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Application of *Cosmos caudatus* Kunth. (*ulam raja*) extract as antibacterial agent in beef and shrimp meats, and its sensory evaluation

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Abstract

The use of chemical preservatives in food products to inhibit the growth of microorganisms is widely used nowadays. However, their use has become a concern due to several negative side effects, and when consumers question the safety of the foods they eat. Therefore, the present work was conducted to investigate the potential of plant natural sanitiser from *Cosmos caudatus* Kunth extract to reduce the natural microflora present in raw beef and shrimp meat samples. The present work aimed to investigate the reduction of natural microflora (*B. cereus*, *E. coli*, *Pseudomonas* spp., *S. aureus*, and *L. monocytogenes*) in raw beef and shrimp meat samples following sanitisation with different concentrations of *C. caudatus* extract (0.05, 0.50, and 5.00%) at different soaking times (5, 10, and 15 min). The sanitised samples were further evaluated with sensory acceptability (colour, odour, texture, and overall acceptability) to determine their acceptance level after treatment. Based on the results, the microflora in beef and shrimp meat samples were reduced significantly ($p < 0.05$) started from 0.05% at varied soaking times. The decrease in bacterial populations was proportional to the increase in extract concentrations and soaking times. In sensory acceptability, all cooked samples achieved acceptance level by the panellists at 0.05% after 10 min of soaking time. Food samples treated with 0.05% of *C. caudatus* extract and 10 min of soaking time showed the best combination in terms of bacterial reduction and the level of acceptance by the panellists. Hence, it can be concluded that *C. caudatus* extract has a high potential as a natural-based food sanitiser that can prevent bacterial contamination while maintaining the sensory acceptability of the foods.

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Introduction

Food contamination can occur during post-harvesting processing or even earlier, such as from farms, water irrigation systems, and manure (Hajipour *et al.*, 2021). Without proper decontamination, pathogens will keep multiplying, especially during food preparation and storage, thus spoiling the foods (Gil *et al.*, 2015). For fresh produce, washing with tap chlorinated water is a common practice applied by the households. As recommended by the United States of Food and Drug Administration (USFDA, 1998), the allowable total chlorine in tap water for washing is 50 - 200 mg/L (pH 6.0 - 7.5), with only 1 - 2 min contact times. Chlorine has been reported to have a disinfectant effect on fresh produce (Joshi *et al.*, 2013). However, its ability to produce carcinogenic trihalomethanes when reacting with organic matters has been raising concerns (Chang *et al.*, 2000). Moreover, water with high chlorine residues will leave foods with unpleasant odour, smell, and flavour (Chang *et al.*, 2000). Food sanitisation on meat and poultry has also become a concern since consumers believe chilling and freezing can stop and kill the contaminants, without understanding the ability of bacteria to adjust their intracellular cells and survive in a stress environment. As reported by Al-Nehlawi *et al.* (2014), *Listeria monocytogenes* can spoil poultry sausages during refrigeration by switching their intracellular constituents and growing anaerobically in foods.

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Nowadays, there are many decontamination methods applied on foods such as chlorine, organic acids, bacteriocins, hydrogen peroxide, ozonation, irradiation, and many more. Some of them are applied alone or in combination, depending on their suitability and efficiency. However, these treatments have several limitations such as deterioration in food quality, increased health risks, and high cost (Gerrity and Snyder, 2011). Some of them also lead to organoleptic changes that negatively impact consumers' acceptability (Negi, 2012). Therefore, an alternative method using natural product for food sanitisation is introduced to reduce microbial populations in food sources, as well as prioritise the consumers' demand for safe foods. In this context, plant antimicrobials are gaining wide interest as most of them have a higher level of food safety with GRAS (Generally Recognized as Safe) status (Palou *et al.*, 2016).

In the present work, *Cosmos caudatus* Kunth was used as a natural food sanitiser. This plant is locally known as *ulam raja*, and widely distributed in tropical countries, including Mexico, United States, Central America, South America, Malaysia, and Thailand (Yusoff *et al.*, 2021). Several biological activities of *C. caudatus* have been reported in previous studies (Salehan *et al.*, 2013; Cheng *et al.*, 2015; Ramli *et al.*, 2017). Therefore, the present work aimed to investigate the ability of *C. caudatus* extract as a food sanitiser on the reduction of bacteria in raw beef and shrimp meat samples, as well as their sensory acceptability after treatment.

