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Effect of dried and extrudate of bitter gourd fruit on epithelial microflora in raw chicken legs meat

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Abstract

Plants have been used recently to eliminate bacterial growth in food products. This study was undertaken to test the *in vitro* sanitizing effect of crude extract from bitter gourd (BG) fruit on the growth of native microorganisms in raw chicken leg meat. Hot air dried BG and extrudate extracts at 1% concentration and exposure times of (5, 10 and 15 min) were used to treat the samples using dilution method. Results showed that BG extrudate had a slightly stronger bactericidal activity against the microflora than the B.G. hot air drying treatment, especially, on *E. coli* at all exposure time. Overall, there is no significant difference between the treatments; Total Plate Count (TPC), *Escherichia coli, Bacillus cereus, Staphylococcus aureus.* The best reduction time of microflora by hot air dried extract was at (15 min) except for *B. cereus* was at (5 min) and for extrudate extract was at (5 min) except for *E. coli* was at (10 min). In conclusion, bitter gourd extract could be used as an important natural sanitizer for rinsing raw food matrials such chicken meat.

Introduction

Microbial hazards occur as a result of the survival of the causative agents at any stage of a supply chain. In the USA and UK, foodborne pathogens associated with poultry were responsible for about 10 - 20% of all foodborne disease outbreaks. A common pathogen, *E. coli* serotype 0157: H7 isolated from poultry samples including chicken legs, chicken nuggets and turkey sticks, for instance, have been known to cause serious harm to human such kidney failure and even death. It has been recognized as the cause for food- associated outbreaks since 1982. Another common pathogen is Staphylococcus aureus, where even though does not produce enterotoxin, high growth level may result in the meat products being rejected by the food industry as they are unfit for use or consumption. Bacterial resistance towards the antibiotic use in poultry has been recognized as a health problem in both human and veterinary medicine (Mead, 2004). For optimal microbial removal, physical and washing treatments are the second step after preventing initial contamination (Gómez-López, 2012). Natural antimicrobial compound found in many plants such as cinnamon, oregano and thyme could be used to increase the decontamination efficacy of food (Mith *et al*., 2014). This can serve as substitute for the chemical based treatments widely use in food produce industry as sanitizers in wash, as they are sometimes harmful due to residues (Gil *et al*., 2009). Momordica charantia Linn known as bitter gourd (BG) or peria katak in Malaysia, it is a tropical plant has been used in folklore medicine and in cooking as well (Grover and Yadav, 2004). In order to develop a natural sanitizer for use in rinsing of raw food, this plant extract can be used. This study provided information on antimicrobial activities based on the use of BG (subcontinent phenotype) against the epithelial microbial populations; microbial spoilage flora and pathogenic bacteria found in raw chicken legs meat.

Materials and Methods

Commercial mature BG fruit (dark green color, hybrid subcontinent variety) was purchased from local market in Selangor, Malaysia (Figure 1). BG deseeded fruits BG were sliced and hot air dried in dryer (SMA-112, Smoke Master, Japan) at 45°C overnight and then grounded. A part of it introduced to extruder at temperature of 80°C to obtain the extrudate. The extrusion process was carried out by using a Brabender single screw stand-alone extruder (KE-19/25 D, Brabender, Germany). Fruit powder

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Figure 1. Bitter gourd subcontinent variety

samples in this experiment were in powder form with ≤ 425 µm particle size. The moisture content of BG powders was determined by using the AOAC Official Method 967.03 (AOAC International, 2006). Raw legs chicken (broiler type) meat was bought from retail store in Serdang, Malaysia and kept refrigerated.

For the enumeration of bacteria in chicken legs meat four selective media were used; MacConkey (SMAC) broth was purchased from Merck Company and the agar from Sigma Company for *Escherichia coli*, Total Plate Count Agar (TPC) was purchased from Oxoid, Company. Polymyxin-Mannitol- Egg Yolk-Phenol agar (PMYPA) was purchased from Difco Company for *Bacillus cereus,* Tryptic Soy agar (TSA) was purchased from Merck Company for *Staphylococcus aureus*. These selective media were prepared according to manufactory's instructions.

Extract preparation

The samples of BG ethanol extract were prepared; 1 g of each dried powder of hot air dried BG and extrudate, separately, was extracted with 50 ml of absolute ethanol at room temperature (27°C). Table 1 showed the extracts yield and total saponins of each BG fruit powder. Total saponins content was determined according to Makkar *et al.* (2007).

