Inhibitory effect of commercial Assam green tea infusion in watermelon juice

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Abstract

The consumption of fruit juices is rapidly increasing due to their freshness, nutritional and health benefits. However food poisoning outbreaks associated with fresh unpasteurized fruit juices have been reported. In some studies, watermelon juice has been found to possess high microbial susceptibility compared to other fruit juices due to its relatively low acidity and high sugar (TSS) content. This study aimed to evaluate the inhibitory effect of Assam green tea infusion against four selected potent pathogens in fresh watermelon juice. Two brands of green tea (green tea A and B) were purchased from the Chiang Rai province in Thailand. Based on our previous minimum inhibitory concentration (MIC) test, various concentrations of the tea-watermelon juice mixture; 75, 100, 175 and 250 mg/mL, were prepared for inhibitory testing against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*, with the inoculum size of approximately 8 log CFU/mL and then incubated at 35 °C. The survival of the microorganisms was determined every day for up to 7 days. The pH of the tea-watermelon juice was significantly lower (P ≤ 0.05) than that of the watermelon juice alone. The TSS of the mixtures ranged from 9 to 12 %. Among all of the tea infusions, green tea A exerted the highest inhibitory effect to all the pathogens with *E. coli* was the most resistant strain. The green tea A mixtures reduced the populations of *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli* to an undetectable level within 2, 3, 5 and 6 days, respectively. This study showed that a green tea infusion could be a potential natural preservative which could used to extend the shelf life of other fruit juices by improving their microbiological safety.

Keywords : green tea infusion; pathogen inhibitory; watermelon; food safety

Introduction

The consumption of fruit juices is increasing due to their freshness, high vitamins content and beneficial health diet components (Ashurst, 2005; Berryman, 2007; Rico et al, 2007). However the consumption fresh fruit juices have lead to a growing public health issue due to associated food safety outbreaks (Ray, 2001; Yuste and Fung, 2002; CDC, 2007; Oussalah, 2007). These outbreaks are mainly caused by *E. coli*, different serovars of *Salmonella*, *S. aureus*, *L. monocytogenes*, and *Yersinia enterocolitica*. Watermelon juice is a potential vehicle of foodborne microorganisms compared to other fruit juices due to its neutral pH (pH 5.2 to 6.7) (FDA, 2001; Mosqueda-Melgar et al, 2007). The FDA (2001) and CDC (2007) have reported outbreaks caused by watermelon juice consumption contaminated with *E. coli*, *S. typhimurium*, *L. monocytogenes* and *S. aureus*.

Minimal processing of fruit juices is a potential method to retain the sensorial and nutritional qualities of the juice, while inactivating the harmful pathogenic microorganisms. Novel non-thermal preservation techniques with or without a combination of added preservatives has been applied to inactivate the pathogenic bacteria in watermelon juice without adversely affecting its sensorial and nutritional qualities (Mosqueda-Melgar et al., 2008; Mosqueda-Melgar et al., 2007). However these novel non-thermal preservation methods require a high level of expertise and capital costs. Many studies have examined the need to maintain fruit juice safety while preserving its nutritious and organoleptic attributes for small scale and home-made juices.

The addition of food additives is one of the potential methods to achieve this, however increasing health concerns of artificial preservatives, natural preservatives are now being examined. Green tea is a powerful natural preservative, exhibiting both antioxidant and antimicrobial properties. Researchers have attempted to exploit these properties, especially its microbial inactivation activities of green tea extracts and purified tea catechins (Gramza and Korczak, 2005; Almajano et al, 2008). However there has no report of using tea-water infusion directly into the real food matrix for these purposes. Therefore the aim of this work was to investigate the properties on the tea-water infusion in a liquid model food matrix (watermelon juice) in order to examine the possibility of using this tea infusion in other types of fruit juice products.

Materials and Methods

Microorganisms

All tested microorganisms (*Staphylococcus aureus*; TISTR1466, *Salmonella typhimurium*; TISTR292 and *Escherichia coli*; TISTR780) were obtained from Microbiological Resources Center, at the Thailand Institute of Scientific and Technological Research (TISTR), except *Listeria monocytogenes* was obtained from DMST culture collection, at the Department of Medical Sciences, Thailand. All microorganisms were transferred into a 100 mL nutrient broth (NB) and incubated along with shaking (200 rpm) at 37 °C for 24 hrs. The cell was harvested, washed and re-suspended in 0.1 % sterile peptone water in order to achieve the cell final concentration of 9 log CFU/mL.

