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Effect of Vietnamese coriander *(Persicaria odorata)*, turmeric *(Curcuma longa)* and *asam* gelugor *(Garcinia atroviridis)* leaf on the microbiological quality of *gulai tempoyak* paste

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Abstract

The objective of this study was to determine microbiological quality of *gulai tempoyak* paste (GTP) added with three different leaf; Vietnamese coriander, turmeric and *asam gelugor*. The GTP was cooked for 10 minutes with control temperature (60-70°C) and the leaf were added at 2, 5 and 8 minutes during the cooking time to give exposure times of 8, 5 and 2 minutes of the leaf to GTP. GTP without addition of leaf was treated as control and all the prepared GTPs were stored at 30°C for 2 days before analysed using total plate count (TPC) and yeast and mould count (YMC). The addition of *asam gelugor* leaf to GTP for 5 minutes of the cooking period significantly ($p > 0.05$) reduced TPC ($\log_{10}$ 3.54 CFU/g) compared to Vietnamese coriander ($\log_{10}$ 4.67 CFU/g) and turmeric leaf ($\log_{10}$ 4.70 CFU/g). *Asam gelugor* leaf also showed a significant difference in TPC reduction ($\log_{10}$ 4.44 CFU/g) when added to GTP for 8 minutes compared to Vietnamese coriander ($\log_{10}$ 5.10 CFU/g), but was insignificant to turmeric leaf ($\log_{10}$ 4.71 CFU/g). In conclusion, there are significant effects on microbiological quality of GTP when added with Vietnamese coriander, turmeric and *asam gelugor* leaf at different exposure time based on TPC and YMC.

Introduction

Cooking practices by the Malays frequently requires additions of herbs for several purposes. Herbs are known of their good benefits and healing properties. The abundance of biologically active compounds in these plants, containing ascorbic acid, carotenoids, flavonoids, polyphenols, alkaloids, and polysaccharides, have been well identified for their pharmacological properties such as antioxidant, antimicrobial, antifungal, anti-diabetic, anti-inflammatory, sedative, hypotensive, anti-stress, and anticancer activities (Mauri *et al.*, 1998). Antimicrobial compounds present in foods can improve the microbiological quality of unprocessed or processed foods by reducing microbial growth rate or viability (Beuchat and Golden, 1989). Originally added to change or improve taste, spices and herbs can also enhance shelf -life because of their antimicrobial nature.

Gulai tempoyak is traditional dish that use fermented durian known as 'tempoyak' and mixed together with fresh turmeric, chilies, water, salt and some herbs Vietnamese coriander *(Persicaria odorata)* cooked with freshwater fish. *Gulai tempoyak* is a traditional dish that is usually prepared from scratch but nowadays ready-made pastes are becoming more popular due to busy lifestyles. Consumer demand plays a major role in the modification of our food supply and their demand is currently driven towards foods that are ''natural'' and free of additives, but still safe and convenient to use (Rhodehamel, 1992). The search for more natural antimicrobials have led food scientists to investigate the effectiveness of inhibitory compounds to be apply in processed food such as organic acids, essential oils, bacteriocins, and dried fermentation-based products and also bioactive

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compounds (Lemay *et al*., 2002).

There are many studies that have done on the antimicrobial properties of three leaf used in this study. Antimicrobial activity found in Vietnamese coriander has been studied and reviewed (Jamal *et al*., 2011; Qader *et al*., 2012; Ridzuan *et al*., 2013) whereas Kang-Ju *et al*. (2005); Abd Aziz *et al.* (2011); Sindhu *et al.* (2011) have revealed the antimicrobial compounds in turmeric leaf and Mackeen *et al.* (2000); Zakaria *et al*. (2011) proved that *asam gelugor* leaf have antimicrobial activity. Nevertheless, the application of this three leaf in cooking process is does not study yet. GTP that produced contains high moisture and water activity which promotes to microbial growth. With addition of natural antimicrobial from three leaves perhaps will improve the microbiological quality of the product. It is generally proved that some of natural bioactive compounds could be substantially lost during thermal processing or heat treatment (Azevedo-Meleiro and Rodriguez, 2005). However, food processing also can lead to disruption of the food matrix, increasing the inaccessibility of many bioactive compounds and thus improving the nutritional and microbiological quality of food products (Pellegrini *et al*., 2010). In addition, the attention should also pay to the processing and preparation methods in order to preserve the desirable and availability of antimicrobial properties of foods (Gorinstein *et al.*, 2009).

