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Assessment of Physicochemical, Microbiological and Sensory Attributes of Maldive Fish with the Incorporation of *Garcinia cambogia* (Goraka)

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ABSTRACT

This study was conducted to evaluate the potential use of *Garcinia cambogia* for the improvement of quality attributes of Maldive fish. Maldive fish were prepared using three different incorporation levels of *G. cambogia* as 0%, 1.25%, 1.67% and 2.5%. Sensory results revealed that sample incorporated with 2.5% of *G. cambogia* had the best qualities. *G. cambogia* treated samples showed significant reduction (P<0.05) of histamine content compared to commercial Maldive fish samples. The highest level of 2-thiobarbituric acid-reactive substances (TBARS) was identified in the commercial sample, while the lowest level found in Maldive fish incorporated with 1.25% of *G. cambogia*. Maldive fish incorporated with the highest level (2.5%) of *G. cambogia* showed greater suppression of bacterial count. Further, significantly lower (P<0.05) fungal counts and bacterial counts were observed in all treatments compared to commercial samples and the control. In conclusions, Maldive fish incorporated with 2.5% of *G. cambogia* results enhanced sensory and quality attributes and safe product for consumers.

Key words: *Garcinia cambogia*, Histamine, Maldive fish, Myoglobin content.

INTRODUCTION

Fish is a nutritious food source which comprises of quality proteins, vitamins, essential fatty acids and minerals. Fish consumption has been gradually increased over the years mainly due to the proven health benefits such as being a rich source of omega 3 fatty acids that reduces cardio-vascular diseases and its use as a lean alternative to meat (Das *et al*., 2009; Siscovick *et al*., 2000). In many of the developing countries, smoked and dried fish are traditionally accepted food items and act as the major source of protein. Maldive fish is a hard dried product obtained by salting, hot smoking and drying the flesh of fresh or frozen wholesome fish of the tuna species (Mohamed, 2013). It is widely used as an ingredient for flavoring and thickening of many dishes in Maldive islands and as well as in neighboring countries like Sri Lanka. Maldive fish is a favorite addition to curries and “sambals” (chilly chutneys) to impart a salty and fishy taste and it is a traditional fish product equivalent to the fish sauce in Vietnam and Thailand. The excess harvest of fresh or frozen tuna species such as Skipjack tuna (*Katsuwonus pelamis*), Frigate Mackerel (*Auxis thazard*), Yellowfin tuna (*Thunnus albacores*) and Mackerel tuna (*Euthynnus affinis*) are commonly used to produce Maldive fish (Mohamed, 2013).

Production of Maldive fish is gradually open out for countries like Sri Lanka, India, Japan and other south Asian countries. In Sri Lankan context, Maldive fish production is a seasonal industry which gives the highest profit during the glut season of fish and when the costs of raw materials are low. However, the products failed to satisfy the quality and quantity of local market requirement. Histamine poisoning due to decarboxylation of histidine into histamine, lipid oxidations which decrease the shelf life of the product are the major quality defective reactions. When consider about the microbial quality, the association between smoked fish and fungal growth is remarkable. Salted food can be affected by the halophilic organisms which has a preference for high salty conditions. In order to overcome these quality defects, manufactures tend to use some natural ingredients in the Maldive fish production process. *G. cambogia* is one of the natural ingredients used in the production process of Maldive fish in Sri Lanka (Gunaratne, 1999). It is also called as Malabar tamarind and famous for its antimicrobial, cytotoxic, antioxidant and anticancer properties of the fruit rind due to the activity of different secondary metabolites such as benzophenones, flavonoids and xanthones (Iinuma *et al*., 1998; Koshy *et al*., 2001; Masullo *et al*., 2008). As per the current trends, consumers highly prefer food products with natural preservatives such as spices than the chemical preservatives (Jakhar *et al*., 2018). Thus, the main objective

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of this study was to ascertain the effect of *G. cambogia* on the improvement of physicochemical, microbiological and sensory attributes of Maldive fish.

MATERIALS AND METHODS

Maldive fish samples were prepared using three different incorporation levels of *G. cambogia*, which were Treatment 1, Treatment 2 and Treatment 3 as 1.25%, 1.67% and 2.5% of *G. cambogia* respectively. A control sample was prepared without adding *G. cambogia*. Commercially available Maldive fish samples were obtained from local markets in Sri Lanka for comparision.

Preparation of Maldive fish

Fresh ripened fruits of *G. cambogia* were cut into 4 pieces and seeds along with the seed covering were removed. After that, fruit rinds were allowed to boil for about 1.5-2 h without addition of water. During the boiling, stirring was done until it became a paste.

