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RESEARCH ARTICLE

EFFECT OF GELATIN AND PAPAYA EXTRACT COMBINATIONS ON MICROBIAL AND RANCIDITY DEVELOPMENT OF FRESH BROILER CHICKEN BREAST

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Abstract

Fresh breast chicken cuts were prepared and dipped individually in solutions of gelatin combined with papaya extract in a ratio of 1:1, 2: 1 and 1:2, respectively. The control and treated samples were then dripped dried and packaged individually aerobically in ethylene vinyl acetate bags (EVA) and stored at refrigeration temperature of 4^{o}C for 15 days. pH, microbial (Total plate count, TPC), colour and rancidity (Thiobarbituric acid, TBA) were investigated on first day and every 5 days intervals. The study revealed that the combination treatment gave better results in all parameters measured compared to the control. For single treatment it was observed that gelatin in the mentioned concentration was more effective in slowing both microbial growth and rancidity development. However, in combination treatments, gelatin combined with papaya extract in the ratio of 2: 1 had the best result in this regard. In conclusion, for better avoidance of rapid microbial growth and rancidity development the combination of gelatin and papaya extract in the ratio of 2: 1 is highly recommended.

Introduction:-

Fresh meat is well recognized as one of the most perishable foods. This is because of rapid microbial growth to unacceptable levels which significantly contributing to meat spoilage (Gram et al., 2002 and Fung, 2010) and then the meat becomes unsuitable for human consumption.

Poultry carcasses and cuts are usually contaminated with many types of microorganisms such as *Salmonella* and *Campylobacter* (Simmons *et al.*, 2003), *Clostridium perfringens* (Kessel *et al.*, 2001), *Escherichia coli* 0157 (Kessel *et al.* (2001) and Listeria monocytogenes (Gonzalez-Fandos, 2014). When poultry meat is aerobically stored under chill conditions, spoilage microorganisms such as *Pseudomonas* spp., are normally present (Zhang et a, 2012). The spoilage of refrigerated fresh chicken is partially caused by microbial growth such as Pseudomonas species bacteria which are responsible for the discoloration, off flavours, off odours, gas and slime production (Petrová et al., 2013).

Earlier, papaya extract gave positive effect against bacterial infections (Wimalawansa, 1981). An *in vitro* studies conducted on extracts from skin, flesh, and seeds of both ripe and unripe *papaya* gave antibacterial activities against various microorganisms including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and

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Shigella Flexner (Yismaw, 2008). Gelatin is considered as one of the most popular biopolymers. It is tasteless and colourless solid substance derived from the fibrous protein collagen. It is widely used in meat applications alone or with other medicinal plants because of its unique functional, technological and antimicrobial properties (Tiwari, 2009 and Jayathilakan, 2012). This study was carried out to investigate the effects of gelatin and gelatin combined with papya on microbial and rancidity development of fresh chicken meat.

Materials and methods:-

Preparation of meat samples:-

Recently slaughtered broiler chicken breasts were purchased from local market (Selangor, Malaysia). The meat cuts were prepared by slicing the chicken breasts parallel to the muscles fibres direction to pieces measuring 1 x 10 x 5 cm (thickness x length x width) and surface area of approximately 50 cm^2 and weighing about 80 g using filleting knife.

Preparation of papaya extract:-

The leaves of papaya were collected from papaya farm, Asia Fruits, Selangor, Malaysia. The leaves were transported to extraction laboratory, Universiti Technologi MARA (UiTM). The leaves were then washed with tab water to remove strange materials and spread on clean table and left to dry overnight at room temperature. The semi dry leaves were placed next day in an oven set at 45^oC and left for one week to fully dry. The dry leaves were broken by hands into small pieces and placed in a Waring blender set at high speed for 20 min. and a fine powder of the leaves were obtained. According to modified method of Skandamis (2002) aqueous extraction was conducted using methanol and water in a ratio of 20: 80, respectively. 20 g of the dry blended leaves were placed in 1000 mL flask and the mixture of methanol in water was added in the ratio of 1:20 (blended leaves: mixture of methanol in water, respectively). The flask was covered with aluminum foil and left till the next day. The mixture was filtered through filter paper no.1 using a funnel. The filtrate was collected and rotor vaporized to remove the water. The extract was then freeze - dried using freeze dryer model (Scanvac Coolsafe, RZ 2.5, Germany) and the extract in form of powder of was obtained. The powder of the extract was then placed in a clean dry bottle using paraffin lamination till use.

