

## Potential of fermented papaya beverage in the prevention of foodborne illness incidence

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### Abstract

Foodborne illness is recognized as an emerging infectious disease. The incidence of foodborne infections is common and the majority cases are undiagnosed or unreported. Apart from some diarrhea or minor gastrointestinal problem, some foodborne pathogenic microbes may cause death, particularly to those people with weakened immune system. In this study, we have developed a new fermented papaya beverage using symbiotic culture of yeast and acetic acid bacteria under controlled biofermentation process. An *in-vitro* assessment of fermented papaya beverage against few foodborne pathogenic microorganism was conducted to determine its minimum bactericidal concentration (MBC<sub>>99</sub>). Three types of foodborne pathogen: *Escherichia coli* O157, *Salmonella enterica* serovar Typhimurium ATCC 53648, *Salmonella enterica* serovar Enteritidis (isolated from infectious chicken) were selected. From minimum bactericidal concentration (MBC<sub>>99</sub>) assay, both fermented papaya pulp and leaves beverages have shown 100% killing rate against three selected foodborne pathogenic microbes. Inversely, non-fermented papaya pulp and leaves beverages indicated no inhibition at all. In fact, further dilution of fermented papaya pulp and leaves beverages demonstrated different degree of MBC<sub>>99</sub> and brix value, but the pH value remained less than 3.5. These findings indicated the combination of soluble solid compounds presents in both fermented papaya beverage and product acidity play an important role in the inhibition of pathogenic microorganisms. The preliminary promising results of this work have shown that the great potential of fermented papaya beverages as a preventive measure to reduce the incidence of foodborne illness.

## 1. Introduction

Papaya (*Carica papaya* Linn.) is known as one of the most favorite fruit due to its nutritional, digestive and medicinal properties. It has rich source of phytonutrient content and high antioxidant capacity which contributing to its significant health profile. The flesh part of ripe papaya fruit has sweet and delicate taste, rich with multiple vitamins (C, E and A) and contains an excellent source of folic acid, potassium, copper, phosphorous and iron. Many published clinical evidence had shown the presence of phytochemicals in papaya leaves contributes to its multiple functional role as a remedy for diabetes treatment (Juárez-Rojop *et al.*, 2014), anti-inflammatory effect (Owoyele *et al.*, 2008), immunomodulatory effects (Otsuki *et al.*, 2010), chemopreventive effects (Tan *et al.*, 2014),

antiplasmodial effect (Ahmad *et al.*, 2011) and analgetic activity (Hasimun *et al.* 2014).

Foodborne illnesses, often called as food poisoning, are referring as any illness resulting from the food spoilage of contaminated food, pathogenic bacteria, virus or parasites that contaminated foods, natural toxins from poisonous mushroom (Addis and Sisay, 2015). Onset of foodborne illness is very rapid with food intoxication and will cause very sick to infected people. Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Vibrio vulnificus*, *Clostridium botulinum*, and *Salmonella* infection are ranked as the most dangerous foodborne pathogens (Lim, 2002; MaCabe-Sellers and Beattie; 2004; Addis and Sisay, 2015; Zuraw, 2015). Even though these pathogenic microorganisms may not potentially life-threatening to most healthy person, however, these pathogens can cause a severe and life-threatening

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illness, particularly dangerous to elderly people, pregnant women, children and immunocompromised people, including those with chronic liver disease, cancer or diabetes (MaCabe-Sellers and Beattie, 2004). In recent year, a considerable effort had been taken to find natural antimicrobial remedies that can inhibit the growth the bacterial and fungal growth in food to maintain food quality and shelf life. The use of food as antimicrobial agent has gained attention by the consumers and food industry. The potential of negative impact of synthetic preservatives on health has raised the interest among researchers to produce the natural antimicrobial compounds in foods.

To date, numerous scientifically studies had supported the clinical evidences on the effectiveness of fermented papaya preparation through natural fermentation process had confirmed a favourably effect in modulating immunological, haematological, neuroprotective, anti-inflammatory, antioxidants, prevention of diabetic complication from the progressive oxidative damage (Zhang *et al.*, 2006; Aruoma *et al.*, 2010; Marotta *et al.*, 2012; Raffaelli *et al.*, 2015; Barbagallo *et al.*, 2015). In this study, we have developed a new biofermentation process to produce new fermented papaya phytonutrients beverage using defined starter cultures of yeast and acetic acid bacteria from MARDI's Collection of Functional Food Cultures (CFFC). Our main objective of this work was to investigate the effect of fermented papaya phytonutrients against the growth inhibition of few common foodborne pathogens with the aim to be served as preventive measures in reducing risk of foodborne illness incidence. The non-fermented papaya beverage samples were used as a control to examine the efficacy of fermented papaya phytonutrient against a broad range of foodborne pathogens.