Materials and methods

Plant and food materials preparation

Fresh samples of *C. caudatus* plant, beef, and shrimp were purchased from Pasar Borong Selangor, Malaysia. All food samples were kept in a cooler box (2 - 5°C), and transported to the laboratory in less than an hour after purchase. The food samples were then transferred to a chiller (2 - 5°C) for storage until further analyses. The *C. caudatus* plant was sent to the Institute of Bioscience, UPM for taxonomic identification under the voucher number SK 2668/15. The shrimps had an average size of 2.0 - 2.5 inches each. Their heads were removed to prevent any errors during the analysis. For beef meat samples, they were cut into cubes of approximately 10 g each.

Extraction of C. caudatus

Extraction was performed according to Rukayadi *et al.* (2008) with slight modifications. The *C. caudatus* leaves were collected, dried at room temperature (27°C), and powdered using a heavy-duty Waring blender (Sinclair and Campbell, Scotland, UK). For extraction, 100 g of powdered *C. caudatus* was soaked in 400 mL of absolute methanol (99.8%) (Sigma-Aldrich, Saint Louis, MO), and left for 2 d under room temperature with occasional stirring. The mixture was filtered using Whatman filter paper No.1 (Whatman International Ltd., Middlesex, UK), and concentrated using a rotary evaporator at 50°C until gummy-like crude extract was formed. The *C. caudatus* crude extract was collected and stored in a freezer (-20°C) until further use.

Preparation of selective media

Five types of selective agar were used; Mannitol Yolk Polymyxin Agar (MYP), Chromocult Coliform Agar, Pseudomonas Agar (PSA), Tryptic Soy Agar (TSA), and Fraser Agar for the detection of *B. cereus, E. coli, Pseudomonas* spp., *S. aureus*, and *L. monocytogenes*, respectively. Colony colour and characteristics of bacteria were determined according to Atlas (2010); *B. cereus* (pink-orange colour), *E. coli* (dark blue to violet colour), *Pseudomonas* spp. (greenish colour), *S. aureus* (yellowish colour), and *L. monocytogenes* (black colour surrounding the colony).

Treatment of raw food materials with C. caudatus extract solution

The *C. caudatus* extract solution was prepared by dissolving the extract in 10% DMSO to make a stock concentration of 10%. Then, a series of dilution was prepared at 5.00, 0.50, and 0.05%. For sanitisation, 10 g of either beef or shrimp meat of uniform size were immersed in filtered tap water, deionised water (DIW), and *C. caudatus* treatment solutions (0.05, 0.50, and 5.00%) at different soaking times (0, 5, 10, and 15 min). Filtered tap water and DIW served as controls. Then, the samples were filtered to remove excess solution, and homogenised in stomacher bags (BagSystem, Interscience, France) containing phosphate buffer saline. Serial dilution was performed by taking out 1 mL of homogenised samples into 9 mL of phosphate saline buffer solution (0.85%) to be made up into three dilutions; 10^{-1}, 10^{-2}, and 10^{-3}. Then, 50 µL from each of the dilutions were

pipetted onto selective media agar separately and incubated at 37°C for 24 h. The colonies were counted and calculated using a colony counter (Fisher Scientific, USA).

Sensory evaluation of treated raw food materials

Sensory evaluation was performed according to Brasil *et al.* (2012) with slight modifications. Briefly, all samples were immersed in 0.05, 0.50, and 5.00% of *C. caudatus* solutions at different soaking times (5, 10, and 15 min), while tap water served as control. A group of panellists was presented with five different 5-digit coded samples placed in a random order. The evaluation was conducted for inspection of acceptance testing, in which the panellists were asked to assess the treated samples based on their colour (observation by eyes), odour (smell by nose), texture (touch by finger), and overall acceptability. A 9-point hedonic scale was used with the ratings from 1 (extremely dislike) to 9 (extremely like). Score ≥ 5 was considered acceptable.