Preparation of treatment solutions:-

50 gram of either gelatin (gelatin type B from bovine skin which was purchased from Sigma Co., Malaysia) or papaya extract individually were placed in a 1000 ml beaker. Deionized water was added and the beaker was put in hot water bath to enhance melting. The contents were mixed thoroughly with glass rod individually or together using. The solution then was left to cool down at room temperature. For preparation of combination of gelatin and papaya in a ratio of 1:1 and concentration of 5%, 25 gram from each were taken and the same procedure was followed.

Samples treatment:-

The meat samples were dipped in the prepared solutions individually for 5 min and after that left to drip dry for 15 min. The control sample was dipped in deionized water. Triplicate of each treatment was conducted. The treated samples were then stored at refrigeration temperature for 15 days. Measurements were taken in the first day and every 5 days interval.

pH measurement:-

10 grams of the sample were obtained and homogenized with 90 ml of deionized water (in the ratio of 1:9), using laboratory blender (Waring products Division Torrington, CT, USA.) for 1 min at low speed. Digital pH meter (Tolledo 420 pH meter, Mettler- Instrument, Germany) was used after standardizing it with two-buffer solutions; one in pH 7.0, and the other in pH 4.0. The pH values of the samples were measured on first day (day 0) and every 5 days during storage period at 4^oC. The pH of readings were obtained in the slurry of the samples with the direct insertion of the pH probe electrode of the pH meter. Triplicates measurement were taken from each sample.

Total plate count assessment:-

Total Plates Counts (TPC) of the samples were determined according to the method of Pizato et al. (2015). 10 ± 0.1 grams of were removed from each package using a sterile knife and weight is taken using sensitive balance (4 decimal digits).The sample were then aseptically transferred to a steriled stomacher bag containing 90 mL of peptone water. The samples were put in a stomacher blender 400 (UAC, London, Britain) and homogenized individually for 2 min. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were then prepared. 0.1 mL of each above

dilution was spread on the plate count agar and the plates were incubated at 37°C for 48 hr. TPC were obtained from plates bearing 30 – 300 colonies in all samples. Triplicate measurements were taken and reported as the $\log_{10}$ of the numbers of colony forming units.

Thiobarbituric acid value measurement:-

2-Thiobarbituric acid value was determined using distillation method described by Egan et al. (1981). Thiobarbituric acid (TBA) solution was prepared using acetic acid glacial 90%. 0.2883 g of TBA powder was accurately taken and dissolved in 100 mL acetic acid solution (w/v) was prepared by dissolving TBA powder (w/v). 10 g of the meat were macerated with 50 mL of distilled water for 2 min. and the mixture was then washed into a distillation flask with 47.5 mL of distilled water. 2.5 mL of 4N hydrochloric acid were added to the mixture, together with an anti-foaming agent A (Sigma), and a few glass beads were added. The mixture was placed in a round bottom flask (250 mL) and boiled for 10 min. About 50 mL of the distillate was collected and 5 mL of the distillate obtained were pipetted into a glass-stoppered tube. 5 mL of TBA solution were added to the mixture. The tube was stoppered, shaken well and heated in a boiling water bath for 35 min. A blank tube was similarly prepared using 5 mL of water and 5 mL of the reagent without meat sample. The tubes were then cooled in water for 10 min. A spectrophotometer model (82-2118-00, Cambridge, England) was used for the measurement. The absorbance value of the colour (D) was measured through a 10 mm glass cell against blank sample at light weave length of 538 nm. The TBA value was then calculated as milligrams of as malonaldehyde (MA) per kilogram of sample which is equal to 7.8 x D. Triplicates of measurements were recorded.

