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Quality characteristics of fermented mushroom and vegetable product using a mixed starter of lactic acid bacteria

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KEYWORDS

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ABSTRACT

Lactic acid fermentations can be performed through inoculation in order to ensure consistent product quality and safety. This research investigates the effect of the use of *Lactobacillus plantarum* and *Lactobacillus johnsonii* as a mixed starter in mushroom and vegetables fermentation on its product quality. The fermented product showed pH, titratable acidity and total phenolic content of 4.01±0.02, 1.00±0.03% and 519±11 mg GAE/kg respectively. Lactic acid bacteria counts in the fermented product were between 8.5-9.5 log CFU/g throughout the storage time. The results from this research suggest quality satisfaction of the fermented product in microbial, chemical and sensory after 30-day storage at 4 °C. This study showed that the fermented mushroom and vegetable product has the potential to be used as a probiotic carrier food.

Introduction

Lactic acid bacteria (LAB) play an important role to generate diversity of sensory quality and increase shelf life of highly perishable fruit and vegetables through lactic fermentation (Steinkraus, 1997). Numerous fermented fruit and vegetable products are produced by LAB such as fermented olive, pickled cucumber, sauerkraut, kimchi. Salted conditions are significant in these fermented products because salting provides an appropriate environment for the growth of LAB which imparts acidic flavor (Ray and Didier, 2014). Lactic acid fermentations can be carried out by spontaneous condition or controlled condition through inoculation (Park et al., 2019). Spontaneous fermentation leads to variations of the product quality in contrast to fermentation with starter inoculation which can ensure consistent product quality and safety (Capozzi et al., 2017). Unsuccessful control of lactic fermentation could be a parameter to destroy product quality and therefore using lactic acid bacteria starter is highly considered in fermented food industry. Furthermore, using LAB starter could be an alternative potential to promote

functional property of fermented fruits and vegetables as probiotic carrier food which typically associated with milk or dairy products.

Mushroom has been commonly consumed as a food source due to their nutritional value, umami taste and medicinal properties in oriental countries for over 2000 years (Roupas et al., 2012; Zhang et al., 2013; Ruthes et al., 2015). Pleasant tastes, well-balanced diet, and health-promoting of mushrooms contributed to a worldwide increase in edible mushroom consumption (Feeney et al., 2014). Edible mushrooms can be cooked or processed for specific consumers such as vegetarians, certain patients, e.g. obesity, diabetes, Alzheimer and cardiovascular disease (Feeney et al., 2014; Ng et al., 2017). Among numerous edible mushrooms, *Pleurotus* spp. is second most cultivated in the world (Ng et al., 2017). This oyster mushroom has a high nutritional value and contains several bioactive compounds, including polysaccharides, peptides, dietary fiber, ergosterol, B vitamins, minerals and antimicrobial agents (Tolera and Abera, 2017; Liu et al., 2016; Pisoschi et al., 2018). The *Pleurotus* mushrooms are delicate and

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sensitive to deteriorate after harvest, thus their shelf life is limited to a few days (Tolera and Abera, 2017). Among food processing technology, fermentation is considered as a preservation method that plays an essential role in the fermented food industry, e.g. reduction of time and energy requirements of food processing resulting in the properties of specialized foods (Steinkraus, 2002). By focusing on these facts, the objective of this present study was to evaluate a mixed starter of *L. plantarum* and *L. johnsonii* on product quality of fermented mushroom and vegetables.

Research Methods

Starter culture preparation

L. plantarum and *L. johnsonii* were provided by Faculty of Science, Maejo University and proliferated following the procedure indicated by Choi et al. (2019). A starter culture containing equal proportions of both species was inoculated at approximately 10^6 CFU/g of substrate.

Mushroom and vegetable fermentation process

Mushroom and fresh vegetables (Chinese cabbage, spring onion, carrot, red bell pepper and purple cabbage) were purchased from the market (Lampang, Thailand). The edible parts of mushroom and vegetable were selected, washed and sliced into 3 mm width. Prepared mushroom and vegetables and salt were mixed with starter then transferred to glass vessels (50 g) and tightly packed together to remove air. The fermentation was induced by the mixed starter culture of *L. plantarum* and *L. johnsonii* previously prepared. The study was performed in 3 replications at $32 \pm 2^\circ\text{C}$ for 9 hours.