Statistical analysis

All experiments were performed in triplicates. Data for the analysis of variance (ANOVA) was analysed using the Tukey's test (Minitab 16.0), and $p < 0.05$ was considered as significant. Results were interpreted as mean ± standard deviation (SD). For sensory analysis, individual scores for each treatment were summed and divided by the number of panellists to obtain the mean scores. Data for colour, odour, texture, and overall acceptability were analysed with ANOVA (Minitab 16.0) with $p < 0.05$ accepted as significant.

Results and discussion

Detection of natural bacterial populations in raw food materials using selective media agar

Raw foods often harbour various microorganisms commonly originated from the environment where they grew. These microorganisms will keep growing along the postharvest handling and food processing, which then cause spoilage of the foods if no proper decontamination method is applied (Gil *et al.*, 2015). The growth and survival of these microorganisms with prolong time will spoil the foods, and some could foodborne illnesses to the consumers. As reported by Chang and Fang (2007), *E. coli* O157:H7 and *Salmonella* Typhimurium can survive in shredded lettuce within 10 - 12 days, and impose a potential health risk to consumers. Besides, the growth of bacterial pathogens will also deteriorate the organoleptic properties of the foods, and decrease consumers' preference. Therefore, food sanitisation is important after harvest to minimise the bacterial loads contaminating the foods before food preservation or processing / cooking.

Table 1 shows the bacterial loads in raw beef and shrimp meat samples. Results showed that TPC, *B. cereus*, and *E. coli* were detected in raw beef meat samples in the range of 5 - 7 $\log_{10}$ CFU/g. While in raw shrimp meat samples, the detected bacteria were TPC, *E. coli*, and *S. aureus* in the range of 5 - 6 $\log_{10}$ CFU/g. There was no *Pseudomonas* spp. and *L. monocytogenes* found in both raw beef and shrimp meat samples. The bacterial loads in raw beef meat samples were significantly higher ($p < 0.05$) as compared to those of shrimp meat samples with the difference of almost 3 $\log_{10}$ CFU/g. The difference in their growth environment and source of food might explain this. For raw beef meat samples, the contamination sources are the slaughtering process and soil attachment, in which, the design of the cowshed is usually attached to the soil. Moreover, their foods such as grasses and pellets were also directly attached to the soil. These conditions contributed to the contamination of *B. cereus* in the raw beef meat samples. As stated by Carlin *et al.* (2010), *B. cereus* is well distributed in soil, and can be found in the gastrointestinal tract of eukaryotes, which originated from the food they consumed. This was also supported by Tewari *et al.* (2015) who also found the contamination of *B. cereus* in raw beef meat samples. Milojevic *et al.* (2019) reported the presence of *B. cereus* in raw beef meat samples from retail shops, which was in the range of 3 - 6 $\log_{10}$ CFU/g, lower as compared to those observed in the present work. These variations could have been contributed by good hygiene practices applied by retailers and meat shops (Tewari *et al.*, 2015).

The presence of *E. coli* in both raw beef and shrimp meat samples was contributed by the cross-contamination in the wholesale market. A previous study conducted by Yusoff *et al.* (2015) proved the high microbial populations in wet market raw chicken meat samples as compared to the supermarket. Furthermore, the ability of *E. coli* to survive in various conditions with extended time has also become one of the major concerns in food microbial contaminations. As reported by Abadias *et al.* (2012), *E. coli* can be found surviving in fresh produce for

several days, which can cause illness to consumers. However, no *Pseudomonas* spp. and *L. monocytogenes* were detected in both raw beef and shrimp meat samples. This contradicted Wan Norhana *et al.* (2010) who reported the presence of *L. monocytogenes* in shrimp samples. This might be due to the insignificant level of *L. monocytogenes* that rendered it undetectable, or unfavourable conditions during handling which might have removed it (Lynch *et al.*, 2009).

Table 1. Bacterial loads (log_{10} CFU/g) of raw beef and shrimp meat samples.

