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preliminary communication

Effect of West Indian Bay Leaf (*Pimenta racemosa*) and Turmeric (*Curcuma longa*) Essential Oils on Preserving Raw Chicken Breasts

Running head: Preservative Effects of Bay Leaf and Turmeric Essential Oils

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SUMMARY

Research background. While the use of chemical preservatives in meats may appear to be tremendously advantageous, they have long been purported to increase the risk of incidence of certain types of cancers. Consequentially, many persons have opted for minimally processed alternatives. This consumer shift has placed substantial pressure on the food industry to implement more natural alternatives to these synthetic preservatives in the meat industry. Research on plant extracts as potential agents for food additives is increasing. Considering the bioactive components present in West Indian bay leaf and turmeric essential oils, these oils present promising potential for their use as novel, green preservatives in the meat industry.

Experimental approach. Raw chicken breast samples (28 g) were each treated with different doses (0.5 mL, 1 mL and 1.5 mL) of individual West Indian bay leaf and turmeric and a combination of the two essential oils (1:1 mixture of both oils to make up each of the 0.5 mL, 1 mL and 1.5 mL doses). Physicochemical, microbiological, and sensory evaluations were performed on the fresh and treated samples stored for 14 days at 4 °C.

Results and conclusions. The bay leaf oil exhibited a higher yield and total phenolic content while the turmeric oil had a higher total flavonoid content. The most effective treatments when compared to the control significantly ($p < 0.05$) minimized the pH rise by 13.9 % (bay leaf oil 1.5 mL),

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reduced texture loss by 44.8 % (combination oil 1.5 mL) and reduced protein loss by 98.9 % (bay leaf oil 1 mL). Most treated samples exhibited reduced microbial loads, with the turmeric oil displaying highest efficacy against lactic acid bacteria, yeasts and moulds. Treated samples had significantly higher ($p < 0.05$) final day sensory scores than the control, with the combination 1.5 mL dose proving to be the most effective, as the storage life of the chicken breast sample was extended by 6 days.

Novelty and scientific contribution. This study has shown for the first time, that the essential oils from turmeric and West Indian bay leaf can extend the shelf life of raw chicken breast and highlights the potential of the oils as natural preservative agents in lieu of synthetic alternatives.

Keywords: essential oil; West Indian bay leaf; turmeric; novel preservatives; chicken breast

INTRODUCTION

Raw meat with its high water content, nutrients and close to neutral pH is an ideal environment for microbial proliferation (1). Preservatives in meats such as nitrites and nitrates inhibit microbial growth through their innate bactericidal effects (2). Fresh chickens, upon slaughter, are injected with sodium acetate or sodium lactate to extend shelf-life through their antimicrobial action (3). Preservatives such as nitrates appear advantageous, but their unregulated usage and excessive consumption may adversely affect consumer's health. Nitrates used in meat processing, upon reduction to nitrites can react with available secondary amines and produce carcinogenic nitroso-compounds (NOCs) (4,5). As these deleterious effects have become common knowledge, consumers have begun to limit their intake of highly processed meats and instead focus on minimally processed alternatives (6). This shift in consumer consciousness mandates the food industry to respond and use more natural preservatives.

Some plant essential oils (EO) have been shown to demonstrate quite potent antimicrobial properties with some, such as lemon (*Citrus limon*) and eucalyptus (*Eucalyptus globulus*) EO, used in food coatings and active food packaging, by providing protection against both spoilage and pathogenic microbes (7). While the antibacterial and preservative nature of the more globally popular aromatic plants and herbs have seen them increasingly incorporated by the food industry, the same cannot be said for the utilization of traditional local herbs and spices in small island developing states in the Caribbean.

Pimenta racemosa, commonly referred to as West Indian bay leaf in Trinidad and Tobago, is a tall, aromatic, arboreal plant, native to both the Caribbean and northwestern South America (8). The volatile compounds found in the EO of the *Pimenta racemosa* leaves have been shown in previous studies to consist of various phenols, monoterpenes, sesquiterpenes, diterpenes and esters (9).

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Eugenol has also been shown to be the most abundant of these volatile compounds accounting for 60.4 % to 82.9 % and subsequently, the source of most of the antibacterial potential (8). Eugenol extracted from the essential oil of cloves and cinnamon leaves, possesses potent antibacterial and insecticidal properties (10).

Turmeric (*Curcuma longa*) is a perennial plant with underground rhizomes, which are predominantly oblong, ovate, and short-branched (11). It belongs to the Zingiberaceae family, a close relative of the more widely known ginger, sharing similar physical characteristics and powerful antioxidant, antibacterial and anti-inflammatory properties (12). The numerous bioactive compounds found in turmeric, such as sesquiterpenes, ketones, tumerone, zingiberene, cineole and various curcuminoids are responsible for these biological properties. Additionally, curcumin from previous research is the main constituent curcuminoid present and the primary phytochemical responsible for the biological functions of turmeric (11).

While the antimicrobial effect of these two EOs have been demonstrated previously, the effect of West Indian bay leaf EO as a natural preservative and its effect in combination with turmeric EO has not been previously demonstrated. This study investigated some quality indicators of the EO extracted from the West Indian bay leaf and turmeric as natural preservatives in raw chicken breast samples.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh West Indian bay leaves were collected from a single large tree located at the St. Augustine campus of The University of The West Indies, Trinidad. Approximately 3 kg of the leaves were carefully harvested and rinsed thoroughly under cool running tap water to remove all debris and organisms. The leaves were then placed on Kraft drying paper (150 cm×50 cm) to air dry for 10 days after which 0.95 kg of the leaves were used in the EO extraction process. Fresh turmeric rhizomes (9 kg) were obtained from a Farmer's Market located in Macoya, Trinidad. The rhizomes were thoroughly rinsed with cool running tap water to remove soil and debris and were then left to air dry for 5 days to approximately 82.2 % of their initial weight. After drying, the rhizomes were sliced into thin 0.2 cm slices using a Hobart Slicer (Hobart Corporation 1612, Ohio, USA) and then partially crushed with a wooden mallet to increase the surface area and facilitate the steam distillation EO extraction.

