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RESEARCH ARTICLE

Production of Pickled Mustard Greens (*Brassica Juncea*)

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Abstract

Mustard greens are fresh during the harvesting season but perishable under the prevailing conditions of temperature and humidity as well as lack of adequate storage facilities. An alternative way of preserving surplus mustard greens could be fermented to pickle products. Preservation of the mustard green by fermentation can eliminate the undesired taste and improve flavor of the vegetable. Therefore we explored a lactic fermentation from mustard greens by focusing on the effect of different parameters such as blanching time and temperature as pre-treatment, salt concentration, fermentation time to physicochemical, microbial and sensory characteristics of pickled mustard greens**.** Experimental results revealed that blanching raw mustard green in water heated at 95^{o}C in 10 seconds, 6% salt in 12 days of fermentation was appropriated to get a pleasant pickled mustard greens quality. Mustard greens pickles are considered as one of the health supplements.

Keywords: *Mustard greens, Lactic fermentation, Salt, blanching, Physicochemical, Microbial, Sensory.*

Introduction

Mustard greens (*Brassica juncea* L.) belong to family brassicaceae. It is a vegetable that has large petioles, thick leaves, well wrapped and firm head. It is usually not consumed fresh because the taste of fresh produce is bitter and spicy. Mustard green is rich in phenolic compounds, vitamins, and minerals [1]. Mustard green is one of the best low-calorie cruciferous vegetable, extensively used as a raw vegetable, or as fermented pickle [2].

They are also known for the production of various volatile organic compounds like ketones, aldehydes, esters, alcohols, terpenes and glucosinolates [3]. Glucosinolates enzymatically decomposed compounds accounted for the major proportion in the volatile compounds [4]. These volatile compounds help in pollination by attracting various insects and animals, as well as they protect plants from herbivorous attack [5]. Mustard greens attracted the researchers due to its ability to reduce cardiovascular diseases and some cancers, especially those of the gastrointestinal tract [6].Pickling is one of the ancient ways of food preservation, and it was a possible way of preserving the foods, especially seasonal foods, before the invention of modern preservative machines like the refrigerator.

Pickle is the good source of antioxidants, probiotics, vitamins (vitamin C, A, K, and folate), and minerals (iron, calcium, and potassium). The fermented vegetables or pickle products made with characterized probiotic strain confirms the supplementation of probiotics to the consumers [2].

Mustard greens is an underutilized vegetable crop and still now there is very limited research available regarding to processing of this vegetable into value added product. The mustard greens vegetable, which typically has high fermentable sugar composition, could be exploited as a substrate for lactic fermentation. *Lb. fermentum* play an important role in the mustard green fermentation [7].

Therefore, we utilized this vegetable as subtrate for lactic fermentation. We focused on the effect of different parameters such as blanching time and temperature as pre-treatment, salt concentration, fermentation time to physicochemical, microbial and sensory characteristics of pickled mustard greens.

Material and Method

Material

Mustard greens were cultivated and collected from My Xuyen district, Soc Trang province, Vietnam. They must be cultivated following VietGAP without pesticide and fertilizer residue to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments.

Apart from collecting mustard greens, we also used other materials such as NaCl, $CaCl_2$, NaOH, phenolphthalein, phosphate buffer, onto MRS (de Man, Rogosa, and Sharpe)-agar. Lab utensils and equipments included knife, weight balance, cooker, fermentation vessel, pH meter, buret, stomacher, colony counter, micropippetor.

Figure 1: Mustard greens *(Brassica juncea)*

Research Method

Effect of Blanching Temperature and Time to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

Mustard greens were pre-treated by blanching in water containing 0.1% $CaCl_2$ with different time and temperature (100°C in 5 seconds, 95°C in 10 seconds, 90°C in 15 seconds and 85°C in 20 seconds). Effectiveness of blanching time and temperature in mustard greens fermentation was evaluated on value of pH, total acidity (%), lactic acid bactria (cfu/ml), sensory score.

Effect of Salt Concentration in Fermentation to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

Mustard greens were fermented with different salt concentration (2%, 4%, 6%, 8%). Effectiveness of salt concentration in mustard greens fermentation was based on value of pH, total acidity (%), lactic acid bactria (cfu/ml), sensory score.