Natural microflora / Food sample	TPC	*B. cereus*	*E. coli*	*Pseudomonas* spp.	*S. aureus*	*L. monocytogenes*
Beef	7.34 ± 0.01	6.72 ± 0.07	5.81 ± 0.00	n.d.	n.d.	n.d.
Shrimp	6.11 ± 0.05	n.d.	5.40 ± 0.00	n.d.	5.44 ± 0.01	n.d.

n.d.: not detected.

Reduction in bacterial loads of food materials following treatment with C. caudatus extract solution

The present work demonstrated the sanitising effect of *C. caudatus* extract solution on beef and shrimp meat samples. Tables 2 and 3 show the survival of bacterial populations in beef and shrimp meat samples, respectively following treatment with tap water and different concentrations of *C. caudatus* extract solution ranging from 0.05, 0.50, and 5.00% with different soaking times; 5, 10, and 15 min. The DIW (0.00%) served as control, while treatment with tap water was the common washing method applied by the general households. Results showed no significant reduction ($p > 0.05$) on most bacterial populations treated with DIW. However, some of them indicated some reduction when the soaking time was increased. This might be due to the unfavourable conditions experienced by the bacteria, which then eliminated them slowly, while treatment with tap water caused a significant reduction ($p < 0.05$) on bacterial loads in the tested food samples. Similar findings were reported by Ukuku *et al.* (2004) and Selma *et al.* (2008). However, the use of filtered tap water was reported to produce carcinogenic compounds such as trihalomethanes when they react with organic matters (Mazhar *et al.*, 2020). Furthermore, reusing filtered tap water can also be another source of contamination (Gil *et al.*, 2009).

Following treatment with *C. caudatus* extract solution, the viability of bacteria in the food samples were determined. TPC, *B. cereus*, and *E. coli* were detected in the treated beef meat samples (Table 2). This was supported by Ali and Takwa (2010) who reported the presence of *B. cereus* in meat and other food materials with high protein content. These pathogens may contaminate meat surfaces during slaughtering or distribution to retailers (Ali and Takwa, 2010). All bacteria survived in the control treatment except for TPC which was reduced to undetectable levels (< 1 log_{10} CFU/g) after 0.50 and 5.00% treatments for 5, 10, and 15 min. Similar reduction ($p < 0.05$) was found in *E. coli* after 0.05% of treatment for 5 min, and reached an undetectable level after 5.00% of treatment for 5 min.

Among them, *B. cereus* was the most resistant species, and survived better than TPC and *E. coli*. *B. cereus* required a longer time to reach their significant reduction than others, which was 0.05% of treatment for 15 min. This could be explained by the capability of *Bacillus* spp. to form spores under unfavourable conditions since spores have a high tolerance towards extreme conditions (Soares *et al.*, 2012). Moreover, the characters of food constituents also affected the solubility of plant extract solutions, in which high-fat content in meat would be harder for the treatment solutions to penetrate the food matrices as compared to aqueous foods (Kuo and Lee, 2014).

TPC, *E. coli*, and *S. aureus* were detected in the treated shrimp meat samples (Table 3). A significant reduction ($p < 0.05$) in TPC was observed after 0.05% treatment for 10 min. While *E. coli* and *S. aureus* were significantly reduced ($p < 0.05$) after the similar soaking time of 10 min, but at 0.50 and 0.05% treatments, respectively. The reduction in *E. coli* population was about 0.98 log_{10} CFU/g, and 0.65 log_{10} CFU/g in *S. aureus*. All bacteria reached their undetected level at 5.00% treatment. In general, the number of survived bacteria decreased with increasing concentrations of treatment solution. This was supported by Abadias *et al.* (2011) who also reported the reduction of *E. coli* and *Salmonella* after being treated with a higher concentration of vanillin (12 g/L).

Based on the results, it can be concluded that the decrease in bacterial populations in treated beef

Table 2. Bacterial loads (log_{10} CFU/g) of beef meat samples treated with *C. caudatus* extract at different concentrations and soaking times.