The EO extractions of the West Indian bay leaf and turmeric were done via steam distillation from a pilot plant as shown in Fig. S1. The dried leaves (0.95 kg) were placed into the steam distillation drum and the distillation process was left to run for three hours. The same process was used for the sliced and partially crushed rhizomes (7.4 kg). After the run time had elapsed in both extractions, the

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EO obtained were collected in 40 mL amber bottles and then immediately placed in refrigerated storage at 4 °C and until required for experiments.

Total phenolic content and total flavonoid content

The Folin-Ciocalteu colorimetric method (13) was used to measure the total phenolic content (TPC) of the extracted EO. The absorbance was measured at 765 nm and the TPC of the extracted EO was expressed as milligrams of gallic acid equivalents (GAE) per milliliter of sample (mgGAE/mL).

The aluminium chloride colorimetric test (14) was employed to determine the Total flavonoid content (TFC) of each EO expressed in milligrams of quercetin equivalent per milliliter of sample (mgQE/mL). Absorbance was read at 415 nm and a UV-Visible Spectrophotometer (Thermo Scientific Evolution 60S, Massachusetts, USA) was used for both TPC and TFC determinations. The chemicals used for the analyses were of reagent grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation and treatment of samples

Raw, bone-in chicken breasts (2.0 kg) were purchased from a Poultry depot located in St. Augustine, Trinidad and were immediately placed in an insulated cooler before arrival at the laboratory. After rinsing with potable water, the breasts were cut to obtain two main sample sets, one triplicate set, each of eleven 28 g samples (for quantitative analyses), and another set of ten 28 g samples (for sensory analysis). The ten samples from the sensory analysis set were labelled as follows: Turmeric 0.5 mL, Turmeric 1 mL, Turmeric 1.5 mL, Bay leaf 0.5 mL, Bay leaf 1 mL, Bay leaf 1.5 mL, Combination 0.5 mL (Bay leaf 0.25 mL + Turmeric 0.25 mL), Combination 1 mL (Bay leaf 0.5 mL + Turmeric 0.5 mL), Combination 1.5 mL (Bay leaf 0.75 mL + Turmeric 0.75 mL) and Stored Control (untreated sample to be stored). Ten samples from the quantitative sample set were labelled similarly with the final eleventh sample functioning as the fresh control breast sample.

Each sample was placed in an individual aluminium foil sheet (12 cm × 12 cm) and according to the sample label, the amount of each corresponding EO was aseptically transferred and dispensed to the entire surface of the samples using a micropipette to ensure consistency in application. Each sample including the control was then wrapped in the aluminium foil sheets and placed in a labelled container and stored at 4 °C for a period of 14 days. The remaining chicken breasts which were not treated with the EO, nor placed in refrigerated storage, served as the fresh, un-stored control samples (day 0 control).

Physicochemical assessment of samples

Colour

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The colour readings of the fresh samples (day 0 control) were recorded with a Konica Minolta Chroma Meter (CR-400, Tokyo, Japan) in the CIELAB colour space values of L^* , a^* , b^* and similarly this process was repeated on day 14 for the treated and stored control samples at 4 °C.

Texture

The texture was expressed in terms of hardness (N) of chicken breast samples using the CNS Farnell QTS Texture Analyser (7113, England, UK). The sample was positioned on the Texture Analyser platform and a full profile analysis was conducted using a TA9 needle probe (1.5 mm diameter) at a constant speed of 1.0 mm/s until a predetermined distance of 15 mm was achieved.

pH

A 5 g sample was placed in a sterile stomacher bag and 50 mL of distilled water was added. The stomacher bag was then placed in a stomacher blender and the sample was allowed to homogenize for a period of 1 min. The bag contents were transferred to a clean 100 mL beaker and the pH of the homogenate was measured using the Hanna Instruments pH 211 Microprocessor pH meter (Rhode Island, USA).

Moisture content

The moisture content of the fresh and refrigerated chicken breast samples (2 g minced) was determined using the convection oven method (Precision Thelco Laboratory Oven 51221144, Colorado, USA). Samples were placed in a pre-heated oven at 198 °C for 1 h 20 min, after which they were cooled in a desiccator to ambient temperature and then weighed. The moisture content of each sample was determined from the following equation (Eq.1)

$$\text{Moisture \%} = ([M \text{ initial} - M \text{ dried}] / M \text{ initial}) \times 100 \quad /1/$$

Where $M \text{ initial}$ is the mass before drying (in g) and $M \text{ dried}$ is the mass after drying (in g).

Protein content

The protein content of the samples was determined using the Kjeldhal method (15). A Gerhardt digestion and distillation system (Gerhardt Analytical Systems, Königswinter, Germany) was used, and the nitrogen and subsequent protein percentages were calculated using the following equations (Eqs. 2 and 3).

$$\% \text{ Nitrogen} = (\text{mL standard} - \text{mL blank}) \times \text{Molarity of acid} \times 1.4007 / \text{weight of sample (g)} \quad /2/$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \quad /3/$$

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Microbiological evaluation

The microbiological analyses of the samples were conducted according to the standard enumeration procedures for total plate count, total yeast and mould and lactic acid bacteria as outlined in the Bacteriological Analytical Manual (BAM) (16).

Preparation of culture media

Dichloran Rose-Bengal Chloramphenicol (DRBC) CM0727; De Man, Rogosa and Sharpe agar (MRS) CM0361 and Total Plate Count (TPC) CM0325 agars (Oxoid Limited, Hampshire, UK) were used to enumerate the yeasts and moulds, lactic acid bacteria, and the total number of aerobes respectively within each sample (fresh and refrigerated). A 20 mL aliquot of each agar, prepared according to the manufacturer's specifications, was poured into 25 mL VWR 100 ×15 mm petri dishes and allowed to set prior to refrigeration at 4 °C for 24 h for subsequent plating of the 11 samples.

Plating of samples and colony enumeration

A 5 g portion of each minced sample (treated and control) was homogenized with 45 mL of diluent (0.85 % NaCl) and was used to create four serial dilutions (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}). Using the spread-plate method, a 0.1 mL of each of the four sample dilutions prepared was added to a separate agar plate. The MRS and DRBC plates were incubated for 72 h at 35 °C and 25 °C respectively whereas, the TPC plates were incubated for 48 h at 35 °C. The plates were then counted using a Reichert Quebec Darkfield Colony Counter (13332500, USA). Plates with more than 300 colonies were deemed too numerous to count (TNTC) while those with less than 30 colonies were deemed too few to count (TFTC). The average number of colonies was used to calculate the colony forming units per gram (CFU/g) of the initial 5 g sample.

