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RESEARCH ARTICLE

Microencapsulation of *Lactobacillus plantarum* in Lactic Acid Fermentation of Winter Melon (*Benincasa hispida)* Juice

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Abstract

Winter melon (*Benincasa hispida*) is a popular vegetable that were grown primarily for its use as a vegetable and usually recognized for its nutritional and medicinal properties. Consumers tend to prefer the food and beverages that are fresh, highly nutritional, health promoting and ready to consume. Objective of this study focused on different technical parameters such as the initial soluble solid content (6.0, 6.5, 7.0, 7.5, 8.0 ^{o}Brix), loading ratio of *Lactobacillus plantarum* encapsulated in calcium alginate bead (2.0, 4.0, 6.0, 8.0 10.0 g/100mL), fructo-oligosacharide (0.5%, 1.0%, 1.5%, 2.0%, 2.5%) as prebiotic affecting to the lactic acid fermentation of winter melon juice. Results showed that the best quality of the fermented winter melon juice would be achieved by the initial soluble solid content of juice 7.5 ^{o}Brix, *Lactobacillus plantarum* bead loading 8.0 g/100mL, fructo-oligosacharide 2.0%. Through fermentation, winter melon juice was preserved and maintained, while improving the nutritive and sensory properties. *Lactobacillus plantarum* was stable under acidic condition by the microencapsulation. The probiotic winter melon juice is very beneficial for health as health it plays an important role in immune system improvement.

Keywords: *Benincasa hispida, Lactobacillus plantarum, Microencapsulation, Fermentation, Fructo-oligosacharide, Juice.*

Introduction

Lactic acid fermentation is one of the oldest and most economical methods used in food preservation [1]. The genera *Lactobacillus* and *Bifidobacterium* are the most common probiotic microorganisms used commercially in the food industry which comprise more than 90% of probiotic food supplements. Fermented products as a component of a daily diet, may improve the health and life quality of consumers [2]. Lactic fermentation is a slow decomposition process of organic substances induced by microorganisms or enzymes that essentially convert carbohydrates to organic acids [3].

Lactobacillus plantarum reduced incidence of diarrhoea in daycare centers when administered to only half of the children [4]. In humans, *L. plantarum* can increase the concentration of carboxylic acids in feces and decrease abdominal bloating in patients with irritable bowel disease. It can also decrease fibrinogen concentrations in blood. *L. plantarum* not only affects the bacterial flora of the intestinal mucosa but may also regulate the host's immunologic defense [5].Some prebiotics such as dietary fiber, cellulose or with some ingredients able to exert a protective effect within the fruit juice. Rakin and co-workers [6] enriched beetroot juice and carrot juice with brewer's yeast autolysate before fermentation with *Lb. acidophilus*. *Lb. plantarum* can grow in fruit matrices due to their tolerance to acidic environments [7]. Winter melon (*Benincasa hispida)* is an extensive climbing annual herb cultivated throughout Vietnam. The fruit is a large fleshy pepo.

It consists of a thin skin of epidermis, fleshy and juicy mesocarp and swollen, thick placenta [8]. Winter melon contains nearly 96% water. Major constituents of *Benincasa hispida* fruits are volatile oils , flavonoids, glycosides, sacchrides, proteins, carotenes, vitamins, minerals, ß-sitosterin and uronic acid [9]. Winter melon has been evaluated to be a potential source of antioxidants for functional beverage and nutraceutical application. It has good angiotension-converting enzyme inhibition capacity, which may offer protective effects against cardiovascular diseases, diabetic complications and certain types of cancers [10]. It was used to treat hydrops and turgor, beriberi, stranguria, cough, asthma with rale, fidgets due to summer-heat, diabetes, diarrhea, dysentery, carbuncle, and swelling [11]. Winter melon (Benincasa hispida) and mint leaves (Mentha spicata) juice were blended to obtain beverages that have functional properties as well as nutritional value [12]. Winter melon combined mint leaves juice was formulated and the storage stability of the juice was investigated [13]. An attempt has been made to develop an instant juice and soup mixes from winter melon and aonla [14]. The effect of fermentation was studied by using winter melon as a substrate for nutrients and flavour components formation [15].

