



EXTRACTION OF POMEGRANATE PEEL AND GREEN TEA LEAVES AND THEIR EFFECTS ON THE MICROBIAL, PHYSICOCHEMICAL, MICROSTRUCTURAL AND SENSORIAL PROPERTIES OF CHILLED-STORED CHICKEN MEAT

(Pengekstrakan Kulit Buah Delima dan Daun Teh Hijau dan Kesannya Terhadap Sifat-Sifat Mikroorganisma, Fizikokimia, Mikrostruktur dan Deria Rasa Daging Ayam yang Disimpan pada Suhu Sejuk)

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Abstract

Pomegranate (*Punica granatum* L.) peel extracts (PPE) and green tea (*Camellia sinensis*) leaves extracts (GTE) have the potential to be the natural preservatives to prolong the chicken meat quality stored in chilling temperature. The first part of this work aimed to determine the effects of solid-liquid extraction (SLE) and ultrasound-assisted extraction (UAE) in extracting the pomegranate peel and green tea leaves. The second part was to determine the microbial, physicochemical, microstructural and sensorial properties of chicken meat applied with the PPE, GTE or PPE+GTE and stored in chill temperature for seven days. UAE method resulted in higher antioxidant activity in PPE and GTE at 74.3% and 70.4%, respectively compared to SLE method at 48.2% and 41.5%, respectively. The GTE inhibited the microbial growth with 5.47 and 5.96 log CFU/g of the chicken meat at the third and seventh day of chilled-storage, respectively. The water holding capacity, pH, lipid peroxidation and texture were not affected by the extracts. GTE affected the chicken meat by increasing the yellowness (b^*), changing the microstructure, and reducing the sensory acceptability. Overall, GTE can be used as a natural preservative for chilled chicken meat, however, further additional treatments are needed to overcome the negative effect on the chicken meat characteristics.

Keywords: chicken meat, green tea leaves, natural antioxidants, pomegranate peel, solid-liquid extraction, ultrasound-assisted extraction

Abstrak

Ekstrak kulit buah delima (*Punica granatum* L.) (PPE) dan ekstrak daun teh hijau (*Camellia sinensis*) (GTE) berpotensi menjadi pengawet semula jadi untuk mengekalkan kualiti daging ayam yang disimpan dalam suhu sejuk. Bahagian pertama kerja ini bertujuan untuk mengenalpasti kesan pengekstrakan pepejal-cecair (SLE) dan pengekstrakan secara-ultrabunyi (UAE) dalam mengekstrak kulit buah delima dan daun teh hijau. Bahagian kedua adalah untuk menentukan sifat mikroorganisma, fizikokimia, mikrostruktur dan deria rasa daging ayam yang diaplikasikan dengan PPE, GTE atau PPE+GTE dan disimpan dalam suhu sejuk selama tujuh hari. Kaedah UAE menghasilkan aktiviti antioksidan yang lebih tinggi pada PPE dan GTE masing-masing pada 74.3% dan 70.4% berbanding kaedah SLE masing-masing pada 48.2% dan 41.5%. GTE menghalang pertumbuhan mikroorganisma masing-masing dengan 5.47 dan 5.96 log CFU/g daging ayam pada hari ketiga dan ketujuh penyimpanan sejuk. Kebolehan memegang air, pH, peroksidaan lipid dan tekstur tidak dipengaruhi oleh ekstrak. GTE mempengaruhi daging ayam dengan meningkatkan warna kekuningan (b^*), mengubah struktur mikro, dan mengurangkan penerimaan analisa deria rasa. Secara keseluruhan, GTE dapat digunakan sebagai pengawet semula jadi untuk daging ayam sejuk, namun, pengolahan tambahan lebih lanjut diperlukan untuk mengatasi kesan negatif kepada ciri daging ayam.

Kata kunci: daging ayam, daun teh hijau, aktioksidan semulajadi, kulit buah delima, pengekstrakan pepejal-cecair, pengekstrakan secara-ultrabunyi

Introduction

Meat, especially chicken meat possesses high percentage of water and abundant nutrients on its surface and is highly susceptible to microbial decay [1]. The spoilage occurs through microbial activities, oxidation of meat protein and lipid [2], especially when stored for a longer time in chilled or room temperature. Recently, plant phenolic compounds which contain natural antioxidant have shown remarkable results when used as food preservative and free radical scavenging agent and at the same time containing health benefits against several diseases [3, 4]. Several abundant plants such as pomegranate fruits and green tea contain high phenolic compounds and may be used as an agent to preserve chicken meat.

Pomegranate fruit (*Punica granatum* L.) is known to be a high source of antioxidants, its peel, in particular, has much higher phenolic compounds compared to other parts [5]. Recent investigations have shown that the pomegranate peel has high phenolic compounds with remarkable medicinal importance [6]. Green tea (*Camellia Sinensis*) is considered the second most consumed beverage around the world after plain water. It has up to 36% phenolic compounds, the amount of which is related to season, variety, and climate [7]. Mitsumoto et al. [8] applied tea catechins and vitamin C on cooked/raw beef and chicken meat patties and showed that green tea was more potent than vitamin C

and extended the shelf life of the meat products even longer. Green tea has also been applied as a preservative in Turkish dry-fermented sausage [9].

Several extraction methods have been used to extract phenolic compounds from these types of plants. These methods vary according to the nature of the plants and their antioxidant activities; the methods can be divided into simple (or conventional) methods and advanced methods [10]. Solid-liquid extraction (SLE) has been used for a long time to extract phenolic compounds from various types of plants [11]. Some important variables in SLE must be considered. For instance, the solvents used in the process such as ethanol, methanol, water or ethyl acetate are strongly dependent on contact time, stirring, and temperature [12]. Of the advanced extraction methods, ultrasound-assisted extraction (UAE) is the most commonly used and preferred in the field of food science because it can easily be used and is more economical compared to other methods [13]. The method uses ultrasonic waves to change the properties of the target plant, interrupt its cell walls and its cavity creation mechanism, causing the release of all the compounds in the cell that cannot be easily extracted. Moreover, UAE uses less solvent, produces a high-quality product, is easy to use, and results in a highly purified product [14].