Sensory evaluation

The treated and control chicken breast samples were evaluated using a 7-point hedonic scale with boundary indications from extremely unacceptable (1) to extremely acceptable (7) on the attributes of odour, colour and appearance at the end of the 14-day storage while the overall acceptability was analyzed every 48 h over the 14-day storage period. The 'overall acceptability' parameter was used to indicate storage life quality with average sensory ratings of less than 3.0, interpreted as sample spoilage. The treated samples and control were evaluated in the morning at 10 am by a panel of 30 semi-trained persons, made up of students at the University of the West Indies, who were not permitted to touch the samples but only to visually observe and smell them. Samples

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were each assigned a random three-character code, were wrapped in aluminium foil and placed in uniform, odourless plastic containers at ambient temperature (25 ± 1 °C).

Statistical analysis

The statistical analysis of the data was carried out using the SPSS statistical software version 29.0 (17) to conduct a one-way ANOVA and Dunnett's Multiple Comparison Tests to determine whether treatment differences were significant. The Tukey multiple range test was also used to compare the results of each treatment group to each other, to determine whether the differences between treatments were significant. Significant differences were established at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Yield and phytochemical content of essential oils

The steam distillation technique employed in this study resulted in the extraction of 20 mL of West Indian bay leaf and 15 mL of turmeric EO representing a 0.02 % and a 0.002 % yield respectively. The total phenolic (TPC) and total flavonoid contents (TFC) of the turmeric EO were determined to be (2.55 ± 0.39) mgGAE/mL and (3.14 ± 0.03) mgQE/mL respectively while the TPC and TFC of the bay leaf EO were (7.27 ± 0.20) mgGAE/mL and (2.22 ± 0.10) mgQE/mL respectively. Phenols and flavonoids form the major classes of the phytochemical composition and a good indicator of how effective an essential oil would be as an antimicrobial agent, can be inferred from their total contents (18). While not much studies have been conducted on the total phenolic content of the West Indian bay leaf essential oils, the phenolic content of the turmeric essential oils was on the lower end of the 2.80-13.40 mgGAE/mL range that was seen in previous research (19). With the values that were obtained for both the total phenolic and flavonoid contents of the West Indian bay leaf and turmeric essential oils, it was expected that the bay leaf oil would demonstrate a higher antimicrobial activity and thus function as a better individual preservative agent as the total phytochemical content (TPC+TFC) was higher than in the turmeric oil.

Effect of EO on sample pH

During meat spoilage, the breakdown of proteins and the formation of ammonia, amines and ammonia from amino acids causes the characteristic rise in meat pH, often reaching values as high as 8.5 (20). The results summarized in Table 1 show that all the treated samples displayed significantly lower ($p < 0.05$) pH values than the stored control on the day at day 14. These findings were in accordance with the results reported in previous studies that showed that the presence of essential oils is able to slow the rate at which meat pH rises as it spoils (21). The 1.5 mL bay leaf EO

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treatment was the most effective as the treated sample showed the lowest pH, 13.9 % less than the stored control. This observation may have been as a direct result of the differences in TPC and TFC values of the turmeric and bay leaf EO. Kaur and Mondal (22) demonstrated that plant species having higher phenolic contents showed greater antibacterial effects than others, even if their TFC were similar. This supported the findings in this study as the higher TPC of the bay leaf EO allowed it to better mitigate the growth of pH-altering proteolytic bacteria than the turmeric EO. This resulted in significantly lower ($p < 0.05$) pH values of the 1 mL and 1.5 mL bay leaf EO samples when compared to the respective turmeric EO samples. No significant difference ($p > 0.05$) was observed at the 0.5 mL level between the oil treatments. Furthermore, as the bay leaf EO dose increased from 1 mL to 1.5 mL, the pH decreased significantly ($p < 0.05$), as the samples probably became exposed to higher levels of phenols which further prevented proteolytic bacterial growth.

This trend was not observed in the turmeric samples as the pH conversely rose significantly ($p < 0.05$) as the treatment dose increased, indicating that perhaps more proteolytic bacterial proliferation occurred with increasing doses, albeit to a lesser degree than in the stored control.

The combination EO treated samples displayed significantly lower ($p < 0.05$) pH values than the stored control and both the 1 mL and 1.5 mL doses performed to a similar degree as with the bay leaf 1.5 mL sample. Individually, the bay leaf EO was more effective than the turmeric EO, but when used in combination, a synergistic effect was observed. At the 1 mL combination dose (0.5 mL each of bay leaf and turmeric EO), the pH was significantly lower ($p < 0.05$) than both the individual 0.5 mL treated samples, while the 1.5 mL combination dose, performed just as effectively as the 1.5 mL bay leaf EO treated sample (highest performing treatment). The results demonstrated that in combination, less of each oil was required to elicit a similar or even better effect than when the oils were used individually.

When EO are used in combination, their effects can sometimes be synergistic and lead to an amplified antibacterial response (23). The amplified effect of the 1 mL and 1.5 mL combination EO mixtures may have led to a greater inhibition of the proteolytic bacteria that typically alters pH levels in meat. No significant difference ($p > 0.05$) was observed when the combination dose increased from 1 mL to 1.5 mL, indicating that the synergistic effect probably diminished as the maximum combined effectiveness peaked at the 1 mL dose. Possibly the increased dose of the less effective turmeric oil may have had a diminishing effect on the bay leaf oil efficacy.

[Table 1 near here]

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Effect of EO on sample texture

As expected, the refrigerated untreated sample exhibited the sharpest decline in average hardness nearing a 50 % decrease compared to the fresh control (Table 1). During spoilage, autolytic breakdown of protein myofibrils and the effects of biofilm formation contribute to an increased softness in meat texture (24). Specifically, the proliferation of bacterial species such as *Lactobacillus spp.*, *Leuconostoc spp.* and *Pseudomonas spp.* probably caused biofilm on meat surfaces which negatively impacted their texture (25).