Statistical Analysis:-

The data obtained were analysed for two way analysis of variance (ANOVA) using Minitab Statistical Software (Minitab Inc., PA, USA) package 16. The results are expressed as mean ± S.D.

Results and Discussion:-

The results of this study during storage period are present in Table 1, Figure 1 Table 2. The pH values of the samples ranged from 5.0 to 7.5. Comparing to the control sample, all treatments have shown decreases in the values of the pH. The value dropped from 5.5 to 5.2, 5.3, 5.1, 5.1 and 5.0 in the sample treated with PS alone, GS alone, combination of PS:GS in (1:1), PS:GS (1:2) and PS:GS (2:1), respectively. The lowest pH value was observed in the sample treated with the combination of PS:GS in the concentration of 2:1. During storage period the pH values of all samples increased gradually till reached the maximum value of 7.5 in control sample. At the end of the storage period the highest pH value was observed followed by samples treated with GS, PS: GS (1:2), PS: GS (1:1), PS and PS: GS (2:1), respectively. Yang et al. (2014) reported that in normal pH meat only few microorganisms such as *C. frigidicarnis*, *C. estertheticum* and *C. tagluense* could be possibly grow substantially. Mountfort et al., (1997) and Spring et al. (2003) reported that the minimum pH values for growth of such microorganisms was above 5.5. Low pH value (< 5.0) would seem to be an indicator of some spoilage microorganisms such as *C. algoriphilum*, *C. frigidicarnis* and *C. algidixylanolyticum* (Broda et al., 1999, Broda et al., 2000 and Shcherbakova et al., 2005). Even so, low pH would seem to play a part in restricting the growth of most of the species on normal pH meat, because most grew well on meat of intermediate pH and all grew well on meat of high pH value.

Table 1:- pH value of the samples during storage at 4°C for 15 days.

Days of storage	Control	PS	GS	PS:GS (1:1)	PS:GS (1:2)	PS:GS (2:1)
pH vales						
0	5.5 ± 0.2	5.2 ± 0.1	5.4 ± 0.1	5.1 ± 0.2	5.1 ± 0.1	5.0 ± 0.2
5	5.8 ± 0.3	5.4 ± 0.2	5.6 ± 0.2	5.3 ± 0.2	5.4 ± 0.3	5.2 ± 0.2
10	6.6 ± 0.2	5.8 ± 0.2	6.2 ± 0.4	5.5 ± 0.4	5.7 ± 0.4	5.5 ± 0.1
15	7.5 ± 0.1	6.0 ± 0.3	6.6 ± 0.3	5.9 ± 0.2	6.1 ± 0.3	5.8 ± 0.3

Note:
PS: Gelatin solution
GS: Papaya solution

The initial TPC of control sample was 3.5 $\log_{10}$ per g and it was decreased to between 3.1 to 3.5 $\log_{10}$ per g in the treated samples. After treatments all samples showed decreases in TPC values. They were in the order control > PS: GS (1:2) > GS > PS > PS: GS (2:1). At the end of the storage time highest microbial growth was observed in

control sample. The value jumped from 3.5 to 6.5 while on the other hand the sample treated with PS: GS (2:1) gave the lowest microbial growth. Shaikh *et al*. (2003) reported that meat samples developed off-odor and discoloration with TPC > 10^6 cfu/g. Also Earlier, It was reported that at 6 -7 $\log_{10}$ cfu/g, meat reached spoilage and it became unsalable (Dainty and Mackey, 1992). In this study it was observed that the TPC at which meat could be considered as spoiled when the microbial reaches 10^6 cfu/g was not reached on last day of storage (day 15). The best treatment that caused good inhibition in microbial growth at the end of the storage period was the sample treated with PS: GS (2:1).