The methods used could influence the phenolic compounds extractions and the extracts obtained could be applied as a natural preservative of raw chicken meat. Therefore, the objectives of this study were 1) to compare the effectiveness of the solid-liquid extraction (SLE) and ultrasound-assisted extraction (UAE) in extracting pomegranate peel and green tea extracts and 2) to determine the physicochemical, microbial and sensorial properties of raw chicken meat applied with the best extract, either pomegranate peel extract (PPE) or green tea extract (GTE) or a combination of both and then stored at 4 °C for seven days.

Materials and Methods

Chemicals and materials

Analytical grade chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA), including the Folin-Ciocalteu reagent, quercetin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ethanol, ethyl acetate, sodium carbonate, potassium acetate, and aluminium chloride. The pomegranate (*Punica granatum* L.) variety native to India, natural dried green tea (*Camellia sinensis*) native to Taiwan, and sodium hypochlorite were purchased from a local market in Seri Kembangan, Selangor. The raw breast chicken meat was purchased from Azli Chicken Meat Whole seller and shipped at 4 °C, 3 hours after slaughter to the laboratory.

Plant samples drying processes

The pomegranate received from the local market was manually peeled and washed with flowing water. The peels were then washed with 20 ppm sodium hypochlorite solution for 15 minutes [15], followed by washing with deionized water. The peels were cut into approximately 1 cm², placed on an aluminium tray with some distance between the peel slices and dried in an air-circulated oven at 45 °C. After that, the dried peel was ground with a Panasonic MX-GM1011 grinder followed by sieving through a 0.425 mm stainless steel sieve. Following that, the sample was immediately stored at -21 °C [16]. Dried green tea was received from the market and then ground using a grinder followed by sieving via a 40 mm mesh stainless steel sieve at room temperature [17, 18].

Solid-liquid extraction process

The phenolic compounds from the dried pomegranate peels and the green tea leaves were extracted using a thermostatic water bath shaker (WNB 14 c/w sloping). The extraction of the pomegranate peel extract was carried out using an ethyl acetate solvent at a 15:1 (w/w) ratio of solvent to sample (dry weight) at 40 °C for 4 hours. The green tea was extracted in 80% acetone solution for 15 minutes at room temperature in a conical flask followed by filtration using a Whatman No. 1 filter. The supernatants of both extracts were vacuum-dried in a rotary evaporator at 40 °C in a dark room until 95% evaporation was reached and the remaining 5% solution was then freeze-dried and stored at -21 °C for further usage. [18,19]. The yield was obtained using equation (1).

Ultrasonic-assisted extraction process

The phenolic compounds from dried pomegranate peel (4 g) were extracted using 200 mL of 70% ethanol solution in an ultrasonic bath set to 60 °C for 30 minutes followed by centrifugation at 4500 rpm for 10 minutes and then filtered using a Whatman No. 1 filter. The supernatant was vacuum-dried in a rotary evaporator at 40 °C in a dark room until 95% evaporation was reached [16, 20]. The remaining 5% solution was then freeze-dried and stored at -21 °C for further usage. The extraction of green tea phenolic compounds was done using an electronic ultrasonic bath at 35 kHz set to 160/640 W input power. Green tea (4 g) was extracted with 400 ml double distilled water at 70 °C. Then, the green tea was placed in an ultrasonic cleaner bath at 80 °C for 20 minutes. After that, it was filtered through Whatman No. 1 filter paper and the extract was cooled at room temperature (25 °C) [17]. Next, centrifugation was done at 4500 rpm for 10 minutes and the tea filtered through a Whatman-1 filter. The supernatant was vacuum-dried in a rotary evaporator in a dark room at 40 °C until 95% evaporation was achieved [16]. The remaining 5% solution was then freeze-dried and stored at -21 °C for further usage.

Total antioxidant activity

Antioxidant activity was measured using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The DPPH (1 mg) was dissolved in 1 mL methanol to

prepare a 1 mg/mL DPPH solution. As the control absorbent, the DPPH solution was further dissolved with 5 mL MeOH solution and observed at 517 nm using a UV-Spectrophotometer. Both PPE and GTE samples (0.05 g each) were dissolved in 5 ml methanol solution. Then, 100 μ L of prepared DPPH (1 mg/ml) solution was added to 100 μ L of the prepared sample solution. This was followed by dilution in 5 mL methanol and incubation at room temperature for 30 minutes and then absorbance at 517 nm UV-Spectrophotometer (Thermo-10D) [21]. The radical scavenging activity can be expressed using equation (2).

Estimation of total phenolic content

The Folin-Ciocalteu colourimetric method was used to determine the phenolic compounds. Samples of PPE and GTE (0.125 g each) were dissolved in 100 mL water. An aliquot of 70 μ L of the prepared sample, 759 μ L of 1.9M sodium carbonate, and 250 μ L of Folin-Ciocalteu were added to a 10 mL test tube. The test tube was filled with distilled water up to 5 mL and vortexed for one minute. It was then incubated for 2 hours in a dark room. The absorbance was detected at 765 nm using a UV-visible spectrophotometer. Gallic acid standard solutions were used to prepare the calibration curve and the result stated in mg/g dry solid [22].

Estimation of total flavonoid content

Calorimetric method of aluminium chloride was used to determine the flavonoid compound. An aliquot (1.25 mL/mg) of the PPE and (3.5 mg/mL) of the GTE were prepared in methanol solution. An aliquot (0.5 mL) of the prepared sample was further dissolved in 1.5 mL of methanol followed by mixing with 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminium chloride and 2.8 mL of distilled water. It was incubated for 30 minutes in dark at room temperature. The absorbance was detected at 415 nm by using UV-visible spectrophotometer. Quercetin standard solution was used for the preparation of a calibration curve and the result was stated as mg/g dry solids [23].