Bacterial species	**TPC**			***Bacillus cereus***			***Escherichia coli***		
Initial load	7.34 ± 0.01^{Aa}			6.72 ± 0.07^{Aa}			5.81 ± 0.00^{Aa}		
Time / Treatment	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Tap water (%)	7.12 ± 0.04^{Aa}	6.52 ± 0.00^{Bb}	5.82 ± 0.14^{Bc}	6.65 ± 0.31^{Aa}	6.52 ± 0.44^{Ab}	5.95 ± 0.81^{Bc}	4.55 ± 0.10^{Cb}	3.90 ± 0.19^{Cc}	3.89 ± 0.50^{ABc}
0.00	7.23 ± 0.24^{Aa}	7.13 ± 1.65^{Aa}	6.66 ± 1.23^{Ab}	6.32 ± 0.07^{ABb}	6.18 ± 0.11^{Ac}	5.84 ± 0.05^{BCd}	5.72 ± 0.00^{Ab}	5.65 ± 0.06^{Ac}	4.11 ± 0.01^{Ad}
0.05	5.51 ± 0.14^{Bb}	5.17 ± 0.01^{Cc}	4.99 ± 0.06^{Cc}	6.43 ± 0.02^{ABb}	6.42 ± 0.07^{Ab}	5.44 ± 0.02^{Cc}	4.87 ± 0.04^{Bb}	4.48 ± 0.10^{BCc}	4.04 ± 0.05^{ABd}
0.50	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Db}	5.23 ± 1.05^{ABb}	5.18 ± 0.18^{Bc}	5.32 ± 0.21^{Cc}	4.54 ± 0.05^{Bb}	4.65 ± 0.05^{Bc}	2.11 ± 0.10^{BCd}
5.00	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Db}	4.58 ± 0.24^{Bb}	2.83 ± 0.09^{Cc}	2.83 ± 0.01^{Dc}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Cd}

Mean ± standard deviation followed by different uppercase superscripts within the same columns are significantly different ($p < 0.05$). Mean ± standard deviation followed by different lowercase superscripts within the same rows are significantly different ($p < 0.05$).

Table 3. Bacterial loads (log_{10} CFU/g) of shrimp meat samples treated with *C. caudatus* extract at different concentrations and soaking times.

Bacterial species	**TPC**			***Escherichia coli***			***Staphylococcus aureus***		
Initial load	6.11 ± 0.05^{Aa}			5.40 ± 0.00^{Aa}			5.44 ± 0.01^{Aa}		
Time / Treatment	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Tap water (%)	5.29 ± 0.07^{ABb}	5.30 ± 0.11^{ABb}	5.18 ± 0.03^{Ac}	5.00 ± 0.00^{Ab}	5.20 ± 0.07^{ABc}	4.89 ± 0.06^{ABd}	5.22 ± 0.03^{Ab}	5.18 ± 0.17^{ABb}	4.85 ± 0.04^{Dc}
0.00	5.52 ± 0.41^{ABb}	5.36 ± 0.26^{Bc}	5.60 ± 0.29^{Ad}	5.47 ± 1.19^{Ab}	5.29 ± 0.49^{Ac}	4.72 ± 0.86^{Bd}	5.11 ± 0.61^{Ab}	5.29 ± 0.45^{ABc}	4.78 ± 0.49^{Bd}
0.05	4.89 ± 0.08^{ABb}	4.72 ± 0.05^{Bc}	4.46 ± 0.40^{Ad}	4.86 ± 0.04^{Ab}	4.81 ± 0.01^{ABc}	4.72 ± 0.04^{ABd}	5.11 ± 0.00^{Ab}	4.79 ± 0.00^{BCc}	4.51 ± 0.01^{BCd}
0.50	4.24 ± 0.19^{BCb}	3.52 ± 0.04^{Cc}	1.83 ± 0.01^{Bd}	4.60 ± 0.03^{ABb}	4.42 ± 0.17^{Bc}	3.00 ± 0.00^{Cd}	5.08 ± 0.23^{Ab}	4.12 ± 0.19^{Cc}	4.18 ± 0.10^{Cd}
5.00	2.60 ± 0.08^{Cb}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Cc}	3.63 ± 1.19^{Bb}	0.00 ± 0.00^{Cc}	0.00 ± 0.00^{Dc}	4.14 ± 1.21^{Bb}	3.11 ± 0.73^{Dc}	0.00 ± 0.00^{Dd}