There are no researches done on addition of plant leaf into GTP and its effect on microbiological quality of the product. Previous study shows that lactic acid bacteria are the predominant microorganisms in *tempoyak* and *Lactobacillus plantarum* was the predominant group in lactic acid bacteria flora (Leisner, 2001). However, heat treatment and cooking process will destroy the lactic acid bacteria because it is most heat sensitive microorganisms. Nevertheless, the effect of addition the plant leaves in the cooked GTP at different time is still unknown. The common storage practice of fermented durian paste (tempoyak) at the market is to place at ambient temperature. This present study was carried out to determine the effect of Vietnamese coriander, turmeric and asam gelugor leaf at different exposure time on microbiological quality of GTP.

Materials and Methods

Preparation of GTP

The *gulai tempoyak* paste (GTP) was prepared according to the recipe adapted from official portal of Temerloh Municipal Council *(http://www.mpt.gov.my/)*, as shown Table 1. All the ingredients were grinded in a blender for 2 minutes excluding durian paste *(tempoyak)*. Basic GTP ingredients (*tempoyak*, salt, chilies and fresh turmeric) and fresh Vietnamese coriander *(Persicaria odorata)*, turmeric *(Curcuma longa)* and *asam gelugor (Garcinia atroviridis)* leaf were purchased from local market at Serdang, Selangor for used in this study.

Table 1. *Gulai tempoyak* paste (GTP) recipe

Ingredients	GTP			
	Control		[1]Added with plant leaf	
	Quantity	Percentage (%)	Quantity	Percentage (%)
Tempoyak	100 g	43.1	100 g	41.3
Fresh Turmeric	20 g	8.6	20 g	8.3
Chillies	10 g	4.3	10 g	4.1
Salt	2 g	0.9	2 g	0.9
Water	100 ml	43.1	100 ml	41.3
[1]Plant leaf	Not Added		10 g	4.1

Note: 1 Plant leaf (Vietnamese coriander, turmeric and *asam gelugor*)

The GTPs were cooked over flame with control temperature 60-70°C and cooked for 10 minutes to reduced ¾ of initial weight or more than 50% weight loss. The leaves were added at 2, 5 and 8 minutes during the cooking time to give exposure times of 8, 5 and 2 minutes of the leaf to GTP. The exposure time in this study was defined as the time of the plant leaf exposed to the cooking period (within 10 minutes). GTP without addition of leaf was treated as control. The GTPs were then placed in air tight container, labelled accordingly and stored at 30°C and analysed using standard method for total plate count (TPC) and yeast and mould count (YMC) after 2 days storage.

Microbiological analyses

In this study, 25 g of the GTPs were placed into stomacher bag and added with 225 ml of diluent buffer peptone water. The sample was mixed and blended in the stomacher for 2 minutes. The homogenate was mixed thoroughly. 1 ml of each sample was pipetted into a universal tube containing 9 ml of buffered peptone water and mixed gently. The spread plate method was used in which a sample dilution (10^{-3}-10-6) was taken by pipetting 0.1 ml of sample homogenate onto plate count agar (Merck, Germany) and potato dextrose agar (Merck, Germany) to determine the total plate count (TPC) and yeast and mould count (YMC), respectively. Each test was performed in duplicate and repeated twice. The results are expressed as $\log_{10}$ CFU/g for all samples.

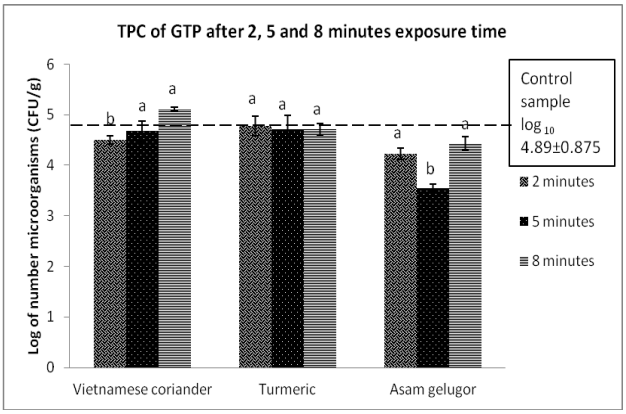
Statistical analysis

The present study was designed using completely randomized design (CRD) as an experimental design. Sampling was done independently and all the analyses were done in duplicate with two readings for each replication. All the data collected were analysed using one-way analysis of variance (ANOVA) and the significant difference at ($P < 0.05$) between treatments were determined using simple t-test. The data obtained were present as mean ± standard deviation and the data analyses were performed using Minitab 16 software. All graphs were performed using Microsoft Excel 2010.