All EO treatments above 0.5 mL significantly reduced ($p < 0.05$) softening when compared to the stored control, indicating that at the 0.5 mL dose, not enough antibacterial potency was exerted by the oils to delay the textural changes in the samples. Only from the 1 mL dose of each treated sample was such an effect first observed as more of the bioactive phytochemical elements could have been present to mitigate changes to texture. Furthermore, while a dose increase from 0.5 mL to 1 mL yielded a significant difference ($p < 0.05$) in the level of hardness retained in the samples, no further significant effect ($p > 0.05$) was observed when the dosages were increased from 1 mL to 1.5 mL, except in the case of the combination treatments, where the 1.5 mL sample retained significantly higher ($p < 0.05$) hardness than the 1 mL sample. Additionally, outside the previously determined ineffective 0.5 mL dose, no statistically significant difference ($p > 0.05$) was observed between the turmeric and bay leaf oil treatments.

The hardness of the 1 mL and 1.5 mL dosed samples were significantly higher ($p < 0.05$) than the stored control and significantly lower ($p < 0.05$) than the hardness of the fresh sample, except for the 1.5 mL combination EO treated sample, which retained 92.7 % of the total hardness of the initial fresh sample. Thus, while there was no observed synergistic effect of the individual oils at the other lower doses, there was a synergistic effect at the highest dose.

Effect of EO on protein content

The protein content of the fresh sample was close to the values of 22.8-23.3 % previously reported (26). Due to microbial action, protein oxidation and autolytic processes occur, whereby the protein content of chicken breast falls as it spoils (27). From Table 1, the stored control had the lowest protein content at the end of the 14-day storage period and fell by 5.63 % from the initial value of the fresh sample.

The treated samples all displayed higher protein contents at day 14 than the stored control but only the protein contents of the 1 mL and 1.5 mL bay leaf EO samples and the 1 mL combination EO samples were significantly different ($p < 0.05$) to the stored control. Furthermore, the 1 mL bay leaf EO sample had the highest protein content of all the treated samples and was not statistically different

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($p > 0.05$) to that of the fresh sample, indicating that it was able to reduce the protein loss of the sample by 98.9 % when compared to the stored control.

The ability of EO to prevent protein oxidation and degradation is largely dependent on their phenolic content (28). From this study, the higher TPC of the bay leaf EO allowed for a better preservation of the protein contents in those samples. Al-Hijazeen (28) showed that as more EO was used on a sample, the rate of protein oxidation decreased as it became exposed to more phenolic substances which effectively mitigated protein content changes. This trend was not uniform with the bay leaf samples as the retained protein initially increased from the 0.5 mL to 1 mL dose but then decreased at the 1.5 mL dose. Conversely, the trend was uniformly observed in the turmeric samples as the values increased with higher doses, however, none of the values obtained were significantly different ($p > 0.05$) from each other.

The effects of the combination EO treatments were not observed to have been amplified as the sample protein contents were not significantly different ($p > 0.05$) than those of the individual oils at any of the corresponding dosage levels. Thus, in terms of protein oxidation, the bay leaf and turmeric EO did not have any synergistic or additive effect when used together.

Effect of EO on moisture content

The fresh sample moisture content of the original fresh chicken breast sample was close to reported values (72-74 %) in a previous study (27). It was expected that by day 14, samples would have decreased rapidly in moisture content due to evaporation during extended storage and the loss of water holding capacity (WHC) that occurs during microbial and autolytic protein degradation as well as to pH changes (29). As shown in Table 1, all samples had a final moisture content in the range of 23.28–28.49 %, a decline of about a third of the moisture content of the original fresh chicken breast sample. All treated samples had higher moisture contents at the end of refrigerated storage compared to the stored control with the moisture content of the 1.5 mL combination EO sample exhibiting highest levels compared to the other treatments.

Heydari *et al.* (30) showed a linear relationship between pH and WHC in that, as the pH decreased, the WHC of the meat decreased too resulting in more water loss and a lower moisture content of the sample. This resulted in protein structure loss that occurred with altered pH which reduced the efficacy of the water binding capacity. However, in this study this expected trend between the pH and moisture content of the samples was not generally observed except for the turmeric oil treatments. As noted previously, only the pH of turmeric samples increased with an increase in the administered doses (0.5 mL to 1.5 mL). This trend of pH increase accompanied by moisture content increase of the turmeric samples was similarly reported by Hedyari *et al.* (30).

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The higher protein content retained in the 1 mL, 1.5 mL bay leaf EO treatments and the 1 mL combination EO treatment were expected to contribute to greater WHC and thus these samples were expected to display higher moisture contents. While this was observed, these higher values were not statistically significant ($p>0.05$). However, the 1.5 mL combination EO treatment exhibited the highest moisture content ($p<0.05$). The effect of the oils on moisture retention was amplified when used together in a 1.5 mL combination dose (0.75 mL each of bay leaf and turmeric EO), compared to the 1 mL and even the 1.5 mL individual doses.

Effect of EO on sample colour

Vital *et al.* (31) showed that the L^* value (lightness) of beef samples decreased throughout storage but samples treated with rosemary and oregano EO had less of a decrease. They attributed this general decrease to structural changes in meat proteins as they undergo oxidation during storage which may increase light scattering, causing a decrease in the overall lightness of the sample. Most likely, the antioxidant and antibacterial effects of the administered oils, lessened the protein structural changes and thus, less of a decrease in the L^* value was observed. A similar trend was noted, as from Table 1, all the treated samples displayed significantly higher ($p<0.05$) L^* values at day 14 when compared to the stored control. For each type of oil treatment, the 0.5 mL dose recorded the lowest L^* values but as the dose increased, the L^* values increased significantly ($p<0.05$) for each oil, with the 1.5 mL bay leaf oil and 1 mL combination EO samples showing the highest values. As more oil was used, less protein structural changes took place which allowed for a diminished degree of light scattering and a higher recorded L^* value. Furthermore, samples with the 1 mL and 1.5 mL combination EO treatments had significantly higher ($p<0.05$) L^* values than the corresponding individual oils suggesting that there was an amplified effect on the lightness of the samples when used in combination.