Chicken meat sample preparation and treatment

Under an aseptic condition, raw chicken breast meat, 3 hours after slaughter, was sliced into sizes of 1.5 cm thick, 2 cm long and 8 cm high portions. The extracts were prepared at 0.02% concentration using sterilized distilled water and applied to the chicken meat at the ratio of 1: 2 (meat: extract). Four treatments were prepared as follows: 1) control (meat: sterilized distilled water), 2) (meat: PPE), 3) (meat: GTE), and 4) (meat: PPE+GTE). The samples were marinated for one minute each, and then wiped using sterilized tissue paper, packed in the aerobic condition in low-density polyethylene bags and stored at 4 °C under aseptic conditions [24]. The analyses were carried out on the zero-day, third-day and seventh-day. The appearance of the chicken meat changed drastically by the higher concentration and longer time of the marination based on the preliminary study. Therefore, lower concentration and shorter time of marination were justified to minimize changes in the appearance of the chicken meat.

Microbial quality evaluation

A plate count agar (PCA) and potato dextrose agar (PDA) were used to measure the total count of bacteria, yeast, and mould. The samples were homogenized (Seward™ Stomacher™, Model 400 Circulator Lab Blender, 110V, England) with sterile peptone water in 10 mg/90 mL, meat/sterile peptone water. The mixture was further diluted with 0.1% peptone water before inoculation to PCA and PDA. Four serial dilutions (10^{-2} - 10^{-5}) were used for each sample in triplicate. After incubation at 35-37 °C for 24-48 hours (PCA) and 25 °C for 72-120 hours (PDA), both plates were read [24].

Determination of water holding capacity

An aliquot of 1 cm long cylinders of the meat sample was weighed and enfolded in filter paper. Then, the sample was centrifuged at $10000 \times g$ for 10 min at 10 °C and the sample was weighed again [25]. The water holding capacity was calculated using the equation (3).

$$\text{Yield (\%)} = \frac{\text{Total weight of the extract}}{\text{Sample total weight before extraction}} \times 100 \quad (1)$$

$$\text{Inhibition (\%)} = (\text{Control absorbance} - \text{Sample absorbance}) / (\text{Control absorbance}) \times 100 \quad (2)$$

$$\text{Water loss (\%)} = (\text{Pre-weight} - \text{Post-weight}) / (\text{Pre-weight}) \times 100 \quad (3)$$

pH analysis

The chicken meat sample (10 g) was homogenized (Seward™ Stomacher™, Model 400 Circulator Lab Blender, 110V, England) in 100 mL distilled water. After filtration, the mixture pH was measured using a pH meter (SevenMulti, Mettler-Toledo GmbH 8603 Scherzenbach, Switzerland) [26].

Lipid peroxidation measurement

A 5 g meat sample was mixed with 25 mL of 7.5% trichloroacetic acid (w/v) including 0.1% ethylenediaminetetraacetic acid (EDTA). It was integrated into a Panasonic grinder MX-GM1011, at 15000 rpm. Later, at room temperature, it was centrifuged at 3600 g for 20 minutes, and purified via a Whatman 4 filter. Then, the Supernatant was combined in a boiling bath for 30 minutes with 5 mL TBA 0.02 mol/L reagent. It was subsequently cooled to room temperature, and the absorbance recorded against a blank sample at 532 nm (5 mL TBA mixed with 5 mL distilled water). In the mg malondialdehyde per kg of meat sample, the result of thiobarbituric acid reactive substances (TBARS) (mg MDA/kg) was shown [26].

Colour analysis

The colour of meat was measured vertically in triplicates on the surface of the meat using a Chroma Meter (CR-410, Japan) to obtain an average value. The results were described as lightness (L^*), redness (a^*) or yellowness (b^*) [26].

Texture profile analysis

The texture profiles (hardness, chewiness, springiness and cohesiveness) of the samples were evaluated using a texture analyzer (Stable Micro Analyzer TA-XT2i, UK). The samples were kept in a closed plastic container and allowed to reach to 25 ± 1 °C before the analysis. A 50 mm flat-bottom aluminium cylindrical probe P/50 SMP was used at 25 °C. All the samples were formed into a cylindrical shape (24 mm diameter, 20 mm height) and compressed by 40% using a double compression

cycle test. The pre-test speed was 1 mm/s, the test speed was 1 mm/s, and the post-test speed was 2 mm/s [27].

Microstructure of meat

The samples were cut into 1 cm × 1 cm × 0.5 cm. dehydrated at 45 °C for 24 hours. Next, the sample was attached to aluminium stubs and then coated with gold. A scanning electron microscope (JEOL JSM-T100 model) was then used to photograph the sample using an accelerated voltage of 1, the micrograph and the video print of the specimen were captured at 300x magnification [28].

Sensory analysis

Every sample was assessed by 30 untrained panelists using a 9-point hedonic scaling test in terms of colour, aroma, flavor, tenderness, juiciness, springiness and overall satisfaction. The ratings were as follows: 1- disliked very much, 2-disliked, 3-moderately disliked, 4-slightly disliked, 5-indifferent, 6-liked slightly, 7- liked moderately, 8-liked, and 9-liked a lot. The chicken meat samples were cooked for 40 minutes at an internal temperature of 120 °C in an electric oven (NN-8655, Panasonic, Osaka, Japan) cooled up to 25 °C, cut into dices (1.0 cm³). The samples were then marked with the three-digit number randomly selected and randomly distributed to all panelists. The panelists tasted the sample one at a time and cleared their mouths with water between each sample [29].