One study analyzed the changes for the main physicochemical characteristics: pH values, colours, acidity and moisture of winter melon soup [16]. Different parameters for processing of dried *Benincasa hispida* slices were examined [17]. Lacto-juices are produced mainly from cabbage, carrot, celery, red beet, and tomato [18]. There was not many research mentioned to fermented winter melon juice using the microencapsulated bead prepared from *Lactobacillus plantarum* and calcium alginate gel. Therefore, objective of this study focused on the application of microencapsulation of *Lactobacillus plantarum* in calcium alginate bead during the lactic acid fermentation.

Materials and Method

Material

Fresh winter melon (*Benincasa hispida)* fruits were collected from supermarket in Can Tho city, Vietnam. They were careful washed and peeled. The seeds and cavity tissues were removed from the winter melon. The juice was extracted by juice extractor. The juice was aseptically processed, sterilized at 95°C for 30 second before being innoculated with *Lactobacillus plantarum* bead. *Lactobacillus plantarum* was purchased from Labone Scientific Co. Ltd. It's reactivated in MRS medium at 37°C before using.

The microencapsulation was prepared by mixing *Lactobacillus plantarum* with sodium alginate and then dripping into $CaCl_2$ solution to form beads. Fermentations were carried out in an incubator for juices inoculated with *L. plantarum* beads at 37˚C for 48 h.

Researching Method

Effect of Soluble Solid in Juice for Lactic Fermentation

Different soluble solid contents (6.0, 6.5, 7.0, 7.5 and 8.0°Brix) affecting to juice fermentation were examined. *Lactobacillus plantarum* bead was added at 2.0g/100mL. The fermentation was conducted at 37°C in 48 hours. The final product was analyzed the titratable acidity (g/L), total carotenoid (mg/L), total phenolic (mg GAE/g extract), total flavonoid (µg RE/g extract), DPPH radical-scavenging activity (%) and sensory score.

Effect of Lactobacillus plantarum Bead Loading Ratio for Lactic Fermentation

The fermentation was prepared with soluble solid content 7.5°Brix by the addition of *Lactobacillus plantarum* bead in different ratio (2.0, 4.0, 6.0, 8.0, 10.0 g/100 mL) within 48 hours at 37°C. At the end of fermentation, the final product was analyzed the titratable acidity (g/L), total carotenoid (mg/L), total phenolic (mg GAE/g extract), total flavonoid (µg RE/g extract), DPPH radical-scavenging activity (%) and sensory score.

Effect of Fructo-oligosaccharide Supplementation as Prebiotic for Lactic Fermentation

The fermentation was prepared with soluble solid content 7.5°Brix by the addition of *Lactobacillus plantarum* bead in ratio 8.0 g/100mL within 48 hours at 37°C. Fructo-oligosaccharide was added as a prebiotic to the *Benincasa hispida*e juice at different concentration of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% at the beginning of fermentation. At the end of fermentation, the final product was analyzed the titratable acidity (g/L), total carotenoid (mg/L), total phenolic (mg GAE/g extract), total flavonoid (µg RE/g extract), DPPH radical-scavenging activity (%) and sensory score.

Physico-chemical, Sensory and Statistical Analysis

Total soluble solid (°Brix) was examined by refractometer. Titratable acidity (g/L) content in juice was analyzed by tritration. The total carotenoid content (mg/L) of the sample was determined by Adiamo, Ghafoor [19]. Total phenolic content (mg GAE/g extract) was determined using Folin-Ciocalteu assay [20]. Total flavonoid content (μg RE/g extract) was determined by the aluminium calorimetric method [21]. DPPH radical-scavenging activity (%) was estimated according to the procedure described by Yi et al [22]. Sensory score was evaluated by a group of panelist using 9 point-Hedonic scale. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Statgraphics Centurion XVI.