Storage conditions

Fermented mushroom and vegetable product at the end of the fermentation process was placed in the refrigerator in order to evaluate the storage quality. The storage temperature was controlled at 4°C for 30 days. Sampling was done every 3 days for determination.

Microbiological analyses

Microbiological analyses were carried out on fermented samples taken at 3-day intervals from refrigerated storage (0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days). Ten grams of each replicate were aseptically diluted in sterile Ringer's solution (Merck, Germany) and homogenised for 1 min. Decimal

dilutions were prepared and inoculated using the pour plate technique, into the corresponding media as follows: total aerobic plate count were enumerated on Plate Count Agar (PCA, Merck, Germany) after incubation at 35°C for 24 hours and total lactic acid bacteria on DeMan, Rogosa and Sharpe (MRS agar, Merck, Germany) after incubation at 35°C for 24 hours.

Chemical analyses

pH was measured by digital pH meter (Model C831, Belgium). Total acidity was determined by diluting each 5 g aliquot of sample in 50 mL distilled water and then titrating to pH 8.2 using 0.1 N NaOH (Nielsen, 2017). Titratable acidity was expressed as lactic acid percentage. Total soluble solid content was determined on an Atago hand-held refractometer. Free alpha amino nitrogen (FAN) was quantified by spectrophotometric method (Intaramoree and Chomsri, 2014). The modified method of Bradford (1976) and Spínola et al., (2015) was used to evaluate total phenolic content and total soluble protein content, respectively. The antioxidant activity was determined by modified method of Wongputtisin et al., (2007).

Sensory analysis

Fermented mushroom and vegetable product was evaluated for organoleptic quality. Assessors were experienced in fermented mushroom products. A 30-member panel took part in this study. Assessors were asked to rate the products for appearance, color, odor, flavor, and overall preference on a structured nine-point hedonic scale; 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely (Meilgaard et al., 2016).

Results and discussion

Properties of fermented mushroom and vegetables

Table 1 shows the properties of the fermented mushroom and vegetables at the end of fermentation. The product pH of 4.01 ± 0.02 was correlated with the titratable acidity of 1.00 ± 0.03 due to organic acids produced by LAB. Capability of LAB to lower pH in fermented vegetables was also reported in other fermented vegetables such as kimchi (Lee et al., 2019) and sauerkraut (Xiong et al., 2014). The obtained product in this study was classified as acid food according to Institute of Food Technologists

(2003). Free alpha amino nitrogen (FAN) measurement in this study revealed approximately 1446 mg/kg (184 mg FAN/L) of amino acids in the fermented product which was 2.8 times lower than in the fermented mushroom obtained from spontaneous fermentation as previously reported by Chomsri and Manowan (2019). The low value of FAN in the product obtained from mixed starter fermentation may be the result of LAB taking up amino acids for their growth (Zajšek et al., 2013; Solval et al., 2019) compared with spontaneous fermentation which had lower LAB amount. It is interesting to note that the nitrogen source in substrate of this study was expected to be adequate for maximum LAB growth according to the experiment of Dong et al. (2014). The product showed 1.4 times higher total phenolic

content than the fermented mushroom without vegetable adding (data not shown). This could indicate the positive effect of vegetables in the fermented product. Microbiological analysis of the fermented product showed LAB amount above 6 log CFU/g which suggested that the product could be possible for a probiotic carrier (Sah et al., 2016). The sensory profile of the fermented product is shown in Figure 1. A sensory panel rated the 5-organoleptic attributes of appearance, color, odor, flavor and overall preferences in the range between like moderately and like very much. These results suggest desirable quality of the fermented mushroom and vegetable product.