Statistical analysis

A triplicate system was used to examine the meat samples and the Minitab 17.0 (Minitab Inc., State College, PA, USA) software was used for significant differences ($p < 0.05$). The comparison was made using one-way ANOVA for each factor separately without considering the interaction between factors.

Results and Discussion

Extraction yield, total antioxidant activity, total phenolic content and total flavonoid content of the extracts

The SLE is a simple method for the extraction of phenolic compounds using polar solvents such as methanol, ethanol, acetone, chloroform, and ethyl acetate. Without advanced technologies, it can be applied easily and less expensive, however, due to the chemicals used, this method is less preferred. The UAE method is derived from the technology of ultrasonic processing which uses as an innovative technology in the food industry. It allows sonication through the process of food extraction, which is created by sound waves. Ultrasound wave sound is produced through a process of rarefaction and compression that produces 2 MHz or greater high-frequency sound. It generates cavitation bubbles near the sample tissue, thus disrupting the walls of plant cells and releasing their cell content [30].

Table 1 shows the extraction yield, total antioxidant activity, total phenolic content and total flavonoid content of pomegranate peel extracts (PPE) and green tea extracts (GTE) via the SLE and UAE methods. The UAE method showed significantly ($p < 0.05$) higher yield compared to SLE, i.e., 22.9% and 28.1% yield for PPE and GTE, respectively. The PPE yield in this study is higher than the 22% yield obtained in a previous study, which used pulsed ultrasound-assisted extraction [31], but it is lower than the 45% yield obtained in a similar study [16]. The reason for this quantitative decrease might be that the freeze-drying method used in this study. Because it resulted in a dry product, however, the other study used rotary evaporator for obtaining the final extract, which normally produces a sticky product and is weighty compared to freeze-drying, which contains less water.

The SLE method yielded 1.6% and 22.6% phenolic compounds for PPE and GTE, respectively. The PPE yield in this research is comparable to that of Wang et al. [19], who obtained a 1.04% yield. However, the use of ethyl acetate caused less extraction of phenolic yield

in comparison to other solvents such as methanol and water, albeit with higher antioxidant activity. The GTE yield in the current finding is double that (9.8%) of a previous study [18]. Perva-Uzunalić et al. [32] also extracted a high phenolic yield from GTE using acetone compared to other polar solvents as part of SLE. The UAE method resulted in 74.3% and 70.4% antioxidant activity in PPE and GTE, respectively, which were significantly ($p < 0.05$) higher than the 48.2% and 41.5% antioxidant activity for PPE and GTE, respectively obtained via SLE. The antioxidant activity of PPE in this study is much higher than that of similar studies by Ali et al. [21], i.e., 68.66% and Tabaraki et al. [16] i.e., 67.94%. The antioxidant activity of GTE in this research was less than the 92% obtained by Das and Eun [17], but it was higher than the extraction using ethyl acetate from a study by Gadkari et al. [33], who obtained 42% yield.

The phenolic content of PPE extracted using UAE was significantly higher ($p < 0.05$) than that of GTE, which was 328.7 mg GAE/g sample and 268.8 mg GAE/g sample, respectively. The TPC result of PPE in this work was much higher than the result of similar research i.e., 86 mg GAE/g sample [16], 131.1 mg/g and 161.25 mg/g [20] and 276 mg/g [5]. The TPC result of GTE in this research was also higher than the GTE obtained by Das and Eun [17], i.e. 167 ± 3 g. The SLE method resulted in 389.5 mg GAE/g and 708.7 mg GAE/g of TPC for PPE and GTE, respectively. This value is higher than similar research previously carried out by Drużyńska et al. [18] and Wang et al. [19]. Different cultivars of the same plants could be one of the factors for the different amounts of TPC obtained [5].

The UAE method produced a significantly higher ($p < 0.05$) TFC level compared to SLE, while PPE showed a notably higher ($p < 0.05$) TFC level than GTE. The TFC amount obtained using UAE was 30.0 mg and 6.3 mg QE/g of PPE and GTE, respectively, whereas SLE obtained 7.9 mg and 0.4 mg QE/g for PPE and GTE, respectively. Kanatt et al. [20] reported that PPE extracted via UAE produced of 7.57 mg/g dry weight flavonoids, about three times less than the current research. However, Das and Eun [17], reported 62 mg/g

dry weight of flavonoid in GTE, which was higher than the results of this work. The SLE method resulted in significantly higher TPC from GTE correlated to UAE and the reason might be due to different solutions and methods for the extraction purpose, however, based on the results collected from the antioxidant activity, TPC (from PPE) and TFC analyses, the PPE and GTE extracted using UAE were the best overall, since the target is higher antioxidant treats hence the UAE method was therefore applied on the raw chicken meat for further investigation.

Application of the phenolic extracts on raw chicken meat: Microbial evaluation

Table 2 shows the total bacteria, yeast, and mould counts of raw chicken meat treated with PPE and GTE measured during storage for seven days at 4 ± 1 °C. No changes were observed between treatments on the zero-day of storage, most probably due to immediate analysis after the application of the extracts. The log counts for all the samples were quite high because no pre-treatments were carried out on the samples, which could be contaminated during the slaughtering and handling. On the third day, the GTE notably decreased ($p < 0.05$) the bacterial count to 5.47 log CFU/g, while, PPE decreased it to 5.89 log CFU/g and GTE mixed with PPE reduced the bacterial count to 6.50 log CFU/g compared to the control (6.81 log CFU/g). It showed that GTE and PPE had the potential to slow down the microbial growth, however, the combination of both could trigger the antagonistic effect as compared to individual treatment. On the seventh day, the bacteriostatic effect of the extracts showed no functionality on the chicken meat, however, the GTE still was able to keep the chicken meat below log 6. In common practice, to keep chicken meat at the chilling temperature more than three days is considered as not safe due to the fragility of the chicken meat, however, this study proved otherwise by the addition of GTE, specifically. The yeast and mould analyses on the treated chicken meat showed no significant changes except for an increment on the third day of storage when both PPE and GTE were combined and applied together. As the yeast and mould are more difficult to be inhibited compared to the bacteria, the antagonistic effect of PPE and GTE, and the uneven

distribution of microbial load that occurred naturally in the chicken meat could be affecting the results.