Mean ± standard deviation followed by different uppercase superscripts within the same columns are significantly different ($p < 0.05$). Mean ± standard deviation followed by different lowercase superscripts within the same rows are significantly different ($p < 0.05$).

and shrimp meat samples was proportional to the increase in *C. caudatus* concentrations and soaking times. The presence of flavonoids as major bioactive compounds in *C. caudatus* extract was proven to promote antimicrobial activity, thus reducing the number of bacteria (Yusoff *et al.*, 2014). Ramli *et al.* (2017) also reported the significant reduction of natural microflora in grapefruits after being treated with a higher concentration (0.50%) of *Syzygium polyanthum* solution as compared to the low concentration. However, treatment with a higher concentration of *C. caudatus* extract and a longer soaking period is not recommended as it might affect the nutrient contents, and potentially lowering consumers' acceptability.

Sensory evaluation of beef and shrimp meat samples treated with C. caudatus extract solution

Table 4 shows the results of the sensory evaluation on beef meat samples treated with *C. caudatus* extract solution. Based on Table 4, most of the panellists preferred colour and odour attributes in treated beef meat samples after 0.50% treatment at 15 min. For texture, the panellists were unable to recognise between treated and non-treated beef meat samples, which then yielded insignificant difference ($p > 0.05$). For overall acceptability, changes in treated beef meat samples would be preferred on the limit of 0.50% treatment. Based on the results obtained, beef meat samples treated with *C. caudatus* extract solution was accepted by the panellists in terms of colour, odour, and overall acceptability after 0.50% treatment for 15 min, with no significant difference ($p > 0.05$) was found on the texture of the treated beef meat samples. Surprisingly, treatment for control (tap water) resulted in medium acceptance by the panellists, which indicated that most panellists were unable to differentiate between the treated and non-treated beef meat samples after they were steamed.

Table 5 shows the results of the sensory evaluation on shrimp meat samples treated with *C. caudatus* extract solution. Based on Table 5, there was no significant difference ($p > 0.05$) in the texture and overall acceptability of the treated shrimp meat samples. Findings also revealed that the texture of shrimp meat samples was not altered even after the extreme treatment of 5.00% for 15 min. The most preferred treatment for colour was at 0.50% for 10 min, whereas the odour and overall acceptability were accepted by the panellists until 5.00% treatment for 10 min. Colour and odour are the major factors influencing consumers' preference. In this present work, odour was accepted by the panellists at 0.50% treatment for all soaking times. This happened due to the steaming process that lessened the pungent odour of the treatment solution.

Most of the attributes for shrimp meat samples after treatment received a score of 5, which indicated that the panellists could not differentiate between treated and non-treated shrimp meat samples. Both beef and shrimp meat samples obtained the minimum acceptance for most of the attributes at 0.50% treatment for 15 min. Among the attributes, texture showed no significant difference ($p > 0.05$) between treated or non-treated samples in beef and shrimp meats. Bingol *et al.* (2011) showed the use of lemon juice extract caused a slight reduction in *Salmonella* Enteritidis and *E. coli* in raw meatballs with no significant difference ($p > 0.05$) on sensory attributes. Higginbotham *et al.* (2014) reported the ability of *Hibiscus sabdariffa* extract to cause a reduction in *L. monocytogenes* contamination on beef hotdogs, but no sensory evaluation was conducted. Solomon *et al.* (2014) also reported microbial reduction in *suya* (boneless meat pieces) after being treated with basil extract, and obtained positive feedback from the panellists.

Conclusion

The present work reported the high potential of *C. caudatus* solution as a practical and efficient sanitising agent to minimise the number of bacterial contaminants in beef and shrimp meats. The relative best combination between antibacterial ability and sensory acceptability was achieved after treatment with 0.05% of *C. caudatus* extract for 10 min of soaking time. Future studies on other types of food samples are encouraged to observe the inhibition capabilities of *C. caudatus* sanitiser to other microbial commodities.

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Table 4. Sensory acceptability of beef meat samples treated with *C. caudatus* extract at different concentrations and soaking times.