Preparation of BG extract for treatment

One hundred milligram of each of cabinet-oven dried and extrudate dried extracts was dissolved separately in 1 ml pure DMSO (Dimethyl sulfoxide) to obtain 100 mg/ml of the extracts concentration. Then, deionized water (Braun Medical Industries, Penang, Malaysia) used to prepare final treatment concentration 1% (v/v) by adding 100 µl of the extract to 900 µl of deionized water. A 10% of DMSO that used in BG extract for treatment does not kill the microorganisms.

Preparation of chicken sample with the treatment

Ten grams (4 cubes) of each sample of raw chicken legs meat was weight (Petrová *et al*., 2013) and transferred to sterile test tubes using sterile forceps. The meat sample untreated with the extract solutions used as control was immersed in pure sterile deionized water at 16°C and immediately plated (zero time) (Hecer and Guldas, 2011) to determine its microflora growth viability on the different types of agar; TPC, PMYP, TSA and MacConkey using sterile plastic spreader and incubated overnight at temperature of 37ºC. The samples were soaked in 2 ml of cabinet-oven and extrudate of BG extracts, separately, for a time of 5, 10 and 15 minutes under laboratory conditions. Dilution plate count method was used for microflora viability and initial inoculum were taken by serial dilution using PBS (phosphate buffered saline) to create 10^{-1}, 10^{-2} and 10^{-3} dilution, 0.01 ml (USDA, 2015) from each dilution and plated onto the surface of solid media; PMYP, TSA, MacConkey and TPC using sterile plastic spreader. After 24 hours incubation at 37°C, CFUs/plate was counted (Yousef and Carlstrom, 2003; Yusoff, Sanuan and Rukayadi, 2015). The difference between average final counts to average control counts of ≥ $3\log_{10}$ CFU/g was considered a significant growth reduction (bactericidal activity) (CLSI, 1999). The assay performed in duplicate (n = 2 × 2).

Statistical analysis

The mean and standard deviation (± SD) of duplicate analysis for microflora population (Log_{10} CFU/g) were calculated on each treatment by Microsoft Excel 2010 software. Analysis of variance (ANOVA) was employed in order to analyze the data and Dunnett test was used to do multiple comparisons with a control and Tukey's test to identify the significance at ($\alpha = 0.05$) between treatment's time.

Results and Discussion

The extraction with absolute ethanol at solvent to solid ratio 0.02 g/ml resulted in the presence of a rich yield of saponins content in the hot air dried and extrudate extracts (Table 1). In this study, ethanolic extracts of hot air dried and extrudate with 1% (v/v) concentration were selected based on their minimum inhibitory concentration (MIC) results in previous experiments conducted by the first author (overall MIC was 5.625 mg/ml) and used to treat chicken leg meat samples using a dilution method. Bactericidal activity was defined as a ≥ 3 log10 decrease in count of CFU/g compared to the control at time zero (Saravolatz *et al*., 2013). Results showed that the number of initial microflora existed in refrigerated chicken leg meat sample (control) which was immersed in deionized water of Total

Table 1. The extracts yield and total saponins value of each ethanolic extract of BG samples

BG sample	Wt. of dry material (g)	Moisture content (%)	Wt. of dry extract (g)	Yield (%)	Total Saponins (%)
Hot air dried (Cabinet dryer)	5.00	2.7	0.30	6.00	9.95
Extrudate	5.00	2.3	0.15	3.00	10.00

Wt, weight.

Table 2. Comparison of the effect of ot air dried BG extract at 1.00% concentration on TPC, *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* in chicken meat at different exposure time in (Log_{10} CFU/g)

	Hot air dried BG extract			
Time/ Number of total or species microorganisms	TPC (Log_{10} CFU/g)	*E. coli* (Log_{10} CFU/g)	*B. cereus* (Log_{10} CFU/g)	*S. aureus* (Log_{10} CFU/g)
Control (0 min, 0.00% treatment concentration)*	6.58 ± 0.03[a]	6.70 ± 0.00[a]	6.81 ± 0.05[a]	6.74 ± 0.06[a]
5 min	5.00 ± 0.01[b]	1.92 ± 0.02[b]	3.30 ± 0.43[b]	4.59 ± 0.16[b]
10 min	2.86 ± 0.17[c]	2.91 ± 0.22[c]	3.48 ± 0.67[b]	2.78 ± 0.06[c]
15 min	2.71 ± 0.41[c]	1.80 ± 0.25[b]	4.15 ± 0.35[c]	2.75 ± 0.05[c]

[a, b, c,] Means that share these letters show have no significant different in Tukey's test within the same column; *, control is the initial count of the microorganism before treatment.