Green tea infusion preparation

Two commercial Assam green teas, represented by green tea A and B, were purchased from the market in Mueng district, Chiang Rai. The green tea infusion was prepared as according to Uzunalic et al. (2006). In summary, the green tea was ground and sieved through a sieve with ~500 µm pore size and kept in a sealed plastic bag until used. Ground sample (62.5 g) was

extracted with 250 mL of boiling distilled water for 10 min. The extract was then filtered through a Whatman No. 4 filter paper. The filtrate was collected and the final volume of the filtrate was made up to 250 mL with distilled water in order to get a stock tea infusion concentration of 250 mg/mL. The tea infusion was subsequently sterilized by passing through a membrane filter with the pore size of 0.45 μm.

Watermelon juice preparation

Fresh watermelon (*Citrullus lanatus*), red cultivar, was purchased from a supermarket in Chiang Rai, Thailand. The watermelon was washed, peeled and the flesh was cut into small pieces. The juice was prepared by blending the watermelon flesh using a blender and filtered by four layers sheet clothes to obtain the clear juice. The filtered juice was centrifuged at 10,000 rpm for 15 min at 4 °C. The juice supernatant was collected and autoclaved at 121 °C for 15 min.

Preparation of tea-watermelon juice mixture

Various volumes of the 250 mg/mL tea infusion were aseptically mixed with different volumes of the sterile watermelon juice in order to get the desired concentrations of the tea-watermelon juice mixture (0, 75, 100, 175 and 250 mg/mL) (Table 1). Total soluble solid (TSS) and pH were determined by hand refractometer and pH meter.

Table 1: Preparation of tea-watermelon juice and final juice TSS and pH.

Volume of 250 mg/mL stock tea infusion (mL)	Volume of sterile watermelon juice (mL)	Tea final concentration (mg/mL)	Juice concentration (% v/v)	TSS (Brix)		pH	
				A	B	A	B
0	20	0	100	9 [a]	9 [a]	6.44[a]	6.44[a]
6	14	75	70	9 [a]	9 [a]	6.21[b]	6.18[b]
8	12	100	60	10 [a]	10 [a]	5.99[c]	5.95[c]
14	6	175	30	11 [a]	11 [a]	5.79[c]	5.75[c]
20	0	250	0	12 [a]	12 [a]	5.71[c]	5.69[c]

*[a-d] Means with different superscript letters within a coloum are significantly different at $p \leq 0.05$

Microbial inoculation

The watermelon juice (20 mL) containing different concentrations of green tea infusion was inoculated with 0.2 mL of 9 log CFU/mL bacteria suspension to obtain the inoculation size of approximately 8 log CFU/mL. A control watermelon juice without green tea, was also inoculated and considered a control. All of samples were incubated 35 °C for 7 days.

Microbiological analysis

Microbiological analysis was determined everyday up to seven days. Each day, a serial dilution was prepared with sterile 0.1 % peptone water and 0.1 mL of the diluent. This was spread in duplicate on culture media to determine the population of *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli*, respectively. All plates were incubated at 35 °C for 24 hrs.

Phenolics compounds determination

HPLC (method ISO 14502-2, 2005) was used to determine the catechins, including catechin (C), epicatechin (EC), gallocatechin (GC), catechin gallate (CG), epigallocatechin (EGC), gallocatechin gallate (GCG), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) as well as gallic acid (G) and caffeine (CF) in the tea infusions. A HPLC system Agilent Technology 1100 Series, equipped with a quaternary pump, a degasser, a thermostatic auto-sampler with a reverse phase C18 column and a photodiode array detector (DAD), was used to separate and quantify the phenolics. The binary mobile phase was consisted of 13 % of

acetonitrile and 87 % of trifluoroacetic acid (0.05%). The flow rate was kept constant at 2 mL/min for a total run time of 12 min. One millilitre of tea infusion was filtered through a polytetrafluoroethylene (PTFE) filter and the filtrate was collected in a small amber vial. A 10 μl sample of the filtrate was injected to the HPLC. The concentrations of investigated phenolics were determined, which based on the standard chromatographic data

Statistical analysis

All data were subject to analysis of variance (ANOVA) by SPSS software. The statistical significance of the differences between Assam green tea infusions were statistically significant when $P \leq 0.05$.