Fresh tuna fish (*Katsuwonus pelamis*) was cleaned by removing head, gills, gut and fins. Washed with clean water and 1.5 kg of fish was weighed accurately. Five liter of water was added to a container with *G. cambogia* paste and 250 g of salt was added. The mixture was heated up to about 55-60°C and fish was dipped in the container and boiled for 45 minutes. It was allowed to cool and water was drained off. After that fish was cut into four longitudinal pieces and all the bones were removed. Smoking was done for 18 h and finally sun drying was done for 5-7 days.

Determination of histamine content

Fluorometric method was employed with modifications for the analysis of histamine content (Horwitz and GW, 2005). Histamine content was calculated by using the standard curve.

Determination of TBARS content

The content of TBARS was analyzed using Spectrophotometric method (Horwitz and GW, 2005). TBARS value was calculated using the standard curves prepared with 1,1,3,3-tetramethoxy methane as a precursor of malonaldehyde (Horwitz and GW, 2005).

Determination of myoglobin content

Maldive fish samples were cleaned by removing exterior layer and cut into small pieces. Two grams of sample was accrately weighed and 20 mL of ice cold 40 mM phosphate buffer (pH 6.8) was added to each sample. Then the samples were blended for 30 seconds at high speed. These homogenates were centrifuged at 3000 g using centrifuge (CF 15D2, Himac, Hitachi, Japan). After that, the supernatant of each sample was filtered using a cheese cloth and followed by filtration with whatman No.1 filter paper. The volume of the filtrate was accurately measured. The absorbance values were measured at emmision wave length of 525 nm and 700 nm.

Microbial quality analysis

Total bacterial count and fungal count were enumerated using Nutrient agar (HIMEDIA, Himedia Laboratories Pvt Ltd., India) and Sabouraud's dextrose agar (OXOID, OXOID Ltd., England) respectively by pour plate technique (Downes and Ito, 2001).

Sensory evaluation

A ranking test was done to identify the best level of treatments. The T1(1.25%), T2(1.67%) and T3(2.5%) were compared and all the treatments were presented as both sliced and whole maldive fish forms. Most preffered sample was obtained the rank one and the least prefered sample obtained the rank three.

Statistical analysis

Completely Randomized Design (CRD) was used for the analysis of quality parameters and mean separation was done by Duncan's Multiple Range ($P<0.05$) test. Data were analyzed using SAS software (SAS Inst. Inc., Cary, NC, USA). Sensory attributes were analyzed separately and ranking test was performed using Friedman test using Minitab 14 software.

RESULTS AND DISCUSSION

Physicochemical analysis

Histamine is a protruding food born chemical hazard which is produced due to bacterial decarboxylation of histidine. Most of the fish species used in Maldive fish production is associated with histamine formation; especially fish belong to family Scombridae (Mustafa, 2018; Thadhani *et al.*, 2002). Histamine determination results (Table 1) indicated that all the Maldive fish samples have relatively much lower levels compared to the maximum allowable histamine level of 200 mg/kg (Fletcher *et al.*, 1998). According to the European Legislation, up to a maximum of 200 mg/kg in fresh fish and 400 mg/kg of histamine in fishery products subjected to enzyme maturation in brine are allowable (Visciano *et al*., 2014). Commercial Maldive fish sample gave relatively higher histamine content than other Maldive fish samples and it could be attributed to the poor quality of the fish used for Maldive fish production and improper processing conditions. Since, histamine forming bacteria are mesophilic, a rapid histamine decarboxylation can take place as a result of large proliferation of this bacteria at optimum temperature (26°C) and pH conditions. According to the results, T2 and

Table 1: Mean histamine content (±SE) of Maldive fish samples at different incorporation levels of *G. cambogia* with reference to commercial and control samples.

Sample	Histamine content (mg/kg)
Commercial sample	108 ± 0.36[a]
Control	14 ± 0.01[c]
1.25% of *G.cambogia*	54 ± 0.004[b]
1.67% of *G.cambogia*	3 ± 0.001[c]
2.50% of *G.cambogia*	11 ± 0.02[c]

Column mean values with different superscript letters are significantly different (p=0.05).

T3 samples showed significant reduction of histamine content over both commercial and control Maldive fish samples. It has been reported that the addition of *G. cambogia* to fresh skipjack tuna homogenates, suppress the histamine formation as a result of lowering pH up to 3.2-3.6 due to the presence of hydroxy citric acid (HCA) in *G. cambogia* (Thadhani *et al.*, 2002). According to previous study findings, mean histamine concentration of inner and outer portions of Maldive fish vary from 190 to 90 ppm respectively and our study findings also in line with that values (Gunaratne, 1999).