Plate Count (TPC), *B. cereus, E. coli* and *S. aureus* were 6.58 ± 0.03, 6.81 ± 0.05, 6.70 ± 0.00 and 6.74 ± 0.06 (Log_{10} CFU/g), respectively. This initial counts of the microflora was performed on the selective media and Mueller-Hinton agar media as well. These results showed insignificant difference in number of microorganisms of chicken legs meat with a study carried out by Yusoff, Noor and Rukayadi (2015). However, the highest count of Log CFU/g was *S. aureus,* whereas, this study *B. cereus* showed the highest count.

The overall growth pattern of *E. coli, S. aureus,* TPC, and *B. cereus* respectively, indicated a significant influence of both treatments in decreasing the viable count formation. The antibacterial effect of cabinet-oven BG dried and extrudate extracts at different exposure times of TPC, *E. coli, B. cereus,* and *S. aureus* are listed in Tables 2 and 3, respectively. Hot air dried BG and extrudate treatments found to be bactericidal with an average of a 4.5 and 4.8 log_{10} reduction, respectively, on *E. coli* growth at all immersion time of 5, 10, and 15 minutes, while *S. aureus* showed a 4.0 and 4.2 log_{10} decrease in the number of CFU/g in the samples treated with Hot air dried BG and extracts extrudate, respectively. For TPC, the viable count for both treatments showed a decrease with an average of a 3.8 log_{10} reduction in growth. Survival *B. cereus* in treated chicken sample with hot air dried BG showed bactericidal activity with an average of a 3.4 log_{10} reduction at time of 5 minutes and 10 minutes while *B. cereus* in treated chicken sample with extrudate showed a 3.3 log_{10} reduction at time of 5 minutes.

The above findings demonstrated a low level of resistance of the microflora in the samples to BG extrudate and hot air dried BG extracts. Both extract treatments recorded the highest bactericidal activity against *E. coli* in the chicken samples among the microflora with a 5.2 and a 4.9 log_{10} reduction at time of 10 minutes and 15 minutes, respectively. BG extrudate treatment showed its bactericidal activity against *S. aureus* with a 4.4 log_{10} reduction; TPC with a 4.3 log_{10} reduction; and *B. cereus* with a 3.3 log_{10} reduction at time of 5 minutes. The bactericidal activity of BG extrudate remained stable between 5 minutes and 15 minutes after the application test for *E. coli, S. aureus,* and TPC. On the other hand, the growth of B. cereus which showed the least influence by BG extrudate treatment started to re-grow with not more than a half log at time of 10 minutes and

Table 3. Comparison of the effect of BG extrudate extract at 1.00% concentration on TPC, *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* in chicken meat at different exposure time in (Log_{10} CFU/g)

Time/ Number of total or species microorganisms	BG extrudate extract			
	TPC (Log_{10} CFU/g)	*E. coli* (Log_{10} CFU/g)	*B. cereus* (Log_{10} CFU/g)	*S. aureus* (Log_{10} CFU/g)
Control (0 min, 0.00% treatment concentration)*	6.58 ± 0.03[a]	6.70 ± 0.00[a]	6.81 ± 0.05[a]	6.74 ± 0.06[a]
5 min	2.22 ± 0.95[b]	2.15 ± 0.05[b]	3.48 ± 0.00[b]	2.28 ± 0.12[b]
10 min	3.07 ± 0.50[c]	1.50 ± 0.12[c]	4.01 ± 0.15[c]	2.59 ± 0.25[c]
15 min	2.90 ± 0.84[c]	1.95 ± 0.00[b]	4.31 ± 0.01[c]	2.79 ± 0.07[c]

[a, b, c,] Means that share these letters show have no significant different in Tukey's test within the same column; *, control is the initial count of the microorganism before treatment.

Table 4. Count reduction (Δ log_{10} CFU/g) in chicken legs meat samplesa

BG Extract	Time (min)	Δ Log_{10} CFU/g (TPC)	Δ Log_{10} CFU/g (*E. coli*)	Δ Log_{10} CFU/g (*B. cereus*)	Δ Log_{10} CFU/g (*S. aureus*)
Cabinet-oven	5	1.58	4.78*	3.51*	2.15
Cabinet-oven	10	3.72*	3.79*	3.33*	3.96*
Cabinet-oven	15	3.87*	4.90*	2.66	3.99*
Extrudate	5	4.36*	4.55*	3.33*	4.46*
Extrudate	10	3.51*	5.20*	2.80	4.15*
Extrudate	15	3.68*	4.75*	2.50	3.95*