Similar research on some plant extracts (rosemary and cloves) applied on beef also showed significant inhibition of bacteria after 9 days of refrigerated storage, however, the sample used was cooked [34]. Likewise, GTE has been claimed to decrease microbial spoilage of raw beef meat stored at 4 °C for 9 days [35]. Malviya et al. [36] also proved the antibacterial activities of pomegranate extract and hypothesized its antibacterial effects against gram-positive and gram-negative bacteria. They declared that the antibacterial effects of PPE targeted metabolic toxins or broad-spectrum antimicrobial compounds. Kanatt et al. [20] applied different concentrations (0.01%, 0.05% and 0.1%) of the PPE on the chicken products and found that it is effective against the well-known food inhibitors such as *E. coli*, *S. typhimurium*, *Pseudomonas* spp., *S. aureus* and *B. cereus*. The PPE was also claimed to be effective against Gram-positive bacteria and showed significant result only against *Pseudomonas* spp. of Gram-negative.

The natural phenolic compound has certain antibacterial activity *in vitro* [35], several factors affecting the antimicrobial traits of natural antioxidants and the existing of these factors decrease its efficiencies such as low pH and low solubility. Generally, the phenolic compounds showed their antimicrobial effects when tested on the media, however, their antimicrobial effects are decreased when applied to food [34]. Microbial spoilage usually limits the shelf life of the raw meat. Raw patties have a shelf life of around 7 days based on hygiene and pre-evasion conditions of refrigeration and aerobiosis [35]. The reason for no significant increase in log CFU/g of the control sample on the seventh day of storage time might be proper hygiene and proper refrigeration condition. The fluctuation of microbes is related to appropriate conditions (nutrient, temperature, pH, and water activity), so the growth of bacteria also changes when the optimal condition is altered [37].

Water holding capacity, pH and lipid peroxidation

Table 3 shows the impact of PPE and GTE on the water holding capacity, pH and lipid peroxidation of raw chicken meat stored at 4 ± 1 °C for seven days. The

extracts did not significantly ($p > 0.05$) affect the water holding capacity of meat samples compared to the control. Similar studies confirm the result of this research [38]. However, Morsy et al. [39] found that the nanoparticles of pomegranate peel extract improved the water holding capacity of meatballs during refrigerated storage, however, the pomegranate peel extract was mixed in the meatballs' emulsion, where else in this study the extracts were only applied on the surface. The application of the extracts significantly reduced ($p < 0.05$) the pH of the meat on zero-day compared to the control but did not have any notable effects ($p > 0.05$) on the third and seventh days of storage. The treated meat sample with GTE (5.65 ± 0.05) showed the lowest pH followed by PPE + GTE (5.88 ± 0.04), PPE (6.01 ± 0.11), and the control (6.18 ± 0.08). A similar study confirms the result of this research, i.e., Qin et al. [40], found that pomegranate peel extracts significantly decreased the pH of raw ground pork from pH 5.88 to 5.61. Low pH can inhibit microbial growth, as the optimal level for bacteria to grow is around pH 7.0 [34, 41]. This could be one of the factors that helped in controlling the bacteria growth especially for the sample treated with GTE.

The application of the extracts, however, did not significantly change ($p > 0.05$) the TBARS values of the chicken meats compared to the control and during storage time. Vaithyanathan et al. [24], claimed that dipping samples in PPE had little effect on the TBARS values of the treated samples, but significant ($p < 0.05$) changes were observed on the fourth day of storage. A study on meat products also declared the significant effects of green tea on the inhibition of TBARS value [9]. Turgut et al. [42] stated that PPE inhibited the TBARS value of beef meatball. The less significant results of the TBARS value in this study might be due to the lower percentage of phenolic extracts used, as some mentioned studies applied up to 1% phenolic extract, which is much higher than the 0.02% used in this research. Selection of low concentrate phenolic (0.02%) in this research was considered due to the effect on the chicken meat sensory attributes.

Colour of the chicken meat

Table 4 shows the effects of PPE and GTE on the colour of raw chicken meat stored at 4 ± 1 °C. The result reveals that the phenolic extracts from different sources did not have any significant effect ($p > 0.05$) on the lightness (L^* value) and redness (a^* value) of the chicken meat except a lower L^* value for PPE+GTE-treated sample and higher a^* value for PPE-treated sample. Both changes most probably occurred due to the effect of storage time as the results were detected on the seventh day of storage. The chicken meat had some notable increments ($p < 0.05$) on the yellowness (b^* value) at the zero-day, especially when treated with GTE due to the green tea colour. On the third and seventh day of storage, no changes were observed for the b^* values, however, the values were still higher compared to the control. The colour changes might be minimal but it can influence the panelists' perceptions. Since the TBARS values of this research indicated no significant difference, therefore, it can also be concluded that oxidation did not affect the meat colour.

Texture properties of the chicken meat

Table 5 shows the texture profile analysis (TPA) of the raw chicken meat samples treated with PPE and GTE and stored at 4 ± 1 °C for seven days. In general, no changes ($p > 0.05$) were observed for the hardness, chewiness, springiness and cohesiveness of the treated chicken meats and control within the zero-day and third day of the storage. However, on the seventh day, the hardness values were increased, while the cohesiveness values were decreased for all the samples. The results most probably were affected by the storage time rather than the extracts applied, where it is uncommon to keep raw chicken meat for more than three days in chilling temperature as mentioned earlier. A study supported the finding where plant extracts including green tea extract applied on chicken breast meat resulted in no significant ($p > 0.05$) changes on hardness, cohesiveness, and chewiness [43]. However, uneven results were observed for the chewiness GTE-treated chicken meat and the springiness of the untreated chicken meat, again, most probably due to the effect of the seven days of storage. Nevertheless, it can be concluded that the extracts did not influence the texture profiles of the chicken meat as the applications were only on the surfaces.