Effect of Fermentation time to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

Mustard greens were fermented with different fermentation time (4, 8, 12, 16 days). Effectiveness of fermentation time in mustard greens fermentation was based on value of pH, total acidity (%), lactic acid Bactria (cfu/ml), sensory score.

Physicochemical, Microbial, Sensory Evaluation

The measurement of pH values were performed using pH meter. The total acidity was determined by titrating 10 ml of pickle extract in 50 ml Erlenmeyer flask using 0.1N NaOH and 1% phenolphthalein as the indicator. The total acidity are expressed as lactic acid (AOAC, 2000). Plate count of lactic acid bacteria were conducted following the method as described by Hadioetomo (1993), 10 ml of fermented fluid were diluted in 90 ml phosphate buffer and 1 ml were then pipetted onto MRS (de Man, Rogosa, and Sharpe)-agar and incubated at 37°C for 2 days before counting the colony formed.

Sensory score was based on 9-point hedonic scale.

Statistical Analysis

Data were statistically summarized by Statgraphics Centurion XVI.

Result and Discussion

Effect of Blanching Temperature and time to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

Blanching is a short time heat treatment widely applied before processing (freezing, frying, drying, and canning) to inactivate deleterious enzymes and to destroy various microorganism present in fresh green vegetables. Commonly hot water blanching technique is applied in the food industries and particularly in the processing of green leafy vegetables [8]. Mustard greens were pre-treated by blanching in water containing 0.1% $CaCl_2$ with different time and temperature (100°C in 5 seconds, 95°C in 10 seconds, 90°C in 15 seconds and 85°C in 20 seconds). Effectiveness of blanching time and temperature in mustard greens fermentation was evaluated on value of pH, total acidity (%), lactic acid bactria (cfu/ml), sensory score.

Results were depicted in Table 1. It's clearly noticed that blanching at 95°C in 10 seconds was optimal for mustard greens fermentation. So we selected this value for next experiments.

Table 1: Blanching temperature and time to physicochemical, microbial and sensory characteristics of pickled mustard green

Blanching temperature and time	pH	Total acidity (%)	Lactic acid bacteria (cfu/ml)	Sensory score
100°C, 5 seconds	3.37±0.01^{ab}	0.90±0.01^{ab}	7.11 x 10^6±0.02^{ab}	5.21±0.01^{ab}
95°C, 10 seconds	3.34±0.02^{b}	0.93±0.03^{a}	7.45 x 10^6±0.01^{a}	6.13±0.02^{a}
90°C, 15 seconds	3.40±0.02^{ab}	0.86±0.00^{b}	6.94 x 10^6±0.03^{b}	5.44±0.03^{ab}
85°C, 20 seconds	3.42±0.01^{a}	0.81±0.03^{c}	6.35 x 10^6±0.01^{c}	4.30±0.02^{b}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

The impact of microwave pretreatment on the thermal degradation of color (chlorophylls) in mustard greens was studied. The microwave pretreatment of mustard greens remarkably influenced the retention of chlorophylls in the final dehydrated powder [8].The increase of treatment time caused a vitamin C decrease in samples blanched by boiling water and steam [9].

Effect of salt Concentration in Fermentation to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

In the pickling industry, salt has historically been used for directing the fermentation of cucumbers, radishes, and carrots [10, 13]. Sodium chloride is an essential in food as it improves the preservative, technological and sensory quality of food [14]. NaCl is one of the most commonly employed agents for food conservation, allowing considerable increase in storage time by reducing water activity [15]. Mustard greens were fermented with different salt concentration (2%, 4%, 6%, 8%).

Effectiveness of salt concentration in mustard greens fermentation was based on value of pH, total acidity (%), lactic acid bactria (cfu/ml), sensory score. Results were depicted in Table 2. It's clearly noticed that 6% salt was optimal for mustard greens fermentation. So we selected this value for next experiments.