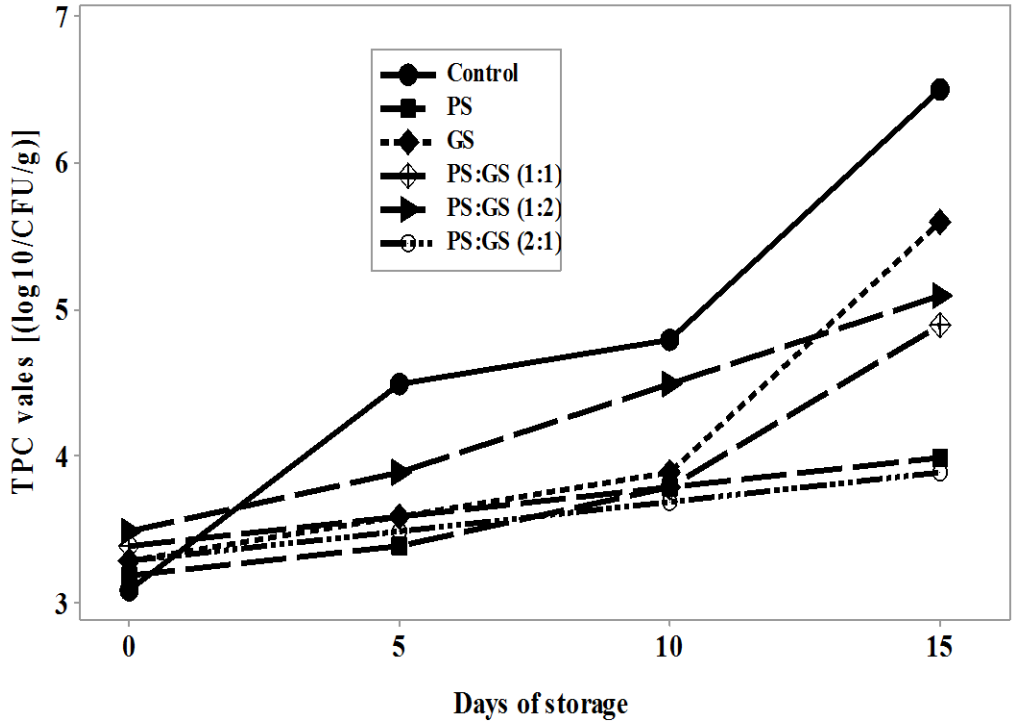

Figure 1: Scatterplot of TPC values of the samples during storage at 4oC for 15 days.

Lipid oxidation showed the same trend similar to pH and microbial growth. TBA increase significantly ($p < 0.05$) in control sample from 0.35 mg MDA/kg reaching a value of 0.68 mg MDA/kg. Earlier, Boles and Parrish (1990) have reported that a warmed-over flavor of meat could be perceived in meat products when TBA values reached above 1.0 mg MDA/kg. In another studies it was reported that a rancid flavor is initially detected in meat products with TBA value of 2.0 (Gray and Pearson (1987). The best treatment that controlled lipid oxidation development in this study was the combination of gelatin and papaya extract in the ratio of 2: 1.

Table 2:- TBA value of the samples during storage at 4^{o}C for 15 days.

Days of storage	Control	PS	GS	PS:GS (1:1)	PS:GS (1:2)	PS:GS (2:1)
TBA vales (mg Malonaldehyde/kg)						
0	0.35 ± 0.1	0.32 ± 0.1	0.34±0.1	0.33±0.2	0.36±0.1	0.34±0.2
5	0.39 ± 0.3	0.35 ± 0.2	0.33±0.2	0.31±0.2	0.39 ±0.3	0.36±0.2
10	0.46 ± 0.2	0.38 ± 0.2	0.42±0.2	0.35±0.4	0.47±0.4	0.38±0.1
15	0.68 ± 0.1	0.45 ± 0.3	0.46±0.1	0.43±0.2	0.51±0.3	0.41±0.2

Note:
PS: Gelatin solution
GS: Papaya solution

Conclusion:-

In conclusion, it was found that all treatments caused great delaying in both microbial and rancidity development of chicken breast. The treatment in the ratio of 2:1 was found to be more effective in this regard. It is highly recommended to treat fresh chicken meat with the latter combination for better results. More researches could be needed to investigate additional details such as the relationship between active materials in papya and specific types of microorganisms.

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