Results and Discussion

The TSS of watermelon juice containing different concentrations of tea infusion was varied ranged from 9 to 12 % (Table 1). This range of TSS is similar to the TSS allowed for commercial watermelon juices (FAO, 2001). The pH of watermelon juice containing tea infusion ranged of pH 5.69 to 6.44, which was significantly higher than that of the control. There was no significant difference in pH between the watermelon juice containing green tea A and B at the same concentration. The pH of watermelon juice decreased when the tea infusion was added and maybe due to phenolic acids in the tea infusion (Hodgson et al., 2004; Somboonvechakarn, 2007).

The reduction of microbial population in the watermelon juice-tea mixture is shown in Figure 1. The results show that the microbial population in watermelon juice without an addition of tea infusion was not significantly different throughout storage. However all microorganisms in the watermelon juice containing the tea infusions decreased dramatically to an undetectable level within 2 to 4 days of incubation. The addition of green tea A to the watermelon juice inactivated *S. aureus* and *L. monocytogenes* within 2 and 3 days, respectively. While the addition of green tea B, showed less inhibitory effect to *S. aureus* and *L. monocytogenes* by taking 3 and 4 days respectively, to exhibit complete inhibition. Both the green tea infusions had similar inhibitory effects with *S. typhimurium* and *E. coli*, requiring only 5 and 6 days respectively, to completely inhibit *S. typhimurium* and *E. coli*. The less inhibition time needed for green tea A and B infusions to inhibit *S. aureus* and *L. monocytogenes* compared to the time needed to inhibit *S. typhimurium* and *E. coli* and suggests that gram positive bacteria exhibit more sensitivity to the tea infusions than gram negative bacteria. These observations are consistent to the results obtained in liquid medium (Kristanti et al., 2008).

Table 2 shows that green tea A had the higher C, EC, and G content than that of green tea B. While, EGC was the only compound in green tea B observed higher than that of green tea A. In regard to the higher inhibitory effect and the higher content of these compounds of green tea A, the results suggest that the microbial inhibitory effect from the green tea is due to the combination effects of C, EC, and G. These results are consistent with other reports which show that the overall inhibitory effects are not the single effects from individual catechins, but a combination effect of all of the catechins. (Gramza and Korczak, 2005; Almajano et al, 2008).
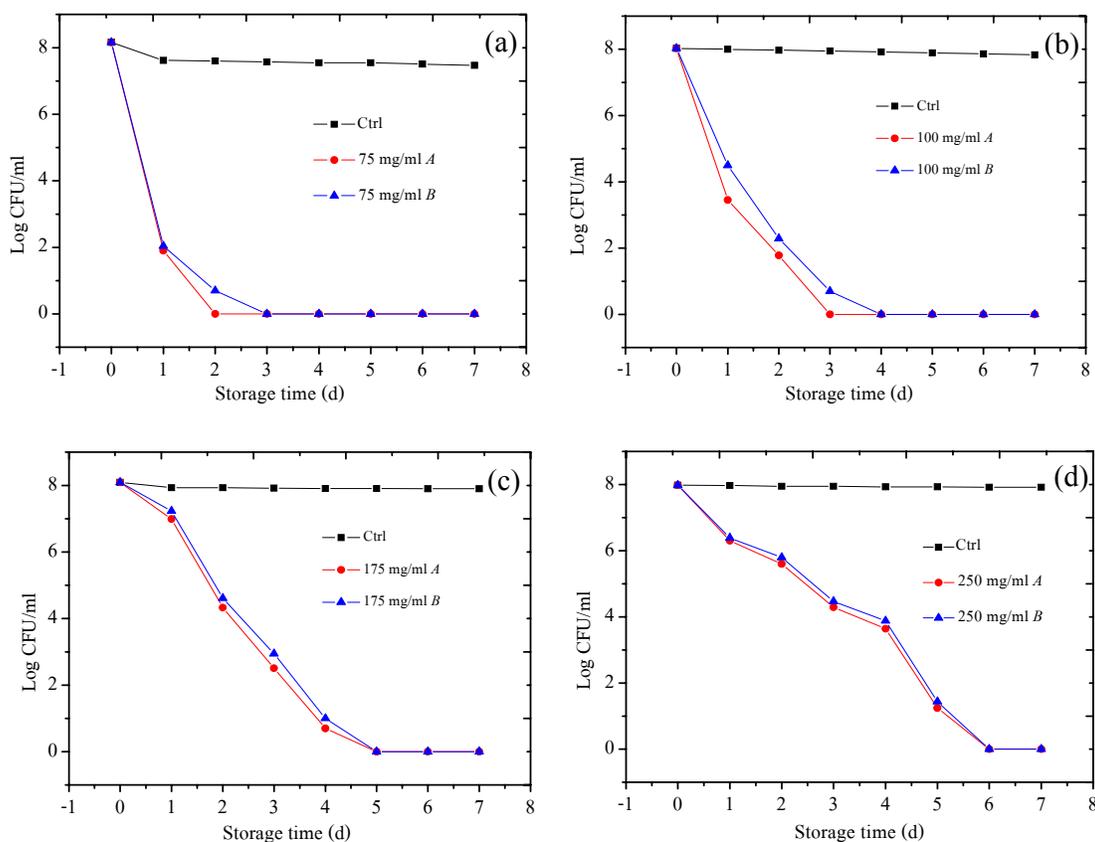
Figure 1: Reduction of (a) *S. aureus* (b) *L. monocytogenes* (c) *S. typhimurium* and (d) *E. coli* in the watermelon juice-tea mixture after 7 days incubation at 35 °C.