[a], count reduction (Δ log_{10} CFU/g) in relation to the total count of log_{10} CFU/g of control sample at time zero; BG, bitter gourd; *, indicate to reductions of ≥ 3 Log_{10} CFU/g (bactericidal activity).

remained stable without showing any increase at time of 15 minutes. Hot air dried BG treatment showed bactericidal activity against *S. aureus* with a 3.9 log_{10} reduction; TPC with a 3.8 log_{10} reduction at time of 15 minutes; and *B. cereus* with a 3.5 log_{10} reduction at time of 5 minutes. Cabinet-oven BG treatment showed better bactericidal activity against *B. cereus* growth at pointed time 5 minutes and 10 minutes than the other treatment. The bactericidal activity of BG extrudate treatment started at time of 5 minutes against the growth of *E. coli, S. aureus,* TPC, and *B. cereus*. Then it remained stable at time of 10 minutes and 15 minutes with all tests except for a slight decrease of the activity in *B. cereus* at 10 minutes and 15 minutes. However, the bactericidal activity of cabinet-oven BG treatment started at time of 5 minutes against the growth of *E. coli* and *B. cereus,* and at time of 10 minutes for S. aureus and TPC before it became stable (Table 4).

In general, hot air dried BG and extrudate treatments showed strong bactericidal activity against the microflora in raw chicken meat. This might be because of presence of relatively high content of saponins in BG powder extracts, and in which might have a non-specific way of action different from other secondary metabolites to show their antibacterial action. The previous studies demonstrated that if saponins content concentration was high enough, the saponins would act as lipophilic compounds in which change bacteria cell membrane fluidity and increase permeability (Wink, 2015).

ANOVA analysis also showed that increasing the exposure time had no significant impact on the overall results of the antibacterial activity of BG extrudate extract which was in contrast to a study carried out by Yusoff, Noor and Rukayadi (2015) that claimed

exposure time had an influence on bacteria growth reduction. However, the claim was in agreement with hot air dried dried extract findings except for isolated *B. cereus*. The first 5 min showed a significant impact of cabinet-oven dried BG extract on *B. cereus* growth reduction with P-value of 0.005 than at other times of 10 and 15 min.

However, the finding which revealed that BG crude extract could be used in decontamination of fresh is similar to the results of using BG seed crude extract with minced meat. Jabeen and Khanum (2014) found that *S. aureus* in fresh minced meat was strongly inhibited by purified peptide in 24h from 8.0 to 3.7 Log. In addition, the results of the concentration 1% (v/v) of the treatments showed success in reducing the high count with Log 6.7 CFU/g of the microflora on the surface of the chicken leg sample to be at low count Log 2 CFU/g.

Thus, it can be concluded that the ethanolic extract was successful in prolonging the freshness of the chicken sample so that it was safe for consumption. Spoilage microflora is present during poultry rearing, production process, storage or preparation, and it can be grown at a relatively low temperature (4°C). Research has shown that an increase in the total bacteria count could influence ammonia content in chicken legs, causing the meat to deteriorate. As a result, the quality of the poultry meat will be affected. This has created an urgent need to ensure a low level of microflora on refrigerated chicken meat at 4°C using natural sanitizer so that the shelf life of the meat parts might be extended. In this study, the extracts succeeded to inhibit organisms that the aetiology of food poisoning recognized as pathogenic bacteria such as *S. aureus* and *E. coli* (Kozačinski *et al.,* 2012). Ozusaglam and Karakoca (2013) found that some foodborne pathogens were susceptible to BG ethanolic extracts and *E. coli* and *S. aureus* among them. This same study also suggested that using BG extract as preservative/sanitizer in food industry. Shortened shelf life of fresh produce is a result of if high microbial populations and then using sanitizing natural agent in the washing process could lower the initial bacterial counts and keep quality and longer shelf life (Zagory, 1999). Thus, BG extracts might have the same effectiveness on removing the native microflora of fresh-cut vegetables as peroxyacetic acid (PPA) does (Vandekinderen *et al*., 2009).

Conclusion

In conclusion, ethanolic extracts hot air dried BG and extrudate succeeded to inhibit the survival of spoilage and pathogenic microorganisms in process wash water such as *Staphylococcus aureus* and *Escherichia coli*. Nevertheless, more studies are needed to develop using BG fruit as a sanitizing agent in wash water sanitizers since the use of low concentration of the extract had eliminated cross-contamination of raw food during washing step. That would have a positive effect towards food safety and it is economical.

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