Table 1. Properties of fermented mushroom and vegetables product

Quality	Values
pH	4.01 ± 0.02
Titrateable acidity (%)	1.00 ± 0.03
Total soluble solids (°Brix)	8.37 ± 0.24
Free alpha amino nitrogen (mg/kg)	184 ± 18
Total soluble protein content (mg/kg)	47.19 ± 1.32
Total phenolic content (mg GAE/ kg)	519 ± 11
Antioxidant activity (% scavenging effect)	37.34 ± 0.19
Ascorbic acid equivalent antioxidant capacity (mg/100g)	16.97 ± 0.03
Tolox equivalent antioxidant capacity (mg/100g)	27.36 ± 0.04
Total plate count (log CFU/g)	8.54 ± 0.03
Lactic acid bacteria count (log CFU/g)	8.48 ± 0.00

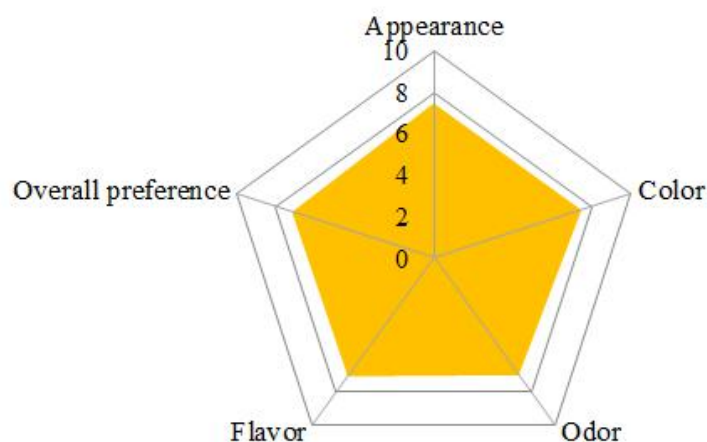


Figure 1. Sensory profile of fermented mushroom and vegetables product

During storage

The total viable and lactic acid bacteria counts during storage period were in the ranges of 8.5-9.5 and 8.5-9.4 log CFU/g in all samples, respectively (Fig.2). Storage times did not significantly influence the viable microbial population in fermented product during 30-day storage at 4°C. There was a remarkable increase in the LAB count shortly thereafter the beginning of the storage to a maximum approximately 9.5 log CFU/g in the sample kept in the refrigerated chamber for 3 days. This might be explained by the fact that LAB could slightly continue multiplying after the terminated time of fermentation. The microbial count after 3-day storage remained consistent until the end of storage time. The stable bacterial count was also reported in kimchi stored in the refrigerator (Moon et al., 2018).

Chemical and organoleptic changes of fermented product during storage

Changes of chemical quality in the fermented product are shown in Figure 3a. At the beginning, the sample had pH, titratable acidity and total soluble solids of 4.01 ± 0.03 , 1.01 ± 0.04 % and 8.37 ± 0.25 °Brix, respectively. The values of pH were gradually decreased during storage time with respect to an increase of acids and total soluble solids. The results described above, agrees very well with this acid increase. Correlation of pH and titratable acidity has also been reported in kimchi fermentation (Choi et al., 2019). Organoleptic results during the 30-day evaluation are presented in Figure 3b-3f. According to the figures, significant difference during storage under the investigated conditions was not obviously observed in samples indicating sensory satisfaction.

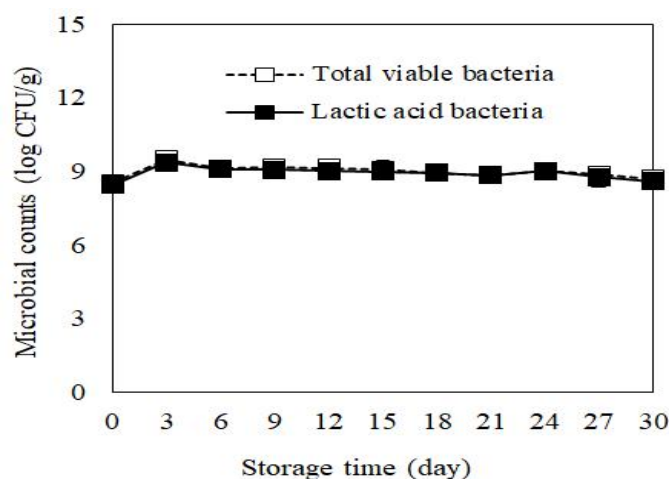


Figure 2. Microbial changes in fermented mushroom and vegetables product during storage at 4°C for 30 days

