and Methods

The *Auricularia auricula* mushroom collected from (Hubei Province China). All required ingredients like spices, vinegar, salt and oil etc collected from local market (Wuhan). All reagents used were of analytical grade.

2.1. Instrumentation

The following instruments were occupied: rotary evaporator model RE-2000A (Yarong Bio-instrument Shanghai, China) water bath model KD-98-IIA Tianjin Taisite Instrument Co., Ltd. spectrophotometer (ZW0310072703 Shanghai China), oven (Model-YX 280 A, Shanghai China.

2.2. Preparation of Mushroom Pickle

Two kg of *Auricularia auricula* was first washed in cold water to remove the foreign matters adhering to surface, then cut 1/4 inch sliced and stem discarded. Blanching was carried out at a temperature of 96–98^{o}C, the proportion by weight of mushrooms to water or solution being 1:5. *Auricularia auricula* was blanched for 5 min in 0.05 percent KMS solution according to the method [22]. The blanched *Auricularia auricula* was washed in cold water for 2-3 times and the extra water drained off and subjected to salt curing process, in which 10 percent sodium chloride is added and kept overnight. The excess water oozed-out of *Auricularia auricula* removed on the next day and was distributing into two jars as (1 kg per jar) i.e. mustard oil, plus vinegar and salt (MOVS) and pickle in vinegar, soft water and salt (SWVS) concentration. Measure quantity of mustard oil heated and cooled. Combined weight of spices added to the heated oil and gently reheats till become brown and cooled. The various spices i.e. turmeric powder (20.0g), black mustard seed powder (rai) 35.0g, red chili powder (10.0g), cumin seed powder (1.5g), carom seed (ajwain) (10.0g), Nigella seed (Kalonji) (10.0g), fennel seed powder (saunf) (1.5g), salt (90g) vinegar (acetic acid 5% solution 100 ml) [23] and oil (mustard oil 200 ml). Mixed the spiced oil with *Auricularia auricula* pieces and add remained oil in jar. Second jar was filled with vinegar and soft water. (Spices & preservatives were mixed to the desired taste and quality of mushroom pickle). The pickles were stored at the ambient temperature 26±4°C for about 3 to 4 weeks for fermentation. Fermenting jars were covered with muslin cloth properly to prevent from contamination from insects and molds and checked every day. Ingredients and their ratio used in the preparation of pickle are listed in table 1, while the flow chart of manufacturing process shown in Figure 1. Both types of pickle were termed as follow:

1. Mustard oil, plus vinegar and salt (MOVS)
2. Soft water, plus vinegar and salt (SWVS)

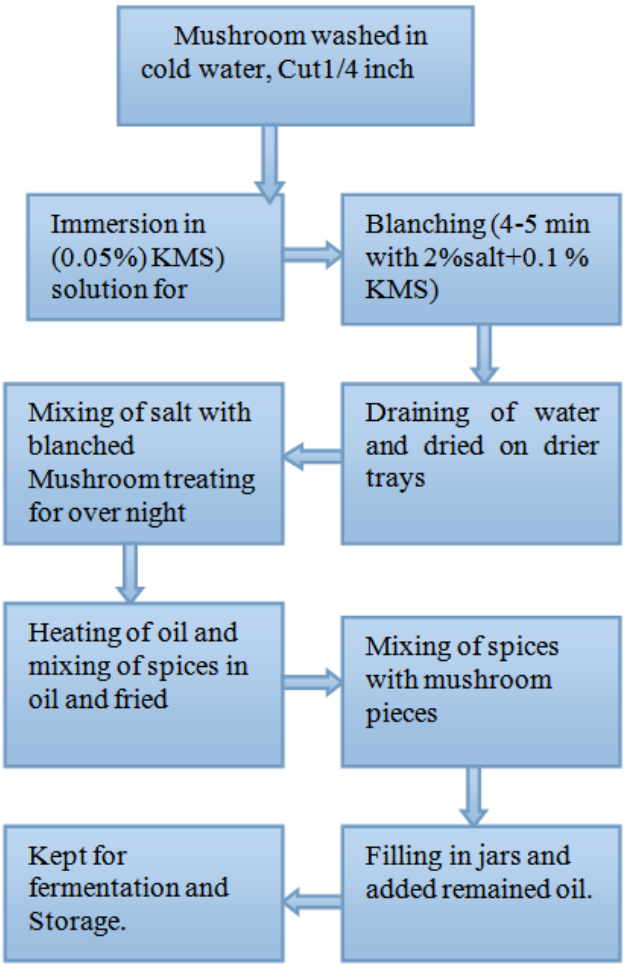

Figure 1. Preparation flow chart of pickle from *Auricularia auricula*

Table 1. Recipe of spices mixture used in the *Auricularia auricula* pickle method by (21)

Ingredients	Percentage
Chili powder	10.0g
Tumeric powder	20.0g
Black mustard seed	35.0g
Funnel,	1.5g
Cumin powder	1.5g
Carom,	10.0g
Nigella seed	10.0g
Common salt	90.0g
Oil (mustard oil)	200.0ml
Vinegar (5%)	100ml

2.3. Sensory Evaluation

For analysis of sensory qualities all samples were evaluated according to the 7 point hedonic scaling method summarized by [24]. The sensory qualities were estimated by 10 members of students and staff of college of Food Sciences and Technology of Huazhong Agricultural University Wuhan China. The group consists of six females and four males and sample product was placed in sensory cups, lids coded, with the appropriate questionnaire description presented to the panelist to evaluate the differences in color, flavor, taste, texture and overall acceptability. The organoleptic scores for the product with a rating 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.

2.4. Microbiological Characteristics

Enumeration of Total plate count (TPC) was accomplished by the method of [25], to evaluate the (TPC) on the stored pickles sample. Appropriate serial dilutions were prepared 10^{-1} to 10^{-5} concentration and 0.1 mL of diluted pickled samples used to spread on media plates using sterile glass spreader. This method was used for the enumeration of total aerobic count, like E.coli, Staphyloc- occal and Salmonella counts on nutrient agar medium and the media were prepared according to the manufacturer's instruction and used for enumeration of total viable bacteria count.

2.5. Chemical Analysis

The polysaccharide was determined by the phenol-sulfuric acid method [26]. Glucose standard curve was used as standard. The absorbance was measured at 490 nm against the blank. The pH measurement of the pickle was determined by using a digital pH Meter (Seven Easy Switzerland, Model 8603) by direct immersion of the electrode in the diluted sample of pickles. Moisture content was determined to the literature of [27].

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. Sensory Evaluation

Sensory evaluation

In the current study, two formulations were estimated for colour, flavour, taste, texture and overall acceptability scores of pickle during the storage period demonstrated in (Figure 2 and Figure 3). The colour, flavor and overall acceptability of the (MOVS) formulation were found to be better as compared with (SWVS) formulation. The panelist scores for pickle formulated with (MOVS) showed 7.0 (very good) for overall acceptability of pickle. Whereas the flavour of pickle was rated slightly lower 6.2 (very good) than the overall acceptability, no significantly different ($P>0.05$) obtained. Result scores showed that the panelists approved the (MOVS) formulated type of pickle. On a seven-point hedonic scale, all sensory results were moderately acceptable, except for the texture (3.0). Whereas the (SWVS) formulated Pickle had lower scored rating among all sensory attributes. In terms of texture and taste, all the samples were rated quite low, indicating a poor preference for the texture and colour. Decrease of texture firmness after blanching and Brining could be related to ultra structural changes has been reported by [28]. The Panelist observation revealed that current sensory results for both types of pickles were not significantly different ($P>0.05$) amongst the storage periods in values of overall acceptability during ambient storage (26°C±4). However pickle formulated with (MOVS) had the highest scores in all sensory attributes and comparatively acceptable as compared to the (SWVS) formulated pickle and the overall acceptability were rated to be good throughout the study period (Figure 3). In the beginning, the texture of the pickles was tough but started to be soft and after 2 months of storage it became soft and results showed a decreasing trend in texture scores. The *Auricularia auricula* pickles changed during the storage period producing sour odor. On current examination, it was observed that the salt, vinegar and oil concentration prevents microbial development in the *Auricularia auricula* pickle results are in agreements with the study on the germinated Bengal gram pickle [29,19]. It was found that pickles became significantly soft after storage, softening in pickle is generally related to an enzymatic action [30]. Progress of dark colour and rancid flavour were observed in catfish pickles during the storage period of 6 months [31,32].