Table 2: Salt concentration (%) to physicochemical, microbial and sensory characteristics of pickled mustard green

Salt concentration (%)	pH	Total acidity (%)	Lactic acid bacteria (cfu/ml)	Sensory score
2	3.34±0.02^{b}	0.93±0.03^{a}	7.45 x 10^6±0.01^{a}	6.13±0.02^{c}
4	3.42±0.01^{ab}	0.92±0.01^{ab}	7.29 x 10^6±0.02^{ab}	7.01±0.01^{b}
6	4.11±0.03^{ab}	0.90±0.02^{ab}	6.85 x 10^6±0.01^{ab}	7.65±0.00^{a}
8	4.35±0.00^{b}	0.87±0.01^{b}	6.17 x 10^6±0.03^{b}	7.11±0.02^{ab}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

The physicochemical characteristics of dry-salted (2% Sodium Chloride [NaCl]) fermented carrots, daikon radish, red cabbage, carrot + daikon radish (mix I), and red cabbage + daikon radish (mix II) were examined during a 14-day fermentation at

room temperature. During the fermentation process, pH of fermented carrot, daikon radish, red cabbage, and mix I and mix II decreased significantly ($p < 0.05$) to 3.99±0.04, 4.17±0.05, 3.76±0.11, 3.74±0.18, and 3.70±0.05, respectively, at the end of fermentation (10 days for carrot and 14 days for other vegetables). Titratable acidity (% as lactic acid) in fermented carrot, daikon radish, red cabbage, and mix I and mix II increased throughout fermentation, and final fermentation day acidity values were 1.39±0.12, 0.78±0.02, 1.54±0.09, 1.2±0.06, and 1.50±0.07%, respectively [16].

Effect Fermentation Time to Physicochemical, Microbial and Sensory Characteristics of Pickled Mustard Green

Naturally occurred lactic acid bacteria from the raw ingredients play an important role in fermentation of pickled mustard green [7]. Mustard greens were fermented with different fermentation time (4, 8, 12, 16 days). Effectiveness of fermentation time in mustard greens fermentation was based on value of pH, total acidity (%), lactic acid Bactria (cfu/ml), sensory score. Results were depicted in table 3. It's clearly noticed that 12 days of fermentation was optimal for mustard greens fermentation. So we selected this value for application.

Table 3: Fermentation time (days) to physicochemical, microbial and sensory characteristics of pickled mustard greens

Fermentation time (days)	pH	Total acidity (%)	Lactic acid bacteria (cfu/ml)	Sensory score
4	$4.11±0.03^{a}$	$0.90±0.02^{b}$	$6.85 \times 10^{6}±0.01^{b}$	$7.65±0.00^{b}$
8	$3.88±0.02^{ab}$	$0.92±0.00^{ab}$	$7.36 \times 10^{6}±0.03^{ab}$	$7.89±0.03^{ab}$
12	$3.35±0.01^{c}$	$0.95±0.00^{a}$	$8.24 \times 10^{6}±0.02^{a}$	$8.25±0.01^{a}$
16	$3.70±0.02^{b}$	$0.83±0.03^{c}$	$6.57 \times 10^{6}±0.00^{c}$	$7.11±0.01^{c}$
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$)				

A study aims to study the lactic acid bacteria (LAB) diversity and their changes during fermentation of the sour pickled mustard green, both in natural fermentation and in starter culture-added fermentation. The control batch without starter culture showed sign of spoilage indicated by increase in pH from 3.33 to 4.44 at day 7 while in the starter culture-added batches the pH remained low throughout the fermentation period.

Analysis of the bacterial diversity revealed succession of the LAB species from *Weissella* spp. to *Lactobacillus plantarum* in naturally fermented batch and from *Weissella* spp. to *Lactobacillus fermentum* in starter culture-added batches [7]. Lactic acid bacteria isolated from fermented mustard greens are potential candidates for supplement to induce immunopotentiating activities [17].

Conclusion

Mustard greens *(Brassica juncea*) is a vegetable with good nutritional attributes but has short shelf-life under the prevailing weather conditions in tropical countries. Mustard greens with their high composition of fermentable reducing sugars such as glucose, sucrose and fructose could serve as substrates for lactic fermentation thus transforming a perishable products to more stable and value added product. Pickled mustard green is one of the most consumed fermented vegetable in Vietnam. Therefore, production of pickle from this vegetable can help increase added values and reduce post-harvest losses.

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