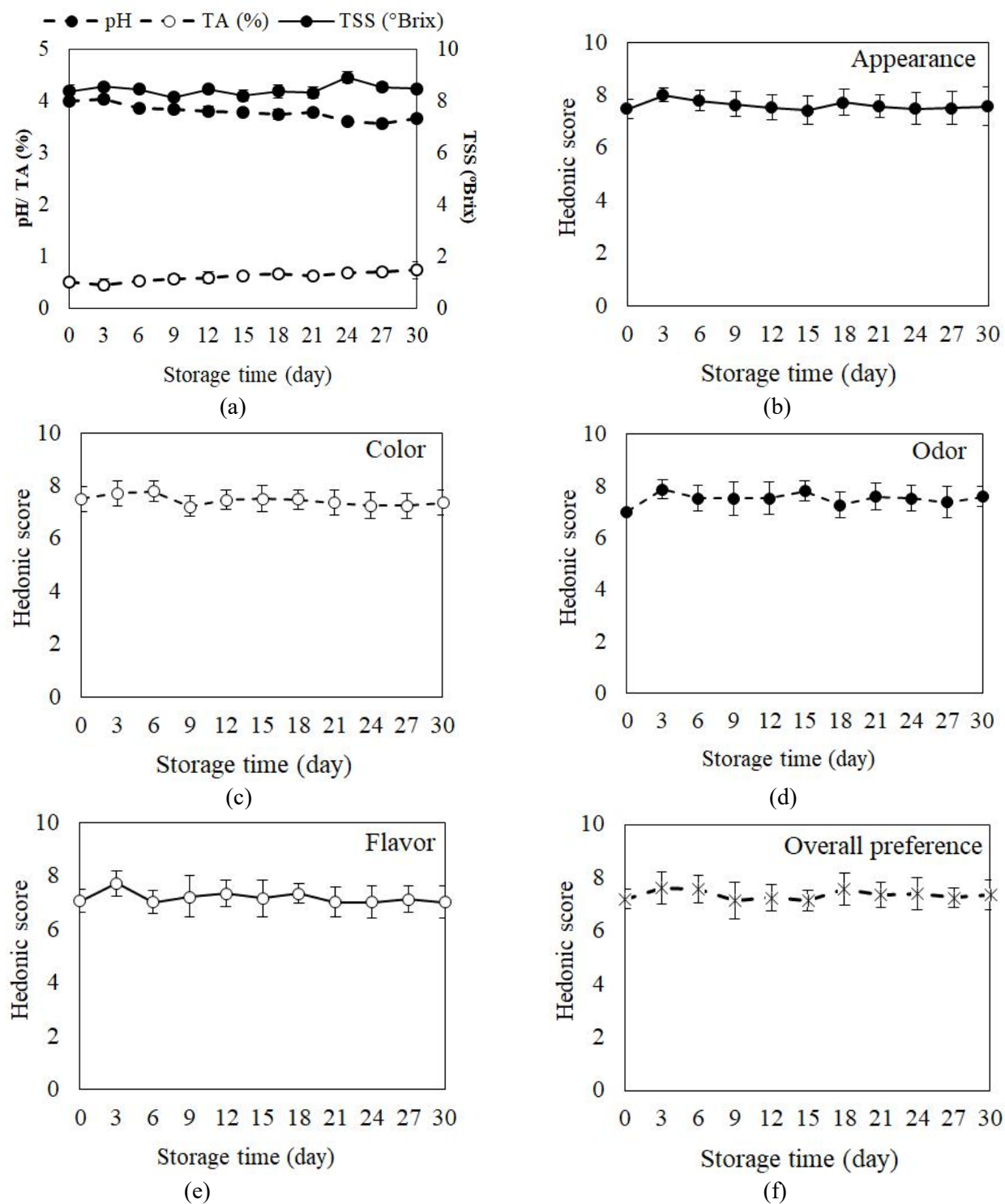


Figure 3. Chemical and organoleptic changes in fermented product during storage at 4°C for 30 days

Conclusion

Results from the present research demonstrated that lactic acid fermentation of mushroom and vegetables by inoculation with LAB produced the desirable product quality. The ability of LAB to increase shelf life of highly perishable mushroom and vegetables through fermentation process was noted in this study. Furthermore, LAB survival throughout the storage period is a plausible evidence to allow further investigation and development of this product for a better fermented functional food.

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Conflict of interest

The authors have no conflicts of interest to declare.

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