Table 2: Phenolics compounds in Assam green teas.

Samples	Catechins (% w/w dry basis)					
	CF	C	EC	EGC	G	EGCG
A	3.44 ± 0.46^a	$0.54 \pm 0.01a$	2.16 ± 0.08^a	2.69 ± 0.45^c	1.21 ± 0.02^b	ND
B	3.33 ± 0.46^a	0.37 ± 0.02^b	1.81 ± 0.19^b	3.37 ± 0.8^a	0.94 ± 0.06^d	ND

*[a-e] Means with different superscript letters within a coloum are significantly different at $p \leq 0.05$

ND = non detectable

The bactericidal effects of green tea in watermelon juice are also a consequence of the lower pH of the juice-tea mixture (Burt, 2004). In addition, the hydrophobicity of green tea increases at low pH, enabling it to more easily dissolved in the cytoplasmic content of the target bacteria, hence the obtained higher bactericidal effect. These observations suggest that the antimicrobial activity of the green tea is a combination effect of the tea phenolic compounds and the low pH of the tea infusions. However the action of the tea infusion against bacteria in watermelon juice was lower than that of the liquid medium. This maybe due to a greater availability of nutrients in the watermelon juice, as compared to liquid medium, which may enable bacteria to repair damaged cells faster in the juice (Gill et al, 2002; Munoz et al, 2009).

Conclusions

The strong microbial inhibitory effect of green tea A (75 mg/mL) and green tea B (100 mg/mL) showed the same tendency as observed in the liquid medium against *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli*. Green tea A was shown to have the stronger inhibitory effects against all pathogens. *E. coli* was the most resistant pathogen as compared to *S. aureus*, *L. monocytogenes* and *S. typhimurium*. This study showed that a green tea infusion can extend the shelf life of watermelon juice up to 7 days. As a result of its almost neutral pH, watermelon juice is more susceptible to microbial spoilage than other fruit juices, which are generally more acidic. This suggests that tea infusions could be a potential natural preservative used to extend the shelf life of fruit juices by improving their microbial safety. However a combination of preservatives could be utilised to enhance the antimicrobial activity against the resistant pathogens *S. typhimurium* and *E. coli*.

References

Almajano, M.P., Carbo, R., Jimenez, J.A.L., and M.H. Gordon. (2008). Antioxidant and antimicrobial activities of tea infusion. **Food Chemistry**, Vol. 108(1), pp. 55-63.