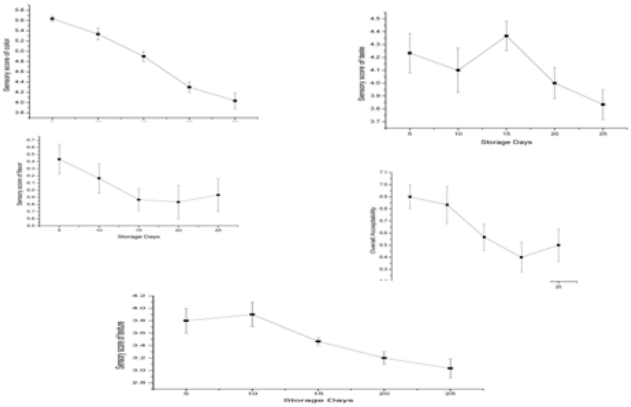

Figure 2. Total mean score of sensory evaluation on Color, flavor, taste, texture and overall acceptability of (MOVS) pickle formulation

3.2. Storage Life

Results of storage study for sensory evaluation of the pickle products at room temperature (26°C ± 4) are shown in Table 2. Colour, flavour, texture, taste and overall acceptability of products were found to be acceptable up to 3 months storage for (MOVS) formulated pickle. Whereas the (SWVS) formulated pickle produced bad smell after two months storage. After 3 months of preservation the significant change was noticed and the pickle remarked as unpleasant to consume. Results revealed that significant changes were observed in the texture and colour. The change occurred perhaps due to

fermentation in existence of fungus (mold and yeast) [33]. The excellent shelf life of the (MOVS) pickle formulated may be the presence of high quantity of oil on the top level of the pickle or due to appropriate packaging [34].

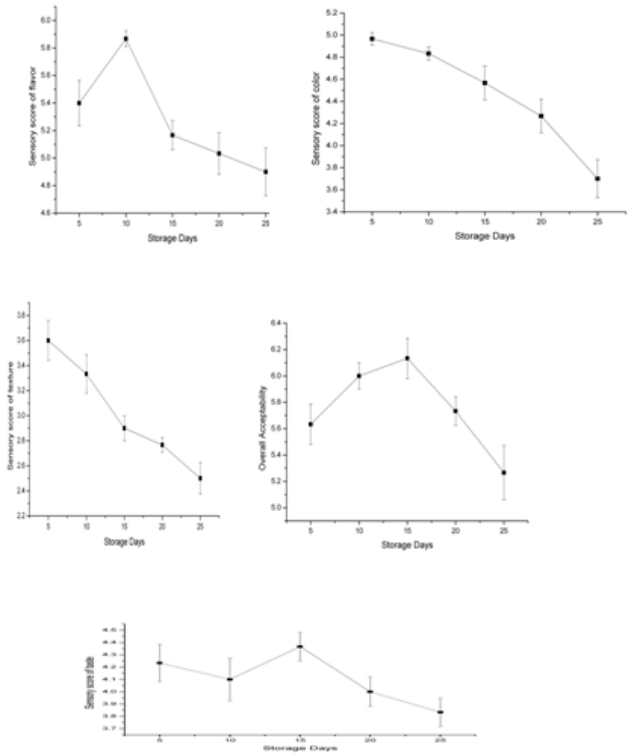

Figure 3. Total Mean score of sensory evaluation on Color, flavor, taste, texture and overall acceptability of (SWVS) pickle formulation