## 2. Materials and methods

### 2.1 Materials

Papaya (*Carica papaya* Linn.) with the variety of Sekaki was bought from local papaya farm. Both papaya leaves and fruit were washed clean before proceeding to fast drying process to produce fruit powder and granule from the leaves and pulp part, respectively. The dried fruit powder and granule were packed in the aluminium bag and stored at the temperature of 4°C prior to fermentation process. Two strains of pathogenic microorganisms: *E. coli* O157 and *Salmonella enterica* serovar Typhimurium ATCC 53648 were bought from American Type Culture Collection, whereas, *Salmonella enterica*

serovar Enteritidis was isolated from infectious chicken.

### 2.2 Preparation of fermented beverage samples from dried papaya samples

A total of 2.5% (v/w) papaya leaves powder and 5% (w/v) papaya pulp granule was prepared as non-fermented papaya leaves and papaya pulp beverage samples, respectively. Another set of fermented papaya leaves and pulp beverage samples was prepared with the same concentration of papaya leaves and papaya pulp suspension samples and were inoculated with 10% of the defined mixed culture of yeast (*Dekkera sp.*) and acetic acid bacteria (*Gluronacetobacter sp.*) from MARDI's Collection of Functional Food Cultures (CFFC) with the colony count of at least  $1 \times 10^8$  CFU/ml and then incubated at 30°C for a duration of 4 days. The pH and Brix value of both fermented papaya leaves and pulp beverage samples were measured using pH meter and refractometer, respectively.

### 2.3 Determination of minimum bactericidal concentration (MBC<sub>>99</sub>)

*E. coli* O157, *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium were cultured onto tryptone soy agar individually and incubated overnight. A loopful of foodborne pathogen culture from each tryptone soy agar was inoculated onto tryptone soy broth and then incubated at temperature of 37°C for 16 h at the agitation rate of 160 rpm to obtain the suspension culture with the initial colony count of  $10^9$  CFU/ml. The MBC<sub>>99</sub> for each microbe was determined using broth microdilution technique as described in Koh *et al.* (2016). Both non-fermented and fermented papaya leaves and papaya pulp beverage was used as an initial working concentration to determine its MBC<sub>>99</sub>. Each well contained 120 µl of the test material in serially descending concentration. A growth control and sterility control were prepared also. A total of 20 µl of inoculum containing foodborne pathogen suspension ( $10^9$  CFU/ml) was added onto each well and was rotated using microtiter plate shaker before incubated at the temperature of 37°C for 24 h. After incubation, the serial dilutions were performed in the eppendorf tube using micropipette and the plate count for each dilution was analyzed in triplicate.

## 2.4 Statistical analysis

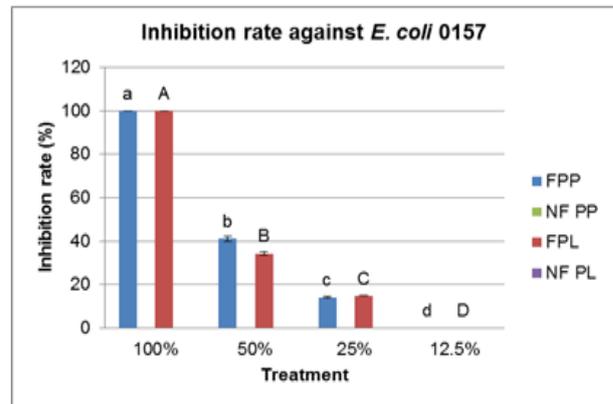
The experimental results were analyzed using SPSS software (SPSS statistics version 16). All data were expressed as the mean  $\pm$  standard deviation of triplicate analysis. A one way analysis of variance (ANOVA) at the 5% significance level was used to determine significant differences ( $P < 0.05$ ) between means.