Results and Discussion

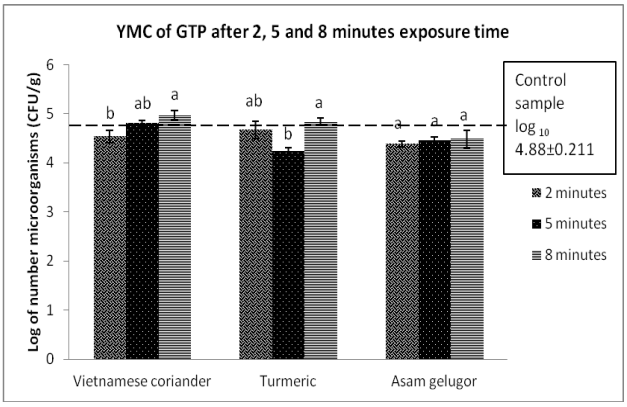
Effect the addition of plant leaf in GTP

The effect of the addition of plant leaf in GTP on TPC is shown in Figure 1. Vietnamese coriander leaf showed a significant reduction of TPC (log_{10} 4.50 CFU/g) when added to GTP for 2 minutes of the cooking time, while asam gelugor leaf reduced TPC (log_{10} 3.54 CFU/g) significantly when added for 5 minutes to GTP cooking time. No significant difference was observed in TPC reduction when turmeric leaf were added to GTP for 2, 5 and minutes of the cooking time. As shown in Figure 2, YMC (log10 4.54 CFU/g) decreased slightly when Vietnamese coriander leaf was added for 2 minutes of the GTP cooking time, while turmeric leaf slightly reduced YMC (log_{10} 4.23 CFU/g) when added for 5 minutes of the GTP cooking time. No significant difference was observed in YMC reduction when asam gelugor leaf was added to GTP for 2, 5 and minutes of the cooking time. The standard microbial load recommended by the International Commission on Microbiological Specification for Food (ICMSF) is $<10^6$ CFU/g per wet weight. Based on previous study, *asam gelugor (Garcinia atroviridis)* leaf have shown to have antibacterial activity compared to turmeric *(Curcuma longa)* and Vietnamese coriander *(Persicaria odorata)* leaf. The antibacterial activity of asam gelugor leaf was strong due to the presence of xanthones and related metabolites that have been implicated for the potent antibacterial activity (Mackeen *et al.*, 2000). *Asam gelugor* (fruits and leaf) also demonstrated various in vitro physiological functions against bacteria while non-toxic effects were reported (Mackeen *et al.*, 2000). Abd Aziz *et al*. (2011) reported that turmeric leaf ethanolic extract exhibited no inhibitory effect on tested bacteria and no previous study has been found on the antimicrobial properties of this plant. Moreover,



Values are presented as mean ± standard deviation. The values of samples followed by same letter (a-b) are not significantly different ($p > 0.05$)

Figure 1. TPC for exposure time of plant leaf in GTP



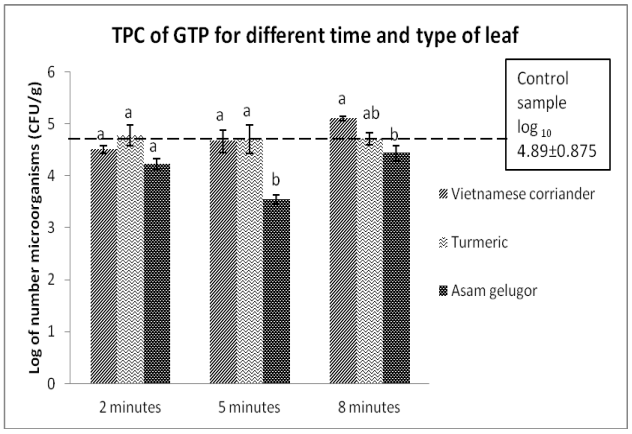
Values are presented as mean ± standard deviation. The values of samples followed by same letter (a-b) are not significantly different ($p > 0.05$)

Figure 2. YMC for exposure time of plant leaf in GTP

the addition of Vietnamese coriander leaf in the GTP has shown slightly lower than control sample for both TPC and YMC. Therefore, this finding could be supported by study conducted by Faridah (2008) where the addition of Vietnamese coriander leaves in refrigerated duck meatballs has shown slightly more effective in preventing microbial growth. Vietnamese coriander leaf could be an alternative natural food preservative to control the microbial growth of GTP since they contain active compounds (aldehydes and terpenes). These compounds have been proven to have strong antimicrobial activity against spoilage microorganisms (Sasangko *et al.*, 2012).