Table 2. Effect of storage on the quality of MOVS and SWVS pickle

Storage period Month	MOVS Pickle formulated			Overall acceptability Remarks
	Colour	Flavour	Texture	
1	No change	Acceptable	Rigid	Acceptable
2	No change	Acceptable	Rigid	
3	No change	Acceptable	Slightly soft	
4	Change	Unpleasant off flavour	Remar-kably Soft	Not acceptable
	SWVS Pickle formulated			Overall acceptability Remarks
	Colour	Flavour	Texture	
1	No change	Acceptable	Rigid	Slightly Acceptable
2	No change	Acceptable	Rigid	
3	Change	Unpleasant off flavour	Soft	
4	Change	Unpleasan, off flavour	Remar-kably soft	Not Acceptable

3.3. Chemical Analysis

The proximate compositions of both forms of pickles formulated are shown in Figure 4a, 4b and 4c. Moisture content of (SWVS) pickle formulated ranged from 80.60% to 86.40 between all treatments investigated; whereas the moisture content for (MOVS) pickle formulated result showed it ranged from the 67.60% to 77.06 %, significant ($p<0.05$) difference were found among both formulation, (SWVS) formulated contained higher moisture content. In the beginning pH was recorded 5.70; pH value was dropped gradually most of the time during processing of pickle.

The pH continuous to decrease to 5.50 to 5.04 and 5.86 to 5.56 for (MOVS) (SWVS) respectively until day 15 and steady at the end of the ending fermentation of pickled. The low pH for the period of storage may be due to the activity of certain types of the bacteria, which is producing acid [35]. In the present study, a decrease in pH was observed in both pickles. Similar decreasing trend in pH throughout storage of pickles reported by several authors [36,37,38,39,31,35]. Study results for the polysaccharide content of both types of pickles are present in Figure 5. In the present study there were significant changes observed in the polysaccharide content during processing at different stages (Figure 5). Mean values were recorded for pickle formulated with (MOVS) were ranged from 3.41% to 2.35% after 5 and 25 days correspondingly. Whereas the pickle formulated with (SWVS) mean values were ranging from the 2.72% to 1.64% after 5 and 25 days respectively. The mean values revealed significant differences ($p<0.05$) between both pickles. The polysaccharides content was different during fermentation process throughout the development of pickle. To date, there is slight information available for the study of the polysaccharide changes in the whole process of the pickled *Auricularia auricula*.

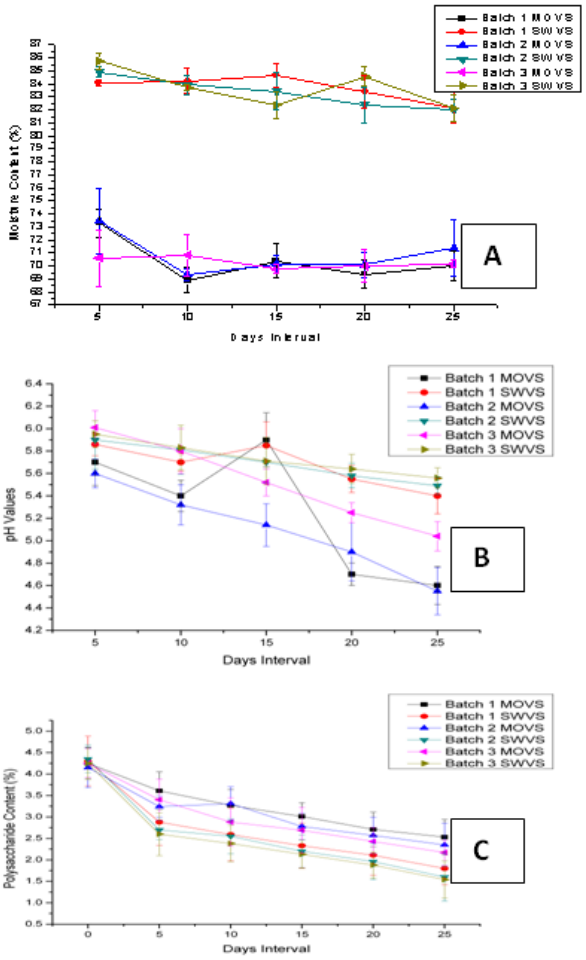

Figure 4. Result for pickles formulated with (MOVS) (SWVS). 4a moisture content, 4b pH, 4c polysaccharide content, all the determinations were done in triplicate and the results were expressed as mean value