## 3. Results and discussion

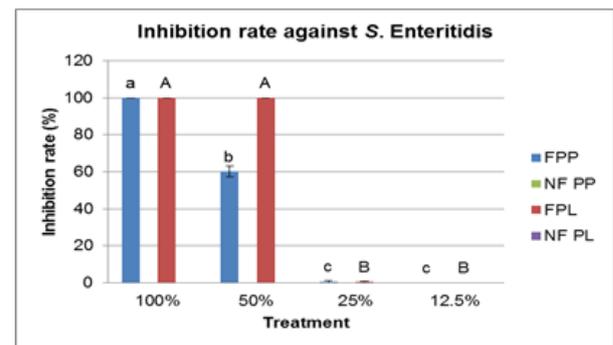
In this study, two types of fermented papaya phytonutrient beverages from the papaya leaves and pulp source were produced using controlled mixed cultures of yeast and acetic acid bacterial fermentation process. With the aid of microbial work, many biochemical changes on nutritive and anti-nutritive components of the papaya leaves and papaya pulp samples are manipulated through microbial enzymatic reactions and metabolisms. These processes can enhance the digestibility, bioavailability of nutrients and, enriched the bioactive components in the fermented papaya products, as being reported in many fermented foods (Russell and Duthie, 2011; Gumienna, *et al.*, 2016). As expected, fermented papaya phytonutrients samples were found more palatable with better aroma and taste than non-fermented papaya phytonutrients samples with the aid of microbial work.

Three types of pathogenic microorganism were selected, which were: a) *E. coli* O157; b) *S. enterica* serovar Enteritidis and c) *S. enterica* serovar Typhimurium to examine the efficacy of fermented papaya leaves and pulp beverage sample against their growth inhibition rate. Different concentration of non-fermented and fermented papaya phytonutrients (papaya pulp and papaya leaves) were studied to determine the  $MBC_{>99}$  against *E. coli* O157, *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium. Generally, non-fermented papaya phytonutrients from both papaya leaves and pulp source showed no growth inhibition at all towards *E. coli* O157, *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium as summarized in Figure 1. Inversely, both fermented papaya leaves and pulp beverage samples exhibited different degree of growth inhibition rate against these specific types of pathogenic microorganisms (Figure 1a, 1b and 1c). In comparison to fermented papaya pulp, fermented papaya leaves beverage exhibited more higher growth inhibition rate against *S. enterica* serovar Enteritidis than *E. coli* O157 and *S. enterica* serovar Typhimurium with the  $MBC_{>99}$  value of 50% (Table

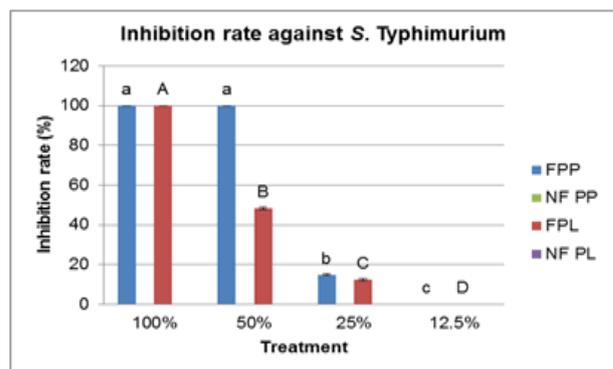
1). On the other hand, fermented papaya pulp showed growth inhibition preference towards *S. enterica* serovar Typhimurium than *E. coli* O157 and *S. enterica* serovar Enteritidis. With the  $MBC_{>99}$  value of 50%, fermented papaya pulp beverage sample was capable to cause 100% killing rate against *S. enterica* serovar Typhimurium.



(a)



(b)



(c)

Figure 1. Inhibition rate against foodborne pathogens: a) *E. coli* O157; b) *S. enterica* serovar Enteritidis and c) *S. enterica* serovar Typhimurium using different concentration of papaya pulp (NF PP), papaya leaves (NF PL), fermented papaya pulp (FPP) and fermented papaya leaves (FPP) beverage samples

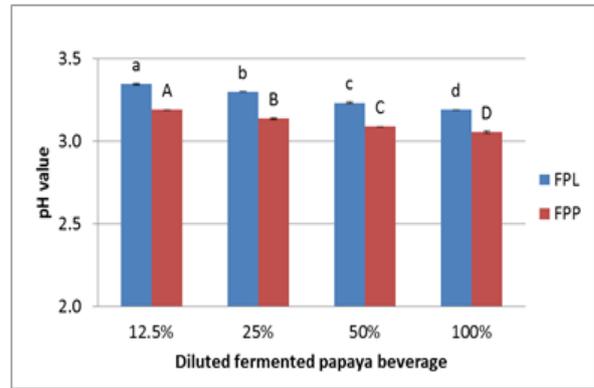
Table 1. Minimum bactericidal concentration (MBC<sub>>99</sub>) of non-fermented and fermented papaya leaves and pulp beverage against foodborne pathogens<sup>a</sup>

