

## Application of betel leaves (*Piper betle* L.) extract for preservation of homemade chili bo

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### Article history

Received: 13 March 2014

Received in revised form:

18 April 2014

Accepted: 22 April 2014

### Keywords

Betel leaves

Chili bo

Aerobic microbial count

Yeast and Mold

Natural preservatives

### Abstract

This study is conducted to investigate the effect of different concentrations of betel leaves extract on color, pH and microbiological in homemade chili bo. The homemade chili bo with different concentrations (0 mg/ml, 0.75 mg/ml, 1.25 mg/ml and 1.75 mg/ml) of betel leaves extract were prepared for analysis. The results showed that the color of chili bo became darker as the concentration of betel leaves extract increased. The extract showed significant in the pH of chili bo after 7 days in which the highest concentration of extract showed the highest value of pH 4.31. The aerobic microbial count was decreased as the concentration of betel leaves extract increased in chili bo. After 7 days of storage, the highest concentration of betel leaves extract showed the highest percentage of reduction (6%), while the control sample showed 2.41% of aerobic reduction. The study also found that the extract contain lesser yeast and mold count (5.22 log CFU/ml) in homemade chili bo compared to the control sample (5.31 log CFU/ml) after 7 days. Betel leaves extract can be considered as natural food preservatives in chili bo to reduce the growth of spoilage microorganism and thus enhance the shelf life of chili bo.

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### Introduction

Chili slurry or locally called as chili bo is a traditional Malaysian food that made either from fresh or dried chili. It is used as a flavoring agent in Malaysian cuisine to increase the spiciness of food. There are many types of chili bo available in the market but all faced the same problem that is easily spoiled by microorganism. Benzoic acid is usually used as a preservative in commercial chili bo to prevent the microbial growth and thus prolong the shelf life of chili bo. The maximum level of benzoic acid permitted by the Malaysian Food Act 1983 in accordance with Codex Alimentarius is 1000 mg/kg. However, according to the studies of Zaini *et al.* (2010), there are nine out of fifteen brands of chili bo in Malaysian market were found to contain exceeded benzoic acid level (5435 mg/kg) that is permitted by the Malaysian Food Act 1983.

Recently, the use of chemical preservative agents such as benzoic acid has arisen a safety concern among the public. Natural preservatives have great demand for their extensive biological properties and bioactive component which had been proved to be useful against a large number of causative agents of disease. Betel leaves or *Piper betle* L. belongs to the genus *Piper* of the family *Piperaceae* have been proved to contain an essential oil called chavicol which has potential antiseptic properties (Majumdar *et al.*, 2003). *Piper betle* is originated from South and

South East Asia. It is usually cultivated in Malaysia, India, Sri Lanka, Indonesia, Philippine Islands and East Africa (Parmer *et al.*, 1997). *Piper betle* has light yellow aromatic essential oil with sharp burning taste. Asian people recognized that the plant has a health benefit. It has been proved that betel leaves extract showed a wide array of activities such as antibacterial, anti-oxidative and anti-hemolytic (Chakraborty and Shah, 2011).

Suliantari *et al.* (2008) had done the research on antibacterial activity of betel leaves extract toward foodborne pathogens using three type of solvents (water, ethanol and ethyl acetate). They found that ethanol extraction produced the best betel leaves extract with strong antibacterial activities against *E. coli* and *Staphylococcus aureus* as compared to ethyl acetate and water extraction. Analysis of components of betel leaves extract with GC-MS revealed that the ethanol extract of betel leaves contained antibacterial components such as chavicol, eugenol, caryophyllene, cylene and chalorene. Furthermore, Hoque *et al.* (2011) revealed that the ethanol extract of betel leaves potentially inhibit the growth of some foodborne pathogens such as *Vibrio cholerae*, *E. coli*, *E. coli* O157:H7, *Shigella dysenteriae* and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of ethanol extract of betel leaves against the five foodborne pathogens was ranged from 0.625 to 0.75 mg/ml. Many researchers have been reported that the betel leaves contain bioactive components that can

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be used as antiseptic, antimicrobial and antioxidant (Bhide *et al.*, 1991; Chen *et al.*, 1995; Ramji *et al.*, 2002).

This study is aimed to investigate the effect of different concentrations of betel leaves extract on the color, pH and microbiological properties in homemade chili bo. The finding of this study can contribute to develop a newly natural antimicrobial agent or preservative in chili bo as a replacement of chemical preservative such as benzoic acid to inhibit the growth of spoilage microorganism and thus prolong the shelf life of chili bo.

## Materials and Methods

### Plant materials

Fresh leaves of *Piper betle* L. and dried chilies with no apparent physical damage were purchased from Pasar Borong Seri Serdang, Selangor, Malaysia.

### Preparation of sample extracts

The betel leaves were washed with distilled water to remove all contaminants. The samples were then cut into small pieces and dried at 40°C for 24 hours in a smoke oven. The dried samples were ground into fine powder using a dry blender and packed in an airtight container for storage until analysis.