3.4. Morphology and Bacterial Count

The microbial of *Auricularia auricula* pickles is shown in Table 3. The total no. of viable bacteria was calculated by multiplying the colony forming unit (cfu) by dilution number. The total bacterial count for (SWVS) pickles formulated were counted and that ranged from 144x10^1 to 39x10^3 (cfu/g), While (MOVS) pickle showed the 94x10^1 to 35x10^2 (cfu/g) batch 1, while (cfu/g) count for batch 2 and batch 3 are showed in Table 3 respectively. It is reported that the low acid meat pickle had a gradual increase in microbial count by increased time [40]. It is reported that pickled covering oil and sugar as well as salt prevent microbial contamination and longer shelf life might be due to the presence of higher amount of oil on the top layer of the pickle or due to proper sealing of the cap [41]. Furthermore, reported [18] that pickle products would deteriorate quickly, protection is required against the act of moulds, which metabolize the acid developed and permit the proceed of other microorganisms. While in cold storage fermented and pickled foods could be expected to remain stable for several months.

Bacterial colonies were distinguished and recognized as explained by the literature of [42]. The results of Gram's staining showed that difficult to understand the microbial ecosystem due to a lack of knowledge in the area of the pickled *Auricularia auricula*. In fact, most of the bacteria found in the pickling process were belongs to bacillus family but it not easy to identified structures of particular species morphological figure are illustrated in (Figure 5).

Table 3. Total Plate Count (CFU) and identification of species from both pickles

Batch	Colony-Forming Units (CFU/g)
(SWVS) 1	144x10^1 to 39x10^3
(MOVS) 1	94x10^1 to 35x10^2
(SWVS) 2	156x10^1 to 45x10^3
(MOVS) 2	109x10^1 to 44x10^2
(SWVS) 3	136x10^1 to 49x10^3
(MOVS) 3	101x10^1 to 41x10^2

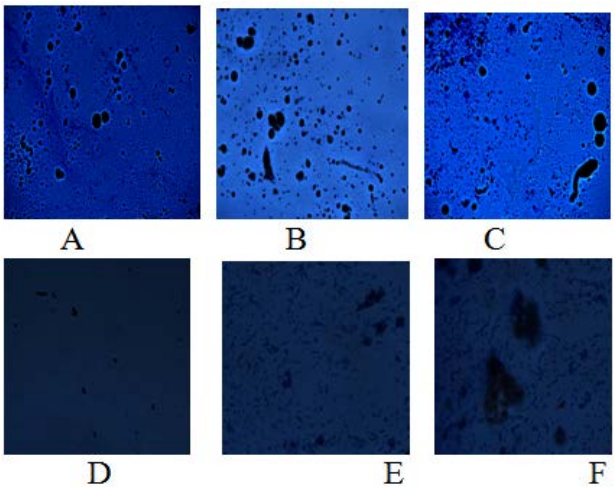

Figure 5. Scanning electron microscope structure photographs of tested colonies by gram staining test of (a, c,) SWVS, (b, d, e, f) MOVS

3.5. Conclusion

The present study was an effort to develop a formulated pickle from *Auricularia auricula*, 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. From the storage study at room temperature of formulated pickle for shelf life observation, it was found that the pickle can be stored up to 90 days without any noticeable change in overall acceptability. The formulated pickle can be preserved by using oil and salt as a preservative up to two to three months at ambient temperature then the product became spongy and the colour turned unfavorably darker leading to storage. Further studies require in evaluating the storage conditions on storage stability of the formulated pickle and improving the final product.

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