As meat spoils, one distinguishable change observed is the development of a greenish hue partly due to the microbial production of hydrogen sulphide, hydrogen peroxide, and sulphomyoglobin, which imparts a green appearance to the meat (1). In this study, it was expected that the EO would have prevented, or at least minimized this colour change by inhibiting microbial growth, and that the stored control would have displayed this expected colour change. The a^* values of the samples measured the degree of redness (+ve values) or greenness (-ve values). From Table 1, while the a^* values of all stored samples fell compared to the fresh control, none of the samples exhibited negative values (green hue) indicative of sulphomyoglobin presence. Even though the refrigerated control recorded the lowest a^* value, close to a two-fold fall from the initial value of the fresh sample, it still did not display the green colour that was expected. It could be deduced that the storage time of 14-

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days was insufficient to promote the level of spoilage that was required to form the green sulphomyoglobin.

As noted previously, the level of sample redness all fell, indicating that the initial bright red oxymyoglobin protein pigment present in the fresh sample was converted to the duller, red-brown metmyoglobin pigment during storage. This would have occurred as the iron present in the heme group of the protein-pigment would have become oxidized upon prolonged exposure to the atmospheric oxygen, thereby imparting a colour change from bright-red (oxymyoglobin) to red-brown (metmyoglobin) (32). For this reason, lower a^* values were generally observed among all the stored samples as they got less red. Apart from the 1.5 mL combination sample, the EO samples all had significantly higher a^* values ($p < 0.05$) than the stored control. These results support a previous study that showed the antioxidant characteristics of EO were able to delay the oxidation of the heme group and thus enabled treated meat samples to display more stable colours (28). Although the 1.5 mL turmeric EO treatment displayed the highest a^* values, no clear trend was observed across the administered dosage of each type of oil treatment. Additionally, no clear trend of a synergistic, amplifying effect was observed when the individual oils were used in combination as only the 1 mL combination treatment exhibited a higher a^* value compared to the 1.5 mL treatment.

With respect to the b^* values, all the samples displayed increased values at the end of storage when compared to the fresh sample. However, as the doses increased from 0.5 mL to 1.5 mL for each oil, no distinct, uniform trend was observed as the values were seen to fluctuate. As a result, the L^* and a^* values were used to give insight into how the EO affected the colour of the samples during storage.

Microbiological assessment of samples

Total plate count

The total plate count of the fresh sample as shown in Table 2 was deemed too few to count (TFTC) whereas, at the end of the storage period, the stored control was deemed too numerous to count (TNTC). Similarly, all the turmeric EO samples were deemed TNTC at the end of storage while the majority of the other EO samples were able to display lower total bacterial loads than the stored control sample.

The total phenolic content (TPC) of EO has been shown in previous studies to be the main reason for their antibacterial efficacy (22). Therefore, as the bay leaf EO had a much higher TPC than the turmeric EO, it is understandable that those EO samples all displayed much lower CFU/g values than the turmeric EO samples, with the 1.5 mL bay leaf dose displaying the lowest of all the treatments. As the bay leaf EO dose increased, these CFU/g values further decreased due to a higher

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exposure to the TPC, which exerted a stronger antibacterial effect. The effects of the oils were not observed to have been enhanced when used in combination as the samples did not display lower bacterial loads than the bay leaf EO samples even though the bacterial loads decreased as higher combination doses were used. It can therefore be assumed that the inclusion of the ineffective turmeric EO, may have had a diminishing effect on the potency of the bay leaf oil, when they were used in combination.

[Table 2 near here]

Lactic acid bacteria

From the results seen in **Table 2**, all the treated samples showed much lower lactic acid bacteria (LAB) loads than the stored control which had a load that was deemed as TNTC. This observation is supported by the results of a previous study which showed that the presence of oregano oil (1 %) in combination with modified atmosphere packaging was able to keep the initial LAB load in a sample of chicken breast relatively constant at 3.66 CFU/g even after a storage time of fifteen days, whereas the load in the control sample approximately doubled after only six days (33).

While the total plate count results showed that the turmeric EO was ineffective in decreasing the total aerobic bacterial load of the samples, it was shown to be the most effective against LAB. As shown in **Table 2**, the antibacterial efficacy of the EO was clearly observed as all the treated samples showed much lower LAB loads than the stored control. At each dose, the bay leaf EO samples displayed higher LAB loads than the turmeric EO samples which indicated the former was not as effective in mitigating the LAB growth. As noted earlier, the turmeric EO possessed a higher total flavonoid content (TFC) than the bay leaf EO inclusive of varied curcuminoids (11). Curcuminoids such as curcumin have been shown to be very effective against gram-positive bacteria (34) and so the proliferation of the gram-positive LAB in the chicken breast samples would have been effectively hindered upon exposure to the turmeric EO.

This trend observed wherein the turmeric oil was shown to be the more potent oil treatment can perhaps explain the observed trends in the pH results. While the general trend was that as each EO dose increased and the corresponding pH decreased, this was not observed with the turmeric samples. These samples displayed slightly increasing pH values as more oil was used, and while this may be due in part to the turmeric EO not being as effective as the others in hindering the growth of proteolytic bacteria, it can also perhaps be due to effective inhibition of LAB growth in the sample. As the LAB growth was less in the turmeric samples, this would mean that the LAB-driven decrease in pH would also be lessened during storage and the samples would display slightly higher pH values

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than the others. The combination oils although effective as evidenced by the low bacterial loads, were not observed to have been more effective than the individual oils.

Yeasts and moulds

The ability of EO to delay yeast and mould (YM) growth was noted previously (35), where the presence of the EO, reduced the YM count of treated samples by at least 50 % at the end of storage when compared to control samples. Like the observation for LAB, since YM are usually found in spoilt meat samples, the YM load of the fresh sample was deemed as TFTC while the load of the stored control was deemed as TNTC.

In general, the turmeric EO treatment was the most effective against YM as the highest dose of 1.5 mL was the only one at that level to be classed as TFTC. Gul and Bakht (12) reported that higher doses of turmeric EO significantly reduced the fungal count in treated samples by almost 50 % as compared to untreated samples. Furthermore, turmeric EO has been shown to contain, in addition to flavonoids and phenols, saponins (36). The study also showed that these saponins are active antifungal compounds which could explain why the turmeric oil in this present study was observed to have been so effective against the YM growth in the treated samples. However, unlike what has been previously observed, the 0.5 mL dosed turmeric sample had a lower load than the higher 1 mL dosed sample which was anomalous, possibly resulting from human error. No major synergistic effect was observed when the oils were used in combination.

