Antioxidative properties and *Lactobacillus* population in traditional Sri Lankan pickle during fermentation

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Introduction

In recent years, probiotic products have become a primary choice for the consumers because of their health attributes (Yen-Ping Tan, 2007). Pickle may be a suitable product of carrying probiotic bacterial strains to the host. Pickling is a method of fermentation of vegetables to extend their shelf life. That process consists with the basis of reducing the pH value due to conversion of sugar to acid by lactic acid bacteria (LAB). LAB are greatly important for human health as they help balance human intestinal flora, thereby boosting overall immunity (Amit et al., 2012). Antioxidant compounds in fermented foods play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. There is no reported study done on evaluating the nutritional properties of Sri Lankan vegetable pickles fermented with coconut vinegar. The main objective of this study was to evaluate the population growth of *Lactobacillus*, pH, total phenols and antioxidant activity during fermentation of Sinhala pickle.

Materials and Methods

Sample preparation, determination of pH and *Lactobacillus* population

The samples for the isolation of LAB included the fermented mixed vegetables which was prepared by mixing thoroughly, cleaned and diced vegetables (carrot, red onion, raw papaya, green chili) in coconut vinegar solution, and was allowed to ferment at ambient temperature (32 C±2). The microbial spread plate method was used to isolation of LAB in the MRS media from the pickle sample. The pH of the withdrawn aliquots at every 24h during the fermentation was monitored using a digital pH meter (HI 2211, USA) at 32 ◌ (Shori *et al*., 2011).

Determination of Antioxidant Activity and Total Phenolic Content

Antioxidant activity (AOA) was measured by DPPH radical scavenging activity method according to the method developed by Adak et al. (2013) with little modifications. Total phenol content (TPC) was measured by Folin-Ciocalteu method with some modifications (Kriengsak *et al.,* 2006).

Results and Discussion

Population of *Lactobacillus* in pickle

Traditionally, the fermentation of Sinhala pickle is processed by the LAB. The results showed that the bacteria population increased from an initial number of 8.0997 0.56 Log_{10} cfu/g after 24 hours fermentation at 37 ^{0}C. The maximum bacteria growth was observed after 48 hours fermentation which had significant difference with other ratios of bacteria except 72 hours bacteria count. After three days storage at 37 ^{0}C, the bacteria population decreased but, no significant difference was observed among other storage time. In contrast, the numbers of LAB were much reduced at the end of storage (Table 1).

Table 01: LAB and functional property variation during the storage at 7 days after fermentation

Time	Log 10cfu/g	pH	AOA	TPC
0 h	-	-	174.67 35.82^{d}	11.7353 0.16^{b}
24 h	8.0997 0.56^{c}	3.51000 0.11^{a}	-	-
48 h	9.4248 0.23^{a}	3.5450 0.08^{a}	205.00 9.90^{cd}	13.1281 0.22^{b}
72 h	9.2322 0.16^{a}	3.59000 0.10^{a}	256.82 4.69^{bc}	15.0893 0.58^{a}
96 h	8.7894 0.17^{b}	3.62000 0.08^{a}	537.64 0.96^{a}	12.3382 0.57^{b}
120 h	8.8253 0.21^{b}	3.69500 0.05^{a}	277.60 28.76^{b}	12.1821 1.03^{b}
168 h	8.6262 0.16^{b}	3.47500 0.91^{a}	-	-

Values followed by the same letters are not significant, and different letters indicate significant difference at $p<0.05$

pH variation during fermentation

The results showed that, pH was almost same during the 7 days of storage, and it consists within the optimal pH level for growth of the LAB. A study revealed that various ratios of the probiotic bacteria did not cause significant difference in pH reduction and increase of lactic acid however, various concentrations of vegetables and spices which used caused a marked difference in pH reduction and lactic acid increasing (Tiktiek et al., 2013).

Determination of Antioxidant Activity variation

The statistical results showed that there was a significant difference in DPPH radical scavenging activity during fermentation. The lower the IC_{50} value, the greater is the AOA. Highest antioxidant activity/ the lowest IC_{50} value was recorded in 0 hours extraction. The least activity was recorded in 96 hours after preparation. Difference in scavenging activities might be due to the changes in the phenolic compounds during fermentation. Previous studies have reported that strictly controlled fermentation by some isolated strains of lactic bacteria resulted in no change in the AO potency of final sauerkraut compared to fresh vegetable (Tolonen *et al.*, 2004).

Total Phenolic Content

As the results were shown fresh pickle had TPC of 11.7353 0.16 mg/ml while fermented pickle form had 15.0893 0.58. However, TPC in the pickled form is still considered high as compared to TPC of other fruits in fresh form, obtained from the previous research (Lako *et al.*, 2007). Sample preparation methods and, ingredients which used to prepare the pickle may also influence the results.

Previous studies have demonstrated the strong relationship between TPC and AOA found in different varieties of fermented foods (Kriengsak *et al.,* 2006). However in this study, there was no clear correlation observed.

Conclusions

The present study demonstrated that after second and third day of preparation LAB, and TPC reached to their optimum value and after that they started declining. pH value remained almost constant and fermentation retained 90-95% AO capacity during the 7day of storage at 37 C. With the findings it could be concluded that maximum health benefits could be obtained after second of preparation, and after third day all the properties were started to declining.

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