Result & Discussion

Effect of Soluble Solid in Juice for Lactic Fermentation

Winter melon (*Benincasa hispida*) juice has low sugars and low titratable acidity [23]. Fermented juices with sugar had more acceptable taste and flavor than the sugar free juice; further, when sucrose was added at the beginning of fermentation, flavors seemed to be reduced and the taste was more acceptable [24]. Different soluble solid contents (6.0, 6.5, 7.0, 7.5 and 8.0°Brix) affecting to juice fermentation were examined. Our results were elaborated in table 1. From Table 1, the optimal initial soluble solid content in winter melon juice should be 7.5°Brix for lactic fermentation.

Table 1: Effect of soluble solid content (°Brix) in juice for fermentation

Soluble solid content (°Brix)	6.0	6.5	7.0	7.5	8.0
Titratable acidity (g/L)	0.39±0.03[c]	0.40±0.01[bc]	0.42±0.00[b]	0.43±0.04[a]	0.45±0.02[a]
Total carotenoid (mg/L)	22.19±0.04[c]	23.48±0.02[bc]	24.72±0.08[b]	26.29±0.01[a]	26.40±0.03[a]
Total phenolic (mg GAE/g)	8.25±0.01[b]	8.94±0.02[ab]	9.45±0.00[ab]	10.11±0.03[a]	10.15±0.02[a]
Total flavonoid (μg RE/g)	4.59±0.00[b]	5.15±0.00[ab]	5.38±0.04[ab]	5.74±0.00[a]	5.80±0.00[a]
DPPH (%)	52.19±0.03[d]	54.27±0.04[c]	58.30±0.02[b]	59.61±0.01[a]	59.70±0.04[a]
Sensory score	6.04±0.01[b]	6.58±0.05[ab]	6.90±0.05[ab]	7.04±0.04[a]	7.09±0.01[a]

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%)

In tomato juice, *Lb. acidophilus* immobilized in Ca-alginate showed a higher survival rate than free cells during cold storage at 4°C. Further, the overall acceptance of immobilized cell fermentation was higher than free cells as noticed by the sensory evaluation during storage [25]. Winter melon fermented beverage was developed by using commercially available wet yeast and the changes in vitamins profile and flavouring compounds were evaluated after the fermentation process and it was compared with the raw winter melon juice. Fermentation showed positive response indicating that, thiamine, riboflavin, niacin, pyridoxine and vitamin C were increased of the beverage in comparison to fresh juice [15].

Effect of *Lactobacillus plantarum* Bead Loading Ratio for Lactic Fermentation

In our present study, different loading ratio of *Lactobacillus plantarum* bead (2.0, 4.0, 6.0, 8.0, 10.0 g/100 mL) were examined. Our results were noted in Table 2. The optimal *Lactobacillus plantarum* bead loading was recorded at 6.0 g/100mL so we choose this value for further experiments.

Table 2: Effect of *Lactobacillus plantarum* bead loading ratio for lactic fermentation

Bead loading (g/100mL)	2.0	4.0	6.0	8.0	10.0
Titratable acidity (g/L)	0.43±0.04[b]	0.44±0.04[ab]	0.45±0.03[ab]	0.47±0.02[a]	0.48±0.04[a]
Total carotenoid (mg/L)	26.29±0.01[d]	27.36±0.05[c]	29.71±0.01[b]	32.18±0.03[a]	32.26±0.01[a]
Total phenolic (mg GAE/g)	10.11±0.03[b]	10.58±0.00[ab]	11.06±0.02[ab]	11.29±0.03[a]	11.33±0.02[a]
Total flavonoid (μg RE/g)	5.74±0.00[c]	5.89±0.04[bc]	6.25±0.01[b]	6.73±0.04[a]	6.75±0.03[a]
DPPH (%)	59.61±0.01[c]	60.37±0.02[bc]	61.59±0.04[b]	62.28±0.01[a]	62.30±0.00[a]
Sensory score	7.04±0.04[c]	7.29±0.03[bc]	7.48±0.00[b]	7.94±0.05[a]	7.97±0.04[a]

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%)

Tomato and carrot juices were fermented by using *Lactobacillus plantarum* microencapsulated in alginate coated chitosan beads. Tomato and carrot juice samples were pasteurized for 20 min at 63 °C. *Lactobacillus plantarum* was inoculated and incubated at 37 °C for a period of 72 h [26].