Sensory panel

Odour

Table 3 showed the final day (day 14) odour ratings of the stored control and treated samples, with the control receiving an average odour score rating of 1.0 (unacceptable). With the exception of the 0.5 mL turmeric and 0.5 mL bay leaf EO samples, the other treated samples all received significantly higher ($p < 0.05$) final day ratings than the control. Due to the antimicrobial properties of the oils highlighted previously, the results showed that the EO treatments (except the 0.5 mL bay leaf and 0.5 mL turmeric) could have delayed microbial driven off-odours as similarly reported by Chouliara *et al.* (33). The 1.5 mL dose of each EO generally performed the best for that respective treatment except in the case of the bay leaf EO where the 1 mL dosed samples were rated the highest with a final day average score of 3.4. The pungency and strong odour of the bay leaf EO at the higher 1.5 mL dose could explain the lower rating by the panellists. The turmeric oil samples were the lowest rated of all the treated samples and although an increase in volume of the turmeric EO resulted in a slight increase in the final day scores, the ratings across the doses were not significantly different

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($p > 0.05$).

Heydari *et al.* (30) reported in a similar study, that samples treated with higher EO concentrations generally received higher ratings by panellists. This trend was only observed with the combination EO treatments as the final ratings increased with the increasing doses. The 0.5 mL combination oil sample was ranked with a low score of 1.8 which may have been due to the low volumes of each oil being used (0.25 mL each of bay leaf and turmeric EO) but it was observed to outperform the individual 0.5 mL doses still marginally from the turmeric and bay leaf EO at the end of storage, which both received scores of 1.5. The 1.5 mL combination dose not only was rated the highest of all the treated samples (3.6) but was also rated significantly higher ($p < 0.05$) than the individual 1.5 mL samples, indicating an increase in consumer appeal of sample odour when the oils were used in combination as opposed to individually at the same dose.

[Table 3 near here]

Colour

Like the odour results, the stored control samples had lowest final day ratings among the panellists for colour appeal with an average score of 1.4. Only the 0.5 mL and 1 mL bay leaf samples and 1.5 mL combination EO samples had significantly higher ($p < 0.05$) final day colour ratings than the control. Heydari *et al.* (30) noted that while the control sample in their study was the lowest ranked for colour, the colour ratings for the treated samples increased as more EO were used. This trend was not observed in this study, as no significant relationship between the dose level and the final day colour ratings were observed. The reason for this could be that while the researchers used a colourless EO (lavender oil), this current study used oils that were predominantly yellow in colour. The yellowness of the oils would have imparted a similar yellow hue on the samples and could have influenced the ratings from the panellists. This would explain why the highest doses (1.5 mL) received the lowest rating among the bay leaf and turmeric EO treatments as the amount of yellow imparted on the samples were perceived by the panellists as undesirable and associated with spoilage. Furthermore, as only 0.75 mL of each oil comprised the 1.5 mL combination EO treatment, it was rated highly, as less of the yellow colour was imparted on the samples.

Appearance

As the meat samples underwent spoilage during storage, their appearance was undesirable primarily because of surface slime and mould growth that occurred in some cases. The stored control sample received the lowest final day rating by the panellists with only the 1 mL turmeric, 0.5 mL bay leaf, 1 mL bay leaf and 1.5 mL combination EO samples receiving significantly higher ($p < 0.05$) ratings.

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For the individual bay leaf and turmeric EO treatments, the 1 mL dose level received significantly higher ($p < 0.05$) final day ratings than the other doses. The 1.5 mL doses did not receive significantly higher ($p > 0.05$) ratings than the 0.5 mL doses which probably meant that the 0.5 mL was too low to prevent microbial driven appearance changes whereas the 1.5 mL was too high and would have conferred some unideal alterations such as an increased oily texture of the sample by the panellists.

However, similar to the previous sensory results, the 1.5 mL combination EO treatment received the highest final day ratings which were significantly higher ($p < 0.05$) than the highest rated individual oil treatments on the final day. This showed that the use of smaller amounts of the oils in combination (0.75 mL each) were able to effectively reduce the microbial driven changes in appearance without imparting an undesirable oily texture on the samples.

Overall acceptability

The overall acceptability of the samples was perhaps one of the most important sensory parameters analysed as it considered the previous sensory parameters and gave an insight into the overall perception of quality including storage life from the panellists scores on the final day of storage. As expected, the stored control obtained the lowest final day rating of 1.3, indicating that it was perceived by the panellists as not fit for consumption. Furthermore, as seen in [Table 4](#), the stored control samples obtained a spoilage rating of less than 3.0 at day 8 of storage compared to 50 % of the treated samples which received a higher score.

Except for the 0.5 mL and 1.5 mL turmeric EO samples, all the treated samples received significantly higher ($p < 0.05$) final day ratings than the control. This observation is supported by previous work in terms of overall acceptability, whereby meat samples treated with EO were consistently rated significantly higher during extended storage as opposed to untreated control samples (37). The highest rated samples were the 1 mL bay leaf and 1.5 mL combination EO samples which were both rated significantly higher ($p < 0.05$) than the other treatments and were not perceived as spoilt by the panellists at the end of the storage period. With the control deemed spoilt at day 8 by the panellists, these EO treatments extended the storage life of the samples by an additional 6 days. [Table 4 near here]

CONCLUSIONS

The positive results from the study indicate an opportunity for the EO from West Indian bay leaf and turmeric to be used as potential natural meat preservatives. The oils were shown to have significant effects on both the quality parameters and the storage life of stored chicken breast

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samples. With respect to the physicochemical properties of the EO treated samples, although the oils were generally ineffective on moisture content, there was a significant effect ($p < 0.05$) on the other properties by specific treatments. All treatments significantly delayed pH changes while all treatments except the 0.5 mL dose and 1.5 mL combination dose significantly delayed texture and colour changes respectively. The 1 mL bay leaf, 1.5 mL bay leaf and 1 mL combination treatments were the only ones to significantly reduce protein loss.