Table 1. Comparison between ultrasonic-assisted and solid-liquid extraction methods

Measurements	Extracts	Extraction Methods	
		Ultrasonic	Solid-Liquid
Extraction yield (%)	PPE	22.9 ± 2.7 ^{Aa}	1.6 ± 0.25 ^{Bb}
	GTE	28.1 ± 2.05 ^{Aa}	22.6 ± 3.5 ^{Ba}
Total antioxidant activity (%)	PPE	74.3 ± 2.26 ^{Aa}	48.2 ± 0.02 ^{Ba}
	GTE	70.4 ± 1.8 ^{Aa}	41.5 ± 2.6 ^{Bb}
Total phenolic content (mg GAE/g)	PPE	328.7 ± 1.6 ^{Aa}	389.5 ± 0.4 ^{Ab}
	GTE	268.8 ± 1.8 ^{Bb}	708.7 ± 10.5 ^{Aa}
Total flavonoid content (mg QE/g)	PPE	30.0 ± 2.5 ^{Aa}	7.9 ± 0.4 ^{Ba}
	GTE	6.3 ± 1.8 ^{Ab}	0.4 ± 0.4 ^{Bb}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different small letters of the same measurement are significantly different ($p < 0.05$) between rows, means with different capital letters of the varied extraction methods are significantly different ($p < 0.05$) between columns

Table 2. Effects of PPE and GTE on total plate count and total Yeast and Mould count of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
Total Plate Count (Log CFU/g)	Control	5.45 ± 0.29 ^{Ab}	6.81 ± 0.68 ^{Aa}	6.02 ± 0.14 ^{Aab}
	PPE	5.37 ± 0.4 ^{Aa}	5.89 ± 0.54 ^{ABa}	6.21 ± 0.22 ^{Aa}
	GTE	5.27 ± 0.57 ^{Aa}	5.47 ± 0.32 ^{Ba}	5.96 ± 0.13 ^{Aa}
	PPE+GTE	5.5 ± 0.3 ^{Ab}	6.50 ± 0.31 ^{ABa}	6.26 ± 0.34 ^{Aab}
Total Yeast and Mould (Log CFU/g)	Control	5.11 ± 0.32 ^{Aa}	5.16 ± 0.4 ^{Aa}	5.65 ± 0.47 ^{Aa}
	PPE	5.2 ± 0.26 ^{Aa}	5.84 ± 0.55 ^{Aa}	5.95 ± 0.1 ^{Aa}
	GTE	5.23 ± 0.42 ^{Aa}	5.2 ± 0.13 ^{Aa}	5.24 ± 0.47 ^{Aa}
	PPE+GTE	5.19 ± 0.29 ^{Ab}	6.16 ± 0.39 ^{Aa}	5.4 ± 0.16 ^{Ab}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different small letters of the same treatment (effect of the day) are significantly different ($p < 0.05$), Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$)

Table 3. Effects of PPE and GTE on the water holding capacity, pH and lipid peroxidation of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
Water Holding Capacity	Control	31.52 ± 4.98 ^{Aa}	22.47 ± 5.10 ^{Aa}	21.27 ± 3.12 ^{Aa}
	PPE	27.17 ± 5.82 ^{Aa}	21.76 ± 2.68 ^{Aa}	18.55 ± 3.75 ^{Aa}
	GTE	27.56 ± 6.38 ^{Aa}	28.53 ± 1.67 ^{Aa}	28.37 ± 3.39 ^{Aa}
	PPE+GTE	29.75 ± 4.37 ^{Aa}	28.33 ± 2.67 ^{Aa}	31.09 ± 4.34 ^{Aa}
pH	Control	6.18 ± 0.08 ^{Aa}	5.86 ± 0.20 ^{Aa}	6.03 ± 0.04 ^{Aa}
	PPE	6.01 ± 0.11 ^{ABa}	6.11 ± 0.34 ^{Aa}	6.02 ± 0.01 ^{Aa}
	GTE	5.65 ± 0.05 ^{Cb}	5.71 ± 0.07 ^{Ab}	5.92 ± 0.05 ^{Aa}
	PPE+GTE	5.88 ± 0.04 ^{Ba}	5.84 ± 0.04 ^{Aa}	5.80 ± 0.26 ^{Aa}

Table 3 (cont'd). Effects of PPE and GTE on the water holding capacity, pH and lipid peroxidation of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
Lipid Peroxidation (TBARS, mg MDA/kg)	Control	0.30 ± 0.10^{Aa}	0.23 ± 0.10^{Aa}	0.35 ± 0.11^{Aa}
	PPE	0.24 ± 0.10^{Aa}	0.18 ± 0.10^{Aa}	0.33 ± 0.02^{Aa}
	GTE	0.34 ± 0.10^{Aa}	0.23 ± 0.10^{Aa}	0.31 ± 0.11^{Aa}
	PPE+GTE	0.21 ± 0.05^{Aa}	0.26 ± 0.11^{Aa}	0.20 ± 0.10^{Aa}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different small letters of the same treatment (effect of day) are significantly different ($p < 0.05$), Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$).