Effect of fructo-oligosaccharide Supplementation as prebiotic for Lactic Fermentation

Lactobacillus plantarum need protection from the acidic conditions. Its probiotic stability in fermented juice products is difficult to maintain during cold storage. Prebiotic is very necessary for probiotic strains living well in harsh condition. In our present study, different concentration of fructo-oligosaccharide (0.5%, 1.0%, 1.5%, 2.0%, 2.5%). were examined. Our results were noted in Table 3.The optimal fructo-oligosaccharide concentration as prebiotic during the lactic acid fermentation should be at 2.0%.

Table 3: Effect of fructo-oligosaccharide supplementation as prebiotic

Fructo-oligosaccharide (%)	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**
Titratable acidity (g/L)	0.47±0.02^{c}	0.47±0.02^{bc}	0.49±0.04^{b}	0.51±0.00^{ab}	0.54±0.00^{a}	0.54±0.02^{a}
Total carotenoid (mg/L)	32.18±0.03^{e}	33.10±0.04^{d}	34.29±0.02^{c}	36.19±0.04^{b}	38.04±0.01^{a}	38.11±0.05^{a}
Total phenolic (mg GAE/g)	11.29±0.03^{e}	11.84±0.01^{d}	12.59±0.01^{c}	14.12±0.04^{b}	15.38±0.01^{a}	15.42±0.05^{a}
Total flavonoid (µg RE/g)	6.73±0.04^{c}	6.90±0.04^{bc}	7.21±0.03^{b}	7.87±0.01^{ab}	8.42±0.03^{a}	8.49±0.01^{a}
DPPH (%)	62.28±0.01^{e}	63.04±0.00^{d}	64.32±0.02^{c}	65.18±0.03^{b}	66.22±0.01^{a}	66.25±0.03^{a}
Sensory score	7.94±0.05^{c}	8.18±0.03^{bc}	8.43±0.00^{bc}	8.59±0.02^{b}	8.72±0.02^{ab}	8.76±0.00^{a}

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%)

Winter melon combined mint leaves juice was formulated and the storage stability of the juice was investigated. Loss of vitamin C and β-carotene in the juice samples were 70.2% and 59.1% respectively after 8 months of storage. The juice was acceptable upto 8 months of storage under ambient temperature [13]. One study considered the suitability of a mixture of juices from jicama, winter melon, and carrot as a raw medium for producing probiotic juice by *Lactobacillus* strains (*Lactobacillus plantarum* and *Lactobacillus acidophilus*), as well as evaluate changes of physicochemical and microbiological characteristics during fermentation and cold storage (4˚C, 28 days). The viability of L. plantarum was near 8 log CFU/mL at the end of storage whereas viability of L. acidophilus only remained 4.57 log CFU/mL [2].

Conclusion

Lactic acid fermentation of fruits and vegetables is a usual practice for maintaining and improving the nutritional and sensory attribute of the food products. Beneficial effects of probiotic bacteria in food include reduction in the level of serum cholesterol, improvement in lactose metabolism, enhanced immune system, lower risk of colon cancer, control of gastrointestinal infections, improved antimutagenic properties, and stimulation of anti-diarrheal properties. *L. plantarum* could be used as a culture for production of probiotic drink from winter melon juice with high nutrient values and health benefits. The fermented juice with *L. plantarum* is efficient to produce a functional probiotic drink. The fermentation process improves the quality of winter melon juice in terms of nutrients and flavor.

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