The microbiological analyses showed that the oils generally reduced the microbial loads of the samples at the end of storage with the turmeric EO proving more effective than the bay leaf EO against LAB and YM but ineffective in the total plate count analysis. Additionally, the sensory analysis also showed that the final day (day 14) average hedonic ratings of the treated samples were predominantly higher than the control with the 1.5 mL combination EO and 1 mL bay leaf EO treatments proving to be the most effective. These doses were rated the highest for 'overall acceptability' and were shown to have extended the storage life and acceptability of the samples by six more days compared to the stored control. The results of this study clearly demonstrated the meat preservative potential that the bay leaf and turmeric essential oils possessed when used both individually and in combination with each other.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors contributed to the conceptualization of the work. C. John constructed the methodology, performed the formal analysis, investigation and data analysis. R. Maharaj performed the writing, review and editing of the manuscript.

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Table 1. Physicochemical parameters of fresh control sample (day 0 at 28 °C), refrigerated control (day 14) and treated (day 14) chicken breast samples stored at 4 °C

Sample treatment	pH	Hardness/N	Protein content/%	Moisture content/%	L^*	a^*	b^*
Turmeric oil							
0.5 mL	6.78±0.01 ^d	0.58±0.08 ^{cd}	20.93±1.17 ^{ab}	23.80±0.28 ^{ab}	67.88±0.02 ^b	12.54±0.02 ^d	17.15±0.01 ^{cde}
1 mL	6.90±0.01 ^e	0.79±0.07 ^{ef}	21.75±0.78 ^{ab}	24.09±1.54 ^{ab}	72.15±0.10 ^d	12.09±0.03 ^c	16.72±0.03 ^{bcd}
1.5 mL	7.01±0.03 ^f	0.80±0.06 ^{ef}	22.31±0.57 ^{abc}	25.00±0.99 ^{ab}	70.83±0.57 ^c	15.23±0.07 ^h	19.69±0.44 ^g
Bay leaf oil							
0.5 mL	6.82±0.01 ^{de}	0.43±0.02 ^b	21.31±0.27 ^{ab}	24.35±1.20 ^{ab}	67.82±0.02 ^b	14.02±0.01 ^f	18.28±0.01 ^f
1 mL	6.62±0.01 ^c	0.72±0.08 ^{de}	24.88±0.88 ^c	25.58±0.54 ^{ab}	75.65±0.06 ^e	11.93±0.01 ^b	16.36±0.03 ^{bc}
1.5 mL	6.32±0.02 ^b	0.76±0.02 ^{ef}	22.50±0.05 ^{bc}	26.33±0.24 ^{ab}	85.48±0.05 ^g	12.60±0.05 ^{de}	18.63±1.13 ^{fg}
Combination oil							
0.5 mL	6.74±0.01 ^d	0.51±0.02 ^{bc}	20.94±1.19 ^{ab}	23.29±0.55 ^a	67.81±0.04 ^b	12.68±0.01 ^e	15.64±0.01 ^b
1 mL	6.40±0.01 ^b	0.69±0.04 ^{de}	22.63±0.81 ^{bc}	26.38±0.20 ^{ab}	85.30±0.27 ^g	14.80±0.05 ^g	18.16±0.01 ^{ef}
1.5 mL	6.36±0.01 ^b	0.88±0.02 ^{af}	22.06±0.79 ^{abc}	28.49±1.65 ^b	83.94±0.15 ^f	11.00±0.02 ^a	16.33±0.02 ^{bc}
Fresh control (day 0)	5.91±0.02 ^a	0.95±0.03 ^a	24.94±0.08 ^c	78.32±2.73 ^c	72.22±0.09 ^d	19.62±0.04 ⁱ	14.45±0.18 ^a

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Stored control (day 14) 7.34 ± 0.08^g 0.49 ± 0.02^{bc} 19.31 ± 0.16^a 23.28 ± 0.37^a 60.24 ± 0.08^a 10.90 ± 0.11^a 17.75 ± 0.28^{def}

* Different superscript letters in a column indicate values are significantly different ($p < 0.05$)

Table 2. Microbiological load of fresh control (day 0 at 28 °C), refrigerated control (day 14) and treated (day 14) chicken breast samples stored at 4 °C

Sample treatment	Total plate count/(CFU/g)	Lactic acid bacteria/(CFU/g)	Yeast and mould/(CFU/g)
Turmeric oil			
0.5 mL	TNTC	2.43×10^5	6.95×10^6
1 mL	TNTC	1.09×10^5	1.27×10^7
1.5 mL	TNTC	TFTC	TFTC
Bay leaf oil			
0.5 mL	TNTC	1.01×10^7	1.80×10^7
1 mL	1.66×10^6	1.43×10^5	1.88×10^5
1.5 mL	1.37×10^6	8.5×10^4	1.60×10^5
Combination oil			
0.5 mL	1.27×10^8	4.65×10^6	6.30×10^6
1 mL	2.62×10^7	2.23×10^6	3.60×10^5

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1.5 mL	1.1×10^7	TFTC	5.70×10^4
Fresh control (day 0)	TFTC	TFTC	TFTC
Stored control (day 14)	TNTC	TNTC	TNTC

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Table 3. Final day sensory ratings of chicken breast samples stored at 4 °C

Treatment	Treatment dose	Odour	Colour	Appearance	Overall acceptability
Turmeric oil	0.5 mL	1.5±0.6 ^{ab}	1.5±0.7 ^a	1.4±0.5 ^a	1.5±0.6 ^{ab}
	1 mL	1.7±0.8 ^b	1.8±0.9 ^a	2.1±0.9 ^b	1.9±0.7 ^{bcd}
	1.5 mL	1.9±0.9 ^b	1.5±0.6 ^a	1.6±0.7 ^a	1.6±0.7 ^{abc}
Bay leaf oil	0.5 mL	1.5±0.8 ^{ab}	2.5±0.9 ^b	2.1±0.8 ^b	2.1±0.8 ^{cd}
	1 mL	3.4±0.9 ^{de}	2.7±0.8 ^b	2.6±0.6 ^c	3.0±0.8 ^e
	1.5 mL	2.9±0.9 ^{cd}	1.9±0.8 ^a	1.8±0.8 ^{ab}	2.4±0.7 ^d
Combination oil	0.5 mL	1.8±0.8 ^b	1.8±0.8 ^a	1.5±0.6 ^a	1.9±0.8 ^{bcd}
	1 mL	2.6±0.8 ^c	1.7±0.8 ^a	1.8±0.7 ^{ab}	1.9±0.6 ^{bcd}
	1.5 mL	3.6±1.0 ^e	2.7±0.6 ^b	3.1±0.8 ^d	3.4±0.9 ^e
Stored control (day 14)		1.0±0.0 ^a	1.4±0.5 ^a	1.4±0.5 ^a	1.3±0.5 ^a