Table 4. The effects of PPE and GTE on the L^* , a^* and b^* values of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
L^*	Control	61.00 ± 4.15^{Aa}	58.82 ± 1.63^{Aa}	58.04 ± 2.50^{Aa}
	PPE	63.58 ± 3.89^{Aa}	60.15 ± 2.40^{Aa}	56.23 ± 3.38^{Aa}
	GTE	58.93 ± 1.07^{Aa}	59.57 ± 3.12^{Aa}	55.65 ± 3.11^{Aa}
	PPE+GTE	63.16 ± 3.32^{Aa}	58.80 ± 2.01^{Ab}	54.88 ± 1.11^{Ab}
a^*	Control	10.69 ± 2.22^{Aa}	10.60 ± 1.75^{Aa}	9.96 ± 2.25^{Aa}
	PPE	9.59 ± 2.03^{Ab}	11.21 ± 0.59^{Aab}	13.28 ± 0.91^{Aa}
	GTE	12.91 ± 0.58^{Aa}	10.21 ± 1.86^{Aa}	12.28 ± 1.71^{Aa}
	PPE+GTE	10.33 ± 1.50^{Aa}	10.90 ± 1.35^{Aa}	12.98 ± 1.05^{Aa}
b^*	Control	7.85 ± 2.70^{Ba}	11.42 ± 3.42^{Aa}	11.00 ± 4.21^{Aa}
	PPE	12.45 ± 1.02^{ABa}	15.40 ± 1.33^{Aa}	15.81 ± 2.91^{Aa}
	GTE	14.15 ± 1.65^{Aa}	14.78 ± 0.61^{Aa}	15.19 ± 1.88^{Aa}
	PPE+GTE	11.63 ± 1.86^{ABa}	13.44 ± 2.16^{Aa}	13.84 ± 0.80^{Aa}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different small letters of the same treatment (effect of the day) are significantly different ($p < 0.05$), Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$)

Table 5. The effects of PPE and GTE on the texture profile of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
Hardness (g)	Control	1546 ± 482^{Ab}	1882 ± 998^{Ab}	19434 ± 4004^{Aa}
	PPE	2946 ± 2121^{Ab}	3969 ± 1376^{Ab}	23930 ± 2498^{Aa}
	GTE	2638 ± 938^{Ab}	2774 ± 15^{Ab}	24035 ± 57^{Aa}
	PPE+GTE	2480 ± 10^{Ab}	2978 ± 91^{Ab}	16377 ± 46^{Aa}
Chewiness (g.mm)	Control	816 ± 236^{Aa}	762 ± 332^{Aa}	1244 ± 980^{Aa}
	PPE	1072 ± 695^{Aa}	1421 ± 465^{Aa}	2680 ± 891^{Aa}
	GTE	1042 ± 339^{Ab}	1125 ± 36^{Ab}	3282 ± 555^{Aa}
	PPE+GTE	1001 ± 231^{Aa}	1288 ± 248^{Aa}	1446 ± 508^{Aa}

Table 5 (cont'd). The effects of PPE and GTE on the texture profile of raw chicken meat stored at 4 ± 1 °C for seven days

Parameters	Treatments	Day 0	Day 3	Day 7
Springiness (mm)	Control	0.74 ± 0.08^{Aa}	0.64 ± 0.08^{Aa}	0.20 ± 0.04^{Bb}
	PPE	0.62 ± 0.05^{ABab}	0.63 ± 0.02^{Aa}	0.30 ± 0.05^{Aa}
	GTE	0.50 ± 0.05^{Bb}	0.60 ± 0.001^{Aa}	0.30 ± 0.003^{ABab}
	PPE+GTE	0.65 ± 0.04^{ABa}	0.60 ± 0.004^{Aa}	0.30 ± 0.03^{ABab}
Cohesiveness	Control	0.71 ± 0.02^{Aa}	0.66 ± 0.03^{Aa}	0.20 ± 0.08^{Ab}
	PPE	0.60 ± 0.08^{Aa}	0.56 ± 0.03^{Aa}	0.30 ± 0.04^{Ab}
	GTE	0.60 ± 0.05^{Aa}	0.66 ± 0.06^{Aa}	0.30 ± 0.03^{Ab}
	PPE+GTE	0.64 ± 0.06^{Aa}	0.66 ± 0.07^{Aa}	0.28 ± 0.03^{Ab}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different small letters of the same treatment (effect of day) are significantly different ($p < 0.05$), Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$)

The microstructure of the chicken meat

Figure 1 reveals the microstructure of the raw chicken meat treated with the extracts on the zero-day. The results showed changes in the microstructure of the chicken meat surfaces treated with phenolic compared to the control. The black holes visible in the control meat sample were filled by the extracts via the marinating process through which the phenolic compounds were absorbed. The surface of the sample treated with PPE appeared to be smoother and clearer than the GTE-treated sample, which looks rough and uneven. The structure of the sample treated with both PPE and GTE was observed to be a little bit rougher similar to the sample treated with GTE but it also had a smooth surface similar to the sample treated with PPE only. It can be concluded that PPE might had smaller particle sizes compared to GTE due to the presence of catechin in GTE. In addition, a study on the effects of marination of grilled chicken with some ingredients on the microstructure of the meat found the same results as this research [28].

Sensory evaluation

The results of sensory evaluation of the treated meat sample using GTE and PPE are shown in Table 6. Thirty

untrained panelists evaluated the cooked chicken meat on the third day of storage using a 9-point hedonic scale. Selection of the third day for the sensory evaluation was due to the safety of panelists based on the microbial results obtained. The data revealed that all samples were not significantly ($p > 0.05$) different in terms of colour, aroma, flavor, tenderness, juiciness, and springiness. However, the overall acceptance scores of the samples were significantly different ($p > 0.05$) where the control sample had the highest score, followed by the samples treated with PPE, PPE + GTE and GTE. Similar research that had studied the effect of applying tea catechins on raw patties of beef and chicken found that the sample treated with tea catechins had significantly decreased sensory scores, especially colour [8]. The reason for the GTE's lowest score might be due to the yellow colour of the green tea, which normally affects the food and alters its normal colour. In addition, the distribution of the particles of the GTE as observed in the microstructure analysis could also influence the panelists. This result also justified the decision of applying lower concentration of the extracts on the chicken meat as sensory results play important roles towards the acceptability of the products.