The ground samples were extracted using 95% ethanol at room temperature for 24 hours. The ethanol fraction was separated using sterilized cheesecloth and filtered through a filter paper (Whatman No. 4) (Hoque *et al.*, 2011). The extract was evaporated using a rotary evaporator under vacuum at 60°C to remove the remaining ethanol solvent completely. The extracts were then added into homemade chili bo and analyzed for color, pH and microbiological test.

### Preparation of chili bo

Dried chilies were soaked in hot water, seeded and drained. The dried chilies were then blended with distilled water. Three different concentrations of betel leaves extract (0.75 mg/ml, 1.25 mg/ml and 1.75 mg/ml) were added into the blended chili slurry, mixed well and kept in sterile airtight containers. The blended chili slurry without the addition of betel leaves extract (0 mg/ml) was used as a control sample. All samples were stored at room temperature.

### Physical analysis

#### Determination of color

The color analysis was carried out according to the method described by Claussen *et al.* (2007) using the Hunter lab instrument. The rates of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) of chili bo samples

were measured.

#### Determination of pH

The pH of each chili bo sample was measured using a digital pH meter (Jenway, 3505) followed AOAC method 981.12 (2012).

### Microbiological analysis

#### Aerobic plate count

Each sample (25 g) was diluted in 225 ml of 0.1% sterile peptone water in a stomacher bag and homogenized for 1 min. Serial dilution of  $10^{-1}$  to  $10^{-6}$  were prepared. Total microbiological counts were enumerated on plate count agar which incubated at 35°C for 48 hours under aerobic condition. The suitable colony counting range is 25-250 (AOAC 966.23). The count was considered as too numerous to count (TNTC) when the number of colonies exceeds 250.

#### Yeast and mold count

Each sample (25 g) was diluted in 225 ml of 0.1% sterile peptone water in a stomacher bag and homogenized for 1 min. Serial dilution of  $10^{-1}$  to  $10^{-6}$  were prepared. Total count of yeast and mold were determined on potato dextrose agar by spreading 0.1 ml of diluents on the agar. The agar was incubated at 30°C for 5 days. The suitable colony counting range is 25-250. The count was considered as too numerous to count (TNTC) when the number of colonies exceeds 250.

#### Statistical analysis

All experiment were carried out in duplicate and the data were expressed as means  $\pm$  standard deviation. A one-way analysis of variance (ANOVA) was performed to calculate significant differences between mean values at a significance level of 95%.

## Results and Discussion

### Effect of betel leaves extract on color of chili bo

The present study analyzed the effect of different concentration of betel leaves extracts on the color of homemade chili bo. Table 1 showed that the color of chili bo became darker when the concentration of the betel leaves extract was increased. It was found that the  $L^*$ ,  $a^*$  and  $b^*$  value tended to decrease when the concentration of betel leaves extract was increased. It is observed that the addition of betel leaves extract decreased the lightness, redness and yellowness of the chili bo. Betel leaves extract was dark green color due to the presence of chlorophyll that give the green color of various crops (Rai *et al.*, 2010). The color

Table 1. Effect of different concentration of betel leaves extract on the color of homemade chili bo

Sample (mg/ml)	Color		
	L*	a*	b*
Control	+89.07 <sup>a</sup>	+2.30 <sup>a</sup>	+4.09 <sup>a</sup>
0.75	+82.67 <sup>a</sup>	+1.63 <sup>a</sup>	+2.02 <sup>a</sup>
1.25	+79.91 <sup>a</sup>	+1.71 <sup>a</sup>	+1.46 <sup>a</sup>
1.75	+79.15 <sup>a</sup>	+1.07 <sup>a</sup>	+2.02 <sup>a</sup>

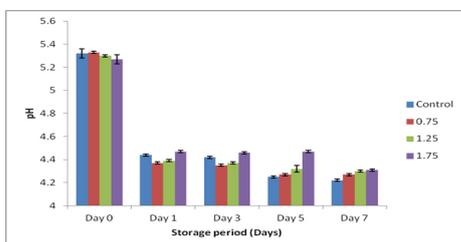


Figure 1. Effect of different concentration of betel leaves extract on pH of chili bo for 7 storage days at room temperature

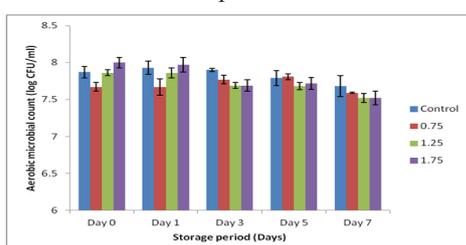


Figure 2. Effect of betel leaves extract concentration on the aerobic microbial counts of chili bo for 7 days of storage

of the extract was largely affected by the presence of chlorophyll content in the betel leaf (Jo *et al.*, 2003). The higher the concentration of betel leaves extract in the chili bo, the greater the chlorophyll contents in the chili bo, and thus the darker the color of the chili bo.