*Different superscript letters in a column indicate values that are significantly different (p<0.05)

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Table 4. Overall acceptability ratings of refrigerated control (day 14) and treated (day 14) chicken breast samples stored at 4 °C

Time/day	Turmeric oil			Bay leaf oil			Combination oil			Control
	0.5 mL	1 mL	1.5 mL	0.5 mL	1 mL	1.5 mL	0.5 mL	1 mL	1.5 mL	
0	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a	7.0±0.0 ^a
2	6.1±0.5 ^{de}	6.4±0.6 ^e	6.2±0.6 ^{de}	5.7±0.5 ^{bc}	5.5±0.6 ^b	5.4±0.5 ^b	5.9±0.6 ^{cd}	5.7±0.6 ^{bc}	5.9±0.6 ^{cd}	5.0±0.5 ^a
4	4.7±0.7 ^{bcd}	5.1±0.6 ^{def}	5.5±0.5 ^f	4.5±0.5 ^{bc}	3.9±0.8 ^{ag}	3.6±0.6 ^g	5.4±0.6 ^{ef}	4.9±0.6 ^{cd}	5.0±0.6 ^{de}	4.3±0.6 ^{ab}
6	3.0±0.6 ^a	3.8±0.6 ^{cd}	4.0±0.6 ^d	4.2±0.6 ^d	3.5±0.8 ^{bc}	2.9±0.8 ^a	4.0±0.8 ^d	4.1±0.7 ^d	4.9±0.6 ^e	3.1±0.4 ^{ab}
8	2.5±0.5 ^{ab}	2.7±0.7 ^{abc}	2.7±0.6 ^{abc}	3.7±0.6 ^e	3.0±0.6 ^{bcd}	2.4±0.5 ^a	3.2±0.7 ^{cde}	3.4±1.0 ^{de}	3.5±1.1 ^{de}	2.3±0.8 ^a
10	2.0±0.6 ^{bc}	2.4±0.7 ^{cd}	2.0±0.6 ^a	2.4±0.7 ^{cd}	2.8±0.5 ^d	2.0±0.6 ^{bc}	2.8±0.4 ^d	2.4±0.6 ^{cd}	3.4±0.7 ^e	1.7±0.5 ^{ab}
12	1.4±0.5 ^a	2.3±0.8 ^b	1.6±0.6 ^a	2.2±0.6 ^b	2.8±0.5 ^c	2.3±0.7 ^b	2.2±0.5 ^b	2.3±0.6 ^b	3.5±0.9 ^d	1.4±0.6 ^a
14	1.5±0.6 ^{ab}	1.9±0.7 ^{bcd}	1.6±0.7 ^{abc}	2.1±0.8 ^{cd}	3.0±0.8 ^e	2.4±0.7 ^d	1.9±0.8 ^{bcd}	1.9±0.6 ^{bcd}	3.4±0.9 ^e	1.3±0.5 ^a

1= extremely unacceptable, 2= moderately unacceptable, 3= slightly unacceptable, 4= neutral, 5= slightly acceptable, 6= moderately acceptable, 7= extremely acceptable. Different superscript letters in a row indicate values that are significantly different ($p<0.05$).

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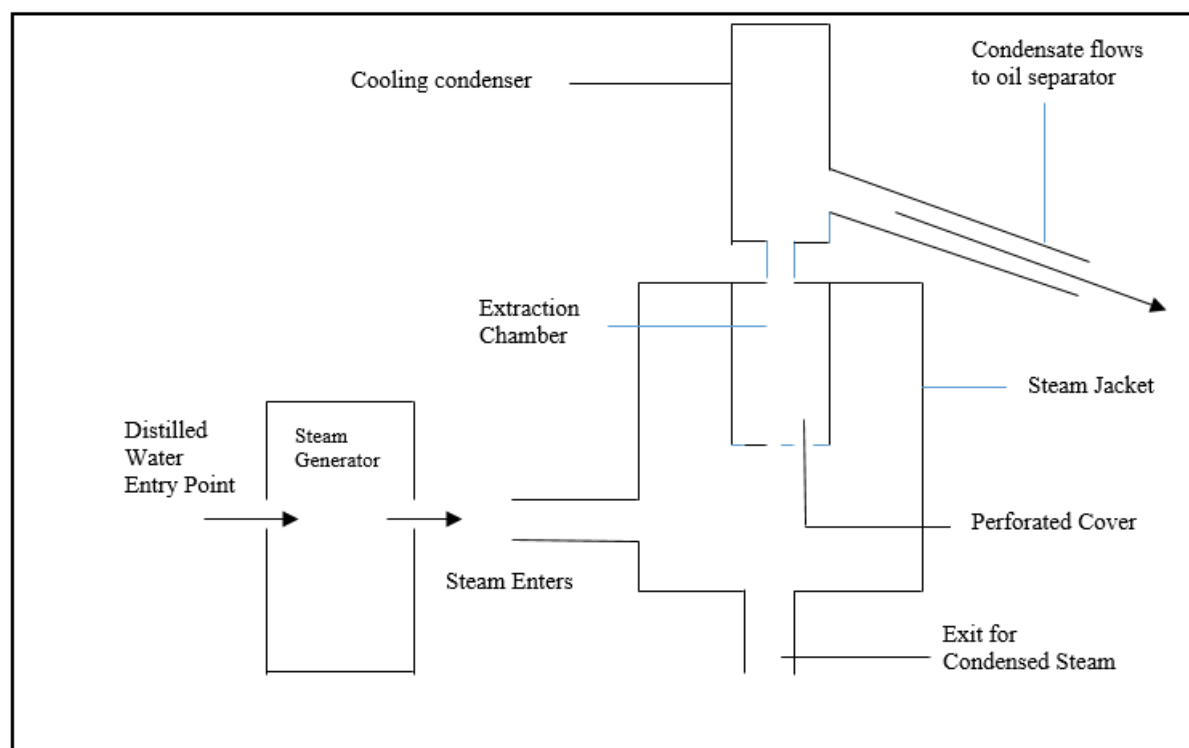


Fig. S1. Diagram of the steam distillation apparatus