Type of Pathogen	Minimum Bactericidal Concentration (MBC <sub>&gt;99</sub> )			
	NF PL	FPL	NF PP	FPP
<i>E. coli</i> O157	NIL	100% ± 0.0A	NIL	100% ± 0.0A
<i>S. enterica</i> serovar Enteritidis	NIL	50% ± 0.0B	NIL	100% ± 0.0A
<i>S. enterica</i> serovar Typhimurium	NIL	100% ± 0.0A	NIL	50% ± 0.0B

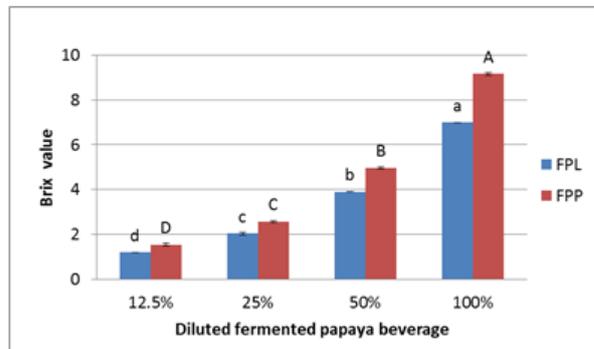
<sup>a</sup>Each value in the Table represents the mean ± standard deviation from triplicate analyses. Mean values with different superscripts in the same row are significantly different at P < 0.05.

Abbreviations: The MBC<sub>>99</sub> is identified by determining the lowest concentration of substrate drink that reduces the viability of the initial pathogens inoculum by 99.9%; papaya pulp (NF PP); papaya leaves (NF PL); fermented papaya pulp (FPP) and fermented papaya leaves (FPP)

Further dilution on both fermented papaya leaves and pulp beverage sample indicated the acidity of fermented papaya product was not the main cause of pathogen inhibition factor as the pH value demonstrated minor change even the fermented papaya product diluted up to 12.5% and the pH value remained below 3.5 (Figure 2a). However, the Brix value which measures the soluble solid content showed significant reduction when the fermented papaya beverage was diluted (Figure 2b). This phenomenon indicated the presence of soluble solid compounds such as soluble organic acids and phenolic acids in both fermented papaya beverages may contribute to its potent inhibition against foodborne pathogens. Past research findings have confirmed that many plant-derived polyphenolic compounds and organic acids with the variety of chemical composition showed their antimicrobial effectiveness against pathogens (Cueva *et al.*, 2010; Stojković *et al.*, 2013; Hawkins, 2014; Gyawali and Ibrahim, 2014). It was believed that the mixed culture of yeast and acetic acid bacteria had broken down the complex structure of phytochemicals in papaya leaves and pulp phytonutrients and changed their metabolite profile into more digestible and bioavailability phytonutrients. The presence of these functional bioactive metabolites in fermented papaya leaves and pulp beverage sample is the main contributing factor of their antimicrobial activity against selected foodborne pathogenic microorganisms when compared with non-fermented papaya leaves and pulp beverage samples.



(a)



(b)

Figure 2. The changes of: a) pH value; b) Brix value when both fermented papaya pulp (FPP) and fermented papaya leaves (FPP) beverage samples were diluted up to 12.5%

#### 4. Conclusions

Both fermented papaya phytonutrients beverage produced from papaya leaves and pulp source have exhibited the promising results on growth inhibition against selected pathogenic microorganisms, which were *E. coli* O157, *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium. This finding indicated the potential of fermented papaya phytonutrients in reducing risk of foodborne illness. We believed that the fermented papaya phytonutrients can be served as a daily nutritional supplement for maintaining good health to minimize food poisoning incidence. Future work will be focused on the study of the metabolite papaya phytonutrient profile changes after fermentation and identify the bioactive compounds that contributing to its growth inhibition against pathogenic microorganisms.

#### Conflicts of interest

The authors declare no conflict of interest.

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