Fish is highly susceptible for lipid oxidation since it contains high level of polyunsaturated fatty acids. It promotes the rancidity and discoloration of fish and fish products (Tsaknis *et al.*, 1999). Hydroperoxides are primary products of lipid oxidation and readily decomposed to produce various TBARS, mostly carbonyl compounds (Ke *et al.*, 1984). The highest level of TBARS value was identified in the commercial sample, while the lowest level present in the Treatment 1 which contained 1.25% *G. cambogia* (Fig 1). All the Maldive fish samples exceed the recommended level of TBARS which is 8 mg MDA/kg except treatment 1, indicating high levels of lipid oxidation (Kocatepe *et al.*, 2014;Özyurt *et al.*, 2009). Higher lipid oxidation levels could be attributed to the bad handling and storage conditions of fish used for Maldive fish production. And also, lipid oxidation of a fish species vary with season and location within the tissue (Flick Jr *et al.*, 1992). All the *G. cambogia* treated samples showed lower level of TBARS compared to both control and commercial sample and it reveals that *G. cambogia* have significant impact upon reduction of lipid oxidation. It has been reported that garcinol and guttiferone k isolated from fruit of *G. cambogia* has exhibit in vitro protective effects against lipid and protein oxidation. Furthermore these compounds cause to reduce the formation of carbonyl groups and thiobarbituric acid reactive substances (Semwal *et al.*, 2015).

Significantly higher myoglobin content was observed in the treatment 3 which is treated with the highest level of *G. cambogia* and no significant difference was observed in between treatment 1 and 2 (Fig 2). Myoglobin is the sarcoplasmic hemeprotein that primarily responsible for the meat color and it is liable to oxidation during the heating and the storage (Suman and Joseph, 2013; Ishikawa *et al.*, 2006). Bailey *et al*., (1990) have reported that, fish contain 65-23 nmol/g of myoglobin in tissue. According to the current study, all the Maldive fish samples comprise of much higher myoglobin content and it implies proper handling and storage temperature of fish used in Maldive fish production (Hui *et al.*, 2004).

Microbiological assessment

Maldive fish samples yielded both bacteria and fungi from all the samples. Commercial sample had the highest total bacterial count and fungal count which could be attributed to unhygienic handling of fish, postharvest delay, improper salting and sun drying processes (Saritha *et al.*, 2012).

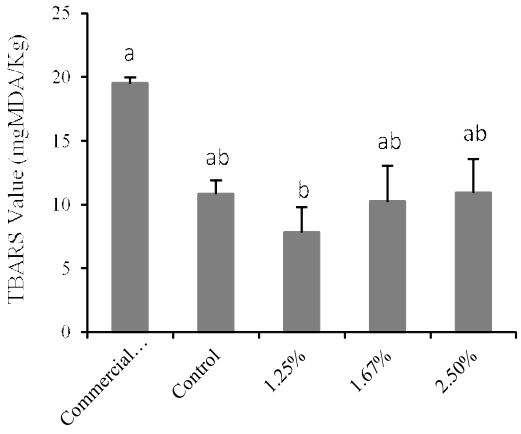

Fig 1: Variation of 2-thiobarbituric acid-reactive substances content in Maldive fish samples at different incorporation levels of *G. cambogia* with reference to commercial and control samples (p=0.05).

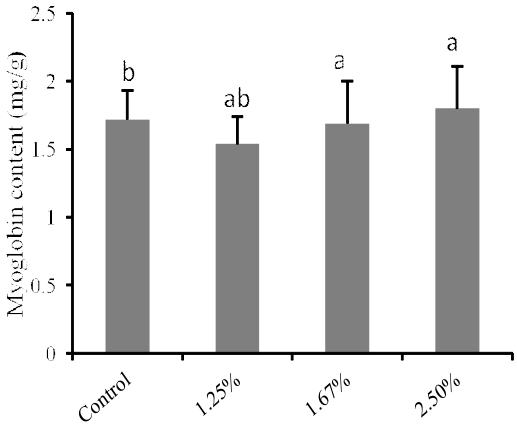

Fig 2: Variation of myoglobin content in Maldive fish samples at different incorporation levels of *G. cambogia* with reference to control sample (p=0.05).

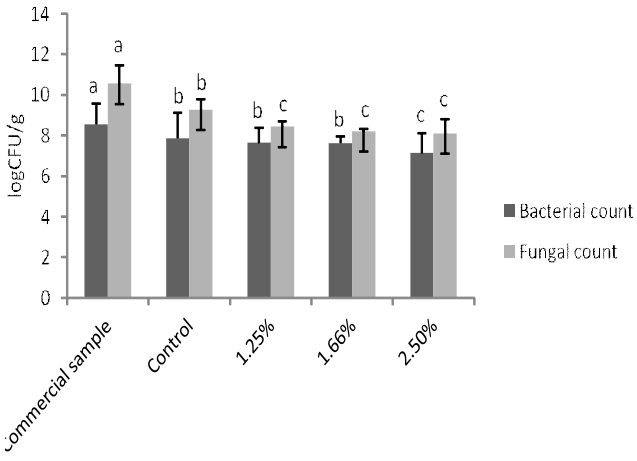

Fig 3: Average bacterial count and fungal count in Maldive fish samples at different incorporation levels of *G. cambogia* with reference to commercial and control samples (p=0.05).