Ashurst, P.R. (2005). **Chemistry and Technology of Soft Drinks and Fruit Juices**. 2nd ed. Wiley-Blackwell Publishing. 374p.

Berryman, D. (2007). Fruit Juice Consumption. 15th IFU (International Federation of Fruit Juice Producers) World Fruit Juice Congress 2007 "Fruit juices, naturally healthy" on June 17-22, 2007 in The Hague (Scheveningen), Netherland, Retrieved October 28, 2008, from http://www.ifu-fruitjuice.com.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods: a review. **International Journal of Food Microbiology**, Vol. 94, pp. 223-253.

Center for Disease control and prevention (CDC). (2007). Annual listing of foodborne disease outbreaks. Retrieved October 28, 2009, from http://www.cdc.gov.

Food and Agriculture Organization (FAO). (2001). Juice and beverage blends. Retrieved October 28, 2008, from http://www.fao.org.

Gill, A.O., Delaquis, P., Russo, P. and R.A. Holley. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. **International Journal of Food Microbiology**, Vol. 73, pp. 83-92.

Gramza, A. and Korczak, J. (2005). Tea constituents (*Camellia sinensis* L.) as antioxidant in lipid systems. **Trends in Food Science and Technology**, Vol. 16, pp. 351-358.

Hodgson, J.M., Chan, S.Y., Puddey, I.B., Burke, V., Ward, N.C. and Croft, K.D. (2004). Phenolic acid metabolites as biomarkers for tea and coffee derived polyphenol exposure in human subjects. **British Journal of Nutrition**, Vol. 91(2), pp. 301-306.

International Organization for Standardization (ISO). (2005). Determination of substances characteristic of green and black tea. Content of catechins in green tea. Method using high-

performance liquid chromatography. ISO 14502-2 2005; International Organization for Standardization (ISO) Geneva

Kristanti, R.A Sampanvejsobha, S.and Punbusayakul, N. (2008). Susceptibility testing of commercial green tea in Chiang Rai against pathogenic bacteria. **Tea international conference on tea production and tea products proceedings**; p.137-144, 26-28 November 2008 in Chiang Rai, Thailand

Mosqueda-Melgar, J., Raybaudi-Massilia, R.M. and Martín-Belloso, O. (2007). Influence of treatment time and pulse frequency on *Salmonella enteritidis*, *Escherichia coli* and *Listeria monocytogenes* populations inoculated in melon and watermelon juices treated by pulsed electric fields. **International Journal of Food Microbiology**, Vol. 117(2), pp. 192-200.

Mosqueda-Melgar,J., Raybaudi-Massilia, R.M. and Martín-Belloso, O. (2008). Combination of high-intensity pulsed electric fields with natural antimicrobials to inactivate pathogenic microorganisms and extend the shelf-life of melon and watermelon juices. **Food Microbiology**, Vol. 25(3), pp. 479-491.

Munoz, M., Guevara, L., Palop, A., Tabera, J. and Fernández, P.S.. (2009). Determination of the effect of plant essential oils obtained by supercritical fluid extraction on the growth and viability of *Listeria monocytogenes* in broth and food systems using flow cytometry. **LWT-Food Science and Technology**, Vol. 42(1), pp. 220-227.

Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes*. **Food Control**, Vol. 18, pp. 414-420.

Ray, B. (2001). **Fundamental Food Microbiology**. 2nd ed. CRC Press. 561p.

Rico, D., Martın-Diana, A.B., Barat, J.B. and Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. **Trends in Food Science and Technology**, Vol. 18, pp. 373-386.

Somboonvechakarn, C. (2007). The effects of green tea extract on soy bread physical properties and total phenolic content. Doctoral dissertation, Faculty of Food Science and Technology, University of Ohio, US.

Uzunalic, A.P., Skerget, M., Knez, Z., Weinreich, B., Otto, F. And Gruner, S. (2006). Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. **Food Chemistry**, Vol. 96(4), pp. 597-605.

Yuste, J. and Fung,. D.Y.C. (2002). Evaluation of *Salmonella typhimurium*, *Yersinia enterocolitica* and *Staphylococcus aureus* counts in apple juice with cinnamon, by conventional media and thin agar layer method. **Food Microbiology**, Vol. 20, pp. 365-370.