Concentration (C)	**Control**	**0.05%**			**0.50%**			**5.00%**		
ET / AT		**5 min**	**10 min**	**15 min**	**5 min**	**10 min**	**15 min**	**5 min**	**10 min**	**15 min**
Colour	5.40 ± 1.76^{a}	5.52 ± 1.64^{a}	4.44 ± 1.64^{a}	5.36 ± 1.96^{a}	4.84 ± 1.86^{ab}	4.64 ± 1.78^{a}	5.20 ± 1.94^{a}	5.60 ± 1.35^{b}	5.52 ± 1.53^{a}	5.40 ± 1.53^{b}
Odour	6.12 ± 1.76^{a}	5.20 ± 2.14^{ab}	5.20 ± 1.66^{ab}	5.60 ± 1.29^{a}	5.20 ± 1.61^{ab}	4.96 ± 1.62^{ab}	5.64 ± 1.11^{ab}	5.60 ± 1.29^{b}	5.00 ± 1.76^{b}	5.88 ± 1.09^{b}
Texture	4.84 ± 2.32^{a}	5.12 ± 1.96^{a}	3.88 ± 1.99^{a}	5.48 ± 1.66^{a}	4.40 ± 2.35^{a}	3.68 ± 2.01^{a}	4.08 ± 1.22^{a}	5.16 ± 1.52^{a}	5.40 ± 1.53^{a}	5.48 ± 1.16^{a}
Overall acceptability	5.36 ± 1.68^{a}	5.00 ± 1.76^{ab}	4.60 ± 1.76^{a}	5.60 ± 1.71^{a}	4.84 ± 1.65^{ab}	4.24 ± 1.64^{a}	4.48 ± 1.42^{ab}	5.48 ± 1.42^{b}	5.28 ± 1.24^{a}	5.52 ± 0.96^{b}

ET = Estimation time; AT = Attributes. Mean ± standard deviation followed by different lowercase superscripts within the same rows are significantly different ($p < 0.05$).

Table 5. Sensory acceptability of shrimp meat samples treated with *C. caudatus* extract at different concentrations and soaking times.

Concentration (C)	**Control**	**0.05%**			**0.50%**			**5.00%**		
ET / AT		**5 min**	**10 min**	**15 min**	**5 min**	**10 min**	**15 min**	**5 min**	**10 min**	**15 min**
Colour	5.76 ± 1.90^{a}	5.96 ± 1.37^{a}	6.52 ± 1.92^{a}	5.40 ± 2.14^{ab}	5.92 ± 1.66^{a}	6.20 ± 1.29^{a}	3.44 ± 1.68^{bc}	6.00 ± 1.19^{b}	6.20 ± 1.19^{b}	5.56 ± 1.58^{c}
Odour	5.12 ± 1.81a	5.04 ± 1.57^{a}	5.12 ± 1.74^{a}	5.08 ± 1.47^{a}	5.60 ± 1.78^{a}	5.80 ± 1.41^{ab}	4.12 ± 1.69^{a}	5.80 ± 1.32^{a}	5.64 ± 1.32^{b}	5.36 ± 1.23^{a}
Texture	5.44 ± 1.94^{a}	5.36 ± 1.70^{a}	5.64 ± 1.82^{a}	5.44 ± 1.16^{a}	5.36 ± 1.82^{a}	5.72 ± 1.21^{a}	4.88 ± 1.48^{a}	5.68 ± 1.18^{a}	5.80 ± 1.19^{a}	5.56 ± 1.26^{a}
Overall acceptability	5.28 ± 1.74^{a}	5.52 ± 1.78^{a}	5.88 ± 1.64^{a}	5.04 ± 1.21^{ab}	5.36 ± 1.87^{a}	6.2 ± 1.22^{a}	5.32 ± 1.44^{ab}	5.72 ± 1.24^{a}	5.80 ± 1.00^{b}	5.76 ± 1.05^{b}

ET = Estimation time; AT = Attributes. Mean ± standard deviation followed by different lowercase superscripts within the same rows are significantly different ($p < 0.05$).

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