#### Effect of betel leaves extract on pH of chili bo

The effect of different concentrations of betel leaves extract on pH of chili bo for 7 days of storage were shown in Figure 1. The pH of all samples was found to be decreased with increasing in the storage time. The pH of the control sample is more acidic than the chili bo that contain betel leaves extract. It is found that the betel leaves extract can decrease the acidity of the chili bo. The higher the concentration of betel leaves extract in the chili bo, the less acidic the chili bo. The variation in the acidity level among the chili bo might be due to the variation in the concentration of acetic acid and lactic acid production by bacteria during storage (Danner *et al.*, 2003).

#### Aerobic microbial count

Aerobic microbial count was conducted to investigate the effect of different concentrations of betel leaves extract on chili bo for 7 days of storage. From the Figure 2, it is found that the addition of betel leaves extract (1.25 mg/ml and 1.75 mg/ml) will decrease the aerobic microbial count in the chili

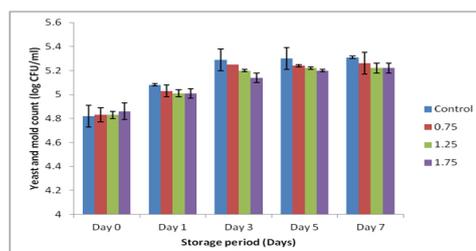


Figure 3. Effect of different concentrations of betel leaves extract on the yeast and mold count of chili bo for 7 days of storage

bo after 7 days of storage. The microbial growth was related to the pH. In general, most microorganisms do not grow at pH values below 4.6, but grow best at pH values around 7.0 (Jay, 1995). The results showed that the aerobic microbial growth decreases as the pH of the sample decreases.

The sample with the highest concentration of betel leaves extract (1.75 mg/ml) reduced the aerobic microbial by 6% from day 0 until day 7, while the control sample which contain 0 mg/ml of betel leaves extract showed 2.41% of the aerobic microbial count reduction. This finding suggests that the higher concentration of betel leaves extract may improve the effectiveness in reduction of aerobic microbial count in chili bo for 7 storage days. Betel leaves contain fatty acids, hydroxyl fatty acids, esters and hydroxyl chavicol which are reported to exhibit antimicrobial activity (Pauli, 2002). In the study of Arambewela *et al.* (2005), it is found that the essential oil from the betel leaves was active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Streptococcus pyrogens*. Hoque *et al.* (2011) also stated that the betel leaves extract which obtained by ethanol extraction has the potential to inhibit the growth of foodborne pathogen. So, it can be said that the betel leaves extract exhibit great antimicrobial activity in chili bo.

#### Yeast and mold counts

Figure 3 shows the effect of different concentrations of betel leaves extract on the yeast and mold count in homemade chili bo. The present study showed that each sample with different concentrations of betel leaves extract did not show any reduction in yeast and mold count during the storage periods even though the pH value is low. This is due to the fact that yeast and mold are relatively versatile to broad acid/alkaline requirement for growth which ranging from pH 2 to above pH 9 (Bandler *et al.*, 1995). Yeast and mold are predominating at low pH values of less than 3.5 (Jay, 1995).

The results showed that the sample with the highest concentration of betel leaves extract (1.75

mg/ml) possessed the lowest yeast and mold load (5.22 log CFU/ml) as compared to the control sample (5.31 log CFU/ml). Furthermore, the percentage of increasing yeast and mold count in chili bo was the least in the sample with the highest concentration of betel leaves extract (1.75 mg/ml) that was 7.41%. The percentage of increasing yeast and mold count in control sample (0 mg/ml), 0.75 mg/ml and 1.25 mg/ml of betel leaves extract in chili bo were 10.17%, 8.90% and 8.07%, respectively. This indicated that the betel leaves extract can delay slightly the growth of yeast and mold in chili bo. Arambewela *et al.* (2005) reported that the ethanol extract of betel leaves contain at least one fungicidal compound that possessed antifungal activity against *Cladosporium* sp.

## Conclusions

Betel leaves extract showed antibacterial activity against aerobic microbial and fungal in the homemade chili bo. The highest concentration of betel leaves extract (1.75 mg/ml) showed the highest percentage of reduction in aerobic microbial (6%) and delayed the growth of yeast and mold (7.41%) in chili bo. Hence, betel leaves extract with the concentration of 1.75 mg/ml has potential for application in chili bo as natural antimicrobial agent or preservative to inhibit the growth of spoilage microorganisms and prolong the shelf life of the product. Several researchers had reported that irradiation technology would improve the color of the extracts of green tea, persimmon leaf and licorice root from dark color to brighter color without any adverse change of physiological functions (Son *et al.*, 2001; Jo *et al.*, 2003). Therefore, it is recommended to conduct irradiation technology on the betel leaves extract in the future research in order to improve the color of betel leaves extract.

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