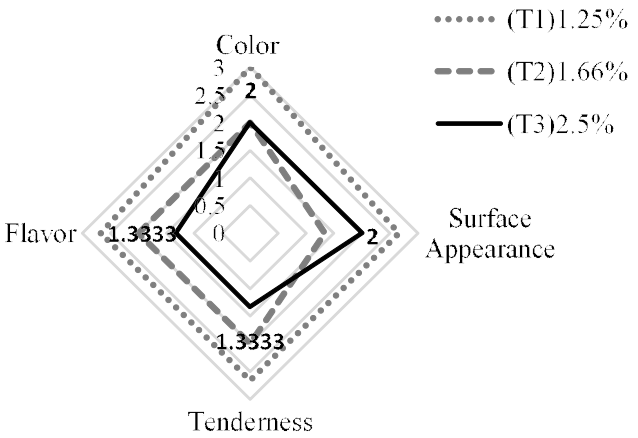

Fig 4: Overall acceptance of the Maldive fish samples at different incorporation levels of *G. cambogia* with reference to commercial and control samples.

Smoked and dried fish products are associated with several food borne bacterial pathogens, toxicogenic fungi and mycotoxins. Higher bacterial counts lead to elevated biogenic amine levels in dried fish products due to the action of histamine producing bacteria such as *Listeria monocytogenes*, *Clostridium botulium*, *Clostridium perfringens*, *Staphylococcus aureus* (Mohamed, 2013). Total bacterial count was significantly reduced in the *G. cambogia* treated samples compared to the control sample. Sample with the highest concentration of *G. cambogia* showed greater suppression of bacterial count than other *G. cambogia* treated samples (Fig 3). Previous studies have proved the *in vitro* antibacterial activity of the fruit rind extracts of *G. cambogia* using hexane, ethyl acetate and ethanol as the extraction solvents. According to the nutritional profile of the *G. cambogia*; availability of xanthones, isoxanthones and garcinol are the responsible compounds for the antibacterial property of *G. cambogia* (Cuesta-Rubio *et al.*, 2005; Iinuma *et al.*, 1998). Fungal count results also gave similar observations as in bacterial count and relatively higher fungal count was observed in all the samples (Fig 3). This is in agreement with the fungal count values reported by Abolagba *et al*. (2011) . Yeast and molds are associated with the spoilage of dry foods especially containing high salt or sugar content. They act as abundant food spoilage agents that can grow over wide range of pH, temperatures and especially at reduced water activity (a_w). Hence, Maldive fish is highly susceptible for fungal contamination as it is a seafood product with low water activity (Williams *et al.*, 2004). The lowest level of fungal count was associated with treatment 3 containing 2.50% *G. cambogia* concentration while the highest count was observed in the commercial sample (Fig 4). Even though the mean count was gradually reduced there was no significant difference in terms of microbial load between T1, T2 and T3. Presence of garcinol, iso-garcinol, benzophenons in *G.cambogia* impart antifungal and antimicrobial effect and thereby reduce the yeast and mold count of Maldive fish with increased levels of *G. cambogia* (Cuesta-Rubio *et al.*, 2005).

Sensory quality analysis

Maldive fish possess unique bright red colour which affect quality and palatability (Becker *et al.*, 2012). T2 and T3 samples obtained the highest colour preference since the bright red color of the Maldive fish samples enhanced with increased *G. cambogia* concentration. Most preferred sample (α=0.05) for the tenderness was T3 (2.5%) which having the highest *G. cambogia* concentration (Fig 4). As the surface appearance, consumers consider combination of various factors such as the characteristic spindle shape, absence of molds and yeast. According to the results the most preferred sample (α=0.05) for the surface appearance was T2 (1.67%) (Fig 4). T3 is selected as the sample which is having the best flavor and it suggests that the increment of the concentration of *G. cambogia* has significantly improved the flavor of Maldive fish. Since *G. cambogia* has a potential to suppress the microbial growth it can be used as natural antimicrobial compound in Maldive fish production and it improves the quality characteristics. According to the sensory and microbiological property analysis, 2.5% of *G. cambogia* is the best level to be incorporated in Maldive fish production.

CONCLUSION

Since *G. cambogia* has a potential to suppress the microbial growth it can be used as natural antimicrobial compound in Maldive fish production and it improves the quality characteristics. According to the sensory and microbiological property analysis, 2.5% of *G. cambogia* is the best level to be incorporated in Maldive fish production.

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