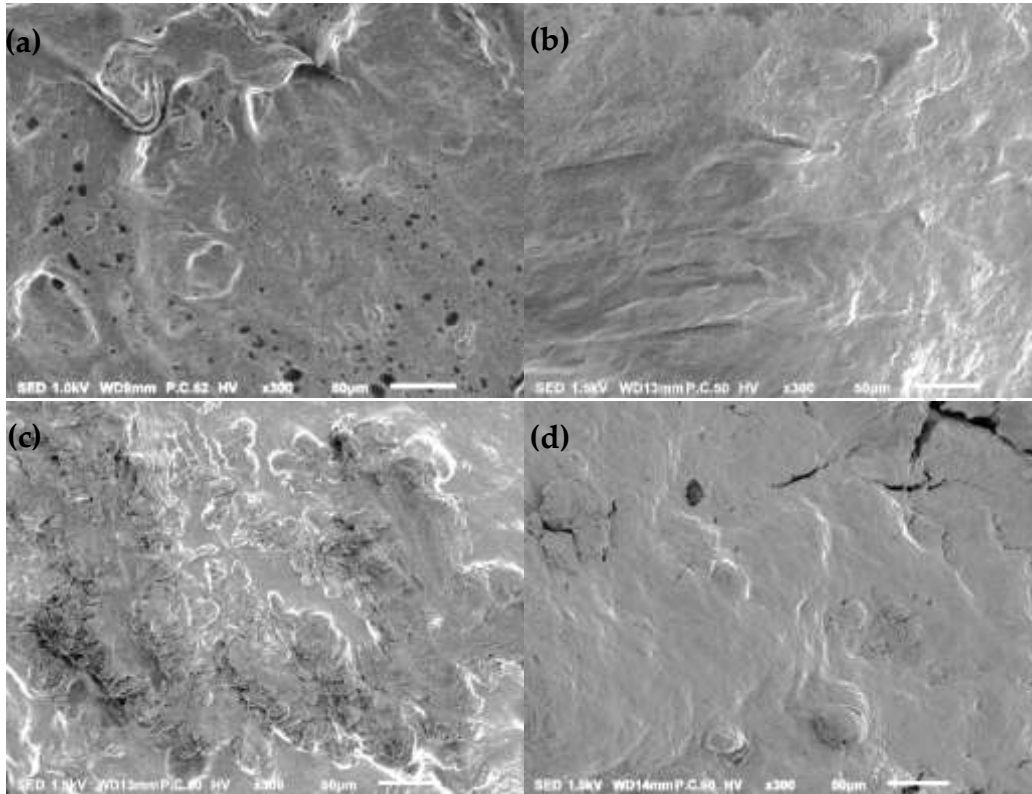


Figure 1. The effects of PPE and GTE on the microstructure of raw chicken meat ($\times 300$): (a) control, (b) chicken meat treated with pomegranate peel extracts (PPE), (c) chicken meat treated with green tea extracts (GTE), (d) chicken meat treated with pomegranate peel extracts and green tea extracts (PPE+GTE)

Table 6. The effects of PPE and GTE on the sensory analysis of raw chicken meat stored at 4 ± 1 °C for three days

Attributes	Treatments			
	Control	PPE	GTE	PPE+GTE
Colour	5.75 ± 1.50^A	5.16 ± 1.40^A	4.91 ± 1.90^A	5.16 ± 2.10^A
Aroma	5.5 ± 1.60^A	5.33 ± 1.6^A	6.08 ± 1.4^A	4.83 ± 1.4^A
Flavor	5.20 ± 1.20^A	4.91 ± 1.80^A	5.00 ± 1.50^A	4.70 ± 1.60^A
Tenderness	6.50 ± 1.60^A	5.91 ± 1.50^A	5.00 ± 1.40^A	5.08 ± 2.00^A
Juiciness	5.16 ± 1.50^A	4.91 ± 1.80^A	4.91 ± 1.40^A	4.91 ± 1.30^A
Springiness	5.75 ± 1.70^A	5.58 ± 1.30^A	5.00 ± 1.40^A	5.08 ± 1.30^A
Overall acceptability	6.41 ± 1.30^A	5.66 ± 1.30^{AB}	4.83 ± 1.20^B	5.58 ± 1.50^{AB}

PPE = Pomegranate peel extracts, GTE = Green tea leaves extracts. Means with different capital letters of the varied treatments (row-wise) are significantly different ($p < 0.05$), Means $\pm$ standard deviation ($n=30$)

Conclusion

The extracts of pomegranate (*Punica granatum* L.) peel and green tea (*Camellia sinensis*) leaves extracted using ultrasound-assisted extraction (UAE) and solid-liquid extraction (SLE) were confirmed of containing phenolic compounds based on their TPC, TFC, and total antioxidant activity. The UAE method is considered a better method because it resulted in a high capacity of radical scavenging activity, the best production of total flavonoids, and the best total yield compared to SLE, even though the total phenolic compounds extracted via SLE were higher than that of UAE. Green tea extract (GTE) was better in inhibiting the microbial growth over seven days of chilled storage compared to pomegranate peel extract (PPE) or a combination of both. However, other physicochemical properties of the chicken meat treated with the extracts such as water holding capacity, pH, lipid peroxidation and texture were not much affected, most probably due to the lower concentration used. The yellowness (b^*) of the chicken meat were higher compared to the control. The microstructure of the treated chicken meat surface also changed significantly especially when GTE was applied. This can also be related to the lower overall acceptability of the GTE-treated chicken meat in the sensory analysis. Therefore, future work should consider this matter as increasing the concentration of the GTE might produce better microbial and physicochemical properties results of the treated chicken meat, however, further additional treatments could help to cater the negative impact on the appearance and acceptance.

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