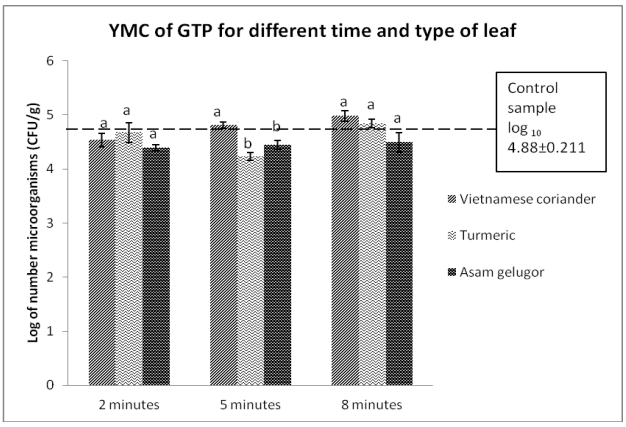
The effect of 2, 5 and 8 minutes exposure of plant leaf during GTP cooking time on microbiological quality

The addition of asam gelugor leaf to GTP for 5 minutes of the cooking period significantly ($p > 0.05$) reduced TPC (log_{10} 3.54 CFU/g) compared to Vietnamese coriander (log_{10} 4.67 CFU/g) and turmeric leaf (log_{10} 4.70 CFU/g). *Asam gelugor* leaf also showed a significant difference in TPC reduction (log_{10} 4.44 CFU/g) when added to GTP for 8 minutes



Values are presented as mean ± standard deviation. The values of samples followed by same letter (a-b) are not significantly different ($p > 0.05$)

Figure 3. TPC of GTP between exposure time and type of plant leaf



Values are presented as mean ± standard deviation. The values of samples followed by same letter (a-b) are not significantly different ($p > 0.05$)

Figure 4. YMC of GTP between exposure time and type of plant leaf

compared to Vietnamese coriander ($\log_{10}$ 5.10 CFU/g), but was insignificant to turmeric leaf ($\log_{10}$ 4.70 CFU/g). No significant difference was observed in TPC reduction when the three leaves were added to GTP for 2 minutes of the cooking time. The addition of Vietnamese coriander leaf to GTP for 5 minutes of the cooking period showed no significant reduction of YMC ($\log_{10}$ 4.80 CFU/g) compared to turmeric ($\log_{10}$ 4.23 CFU/g) and *asam gelugor* ($\log_{10}$ 4.44 CFU/g) leaf. However the YMC reduction demonstrated by both turmeric and asam gelugor leaf were insignificantly different. No significant difference was observed in YMC reduction when the three leaves were added to GTP for 2 and 8 minutes of the cooking time. In this study, suggested that plant leaf can be added at 2, 5 and 8 minutes of the 10 minutes cooking period because the microbial load is still within the recommended range. Previous study indicated that the effects of cooking or exposure time on the antimicrobial compound may be negative, positive or none at all (Chohan *et al*., 2008). Apart from that, the long cooking time produced thermal degradation of bioactive compounds in food compound as described by Martínez-Hernández *et al*. (2012). In this study demonstrated that 8 minutes exposure time shows significantly higher microbial load in all three leaves used for both TPC and YMC. This could be proved that longer exposure time in cooking process will degraded and lost the natural antimicrobial that present in the plant leaves. Martínez-Hernández *et al.* (2012) also added that after cooking period the level of bioactive compound in the food products will be decreased as compared to raw plant samples. However, some research reported that when the plants are heated, the antimicrobial compounds may convert to some form that has little antimicrobial activity due to their instability to heat (Ankri and Mirelman, 1999). Moreover, some other factors should be considered such as present of fat, carbohydrate, protein, salt, and pH reaction influence the effectiveness of these antimicrobial agents in foods (Holley and Patel, 2005). The storage temperature may also influence the effectiveness of antimicrobial of the plant leaf due to the fact that the diffusibility of compounds is related to temperature (Friedman *et al*., 2004).

Conclusions

This study concludes that the addition of *asam gelugor* leaf for 5 minutes of the GTP cooking time inhibit bacterial growth significantly. The result is useful as the GTP is easily produced at home and asam gelugor leaves are easily accessible. This study also provides a basis for further investigation on how to extend the shelf -life of GTP and sensory acceptance using asam gelugor leaf. Furthermore, the results indicate that the application of these three leaves is strongly dependent on the problem to be addressed, considering that each leaf gives varying results on the microbiological quality of the food.

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