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Bioactive Compounds from Selected Fruits Improve Quality and Oxidative Stability of *Wallago Attu* Fish Nuggets

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ABSTRACT

Background: This study determined the potential of fruit powder as natural preservatives in fish nuggets and indicated that fruit powder can successfully be incorporated in them without altering their acceptability. Additionally, a comparative study was carried out to find which fruit powder has the best antioxidant and antimicrobial activity, contributing towards the stability of the nuggets.

Methods: The guava (*Psidium guajava* L.) powder, bael (*Aegle marmelos* L.) pulp powder and dragon fruit (*Hylocereus undatus* L.) peel powders were incorporated at the rate of 1.5% (w/w) respectively in fish nuggets and its effects on various physico-chemical, textural and sensory attributes of products were evaluated against control.

Result: Incorporation of fruit powder significantly (P<0.05) increased emulsion stability, cooking yield, moisture, fat and protein percentage. Textural attributes were improved in treated nuggets, however overall acceptability scores were significantly (P<0.05) higher for control as adjudged by the panelists. Fruit powder was found to slow down the lipid peroxidation of fish nuggets, as measured by thiobarbituric acid (TBA) value. They also maintained the microbial stability of nuggets upto 10 days during refrigerated storage (4±1°C).

Key words: Anti-microbial activity, Anti-oxidative activity, Fish nuggets, Natural antioxidants, Shelf-life.

INTRODUCTION

Owing to its high nutritional quality and consumer demand, the 'Asian sheat catfish' or 'freshwater shark', *Wallago attu* is harvested across tropical Asia (Thella *et al.,* 2018). This fast-growing freshwater catfish belongs to family Siluridae and is reported to be a very good source of protein, high content of water, lipid soluble vitamins, minerals and polyunsaturated fatty acids (PUFAs) of n-3 family (Gupta *et al.,* 2015). The high moisture content (Lilabati and Viswanath, 1996) along with presence of unsaturated fatty acids makes them prone to microbial and chemical deterioration. Lipid oxidation, which affects sensory attributes such as taste, colour, texture and nutritional value, is a major limiting factor in the quality and acceptability of fish and fish products. Researchers are continuously exploring the potential of plants extracts and essential oils of herbs and spices as an alternative to synthetic preservatives in preservation of fish and fish products.

Guava (*Psidium guajava* L.), a 'super food' contains high amounts of dietary fibre, phenolic compounds, ellagic acid and anthocyanins, as well as other bioactive compounds (Miean and Mohamed, 2001). Many functional and bioactive compounds such as dietary fibre, carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids and other antioxidants are present in bael fruit (*Aegle marmelos L. Correa*), which is commonly found in the Indian Peninsula (Charoensidhi and Anprung, 2008). Dragon fruit (*Hylocereus polyrhizus*), which has recently gained popularity as a new source of food ingredients, is also high in antioxidants, fibre, vitamin C and minerals. Till date there have been no reports of the use of guava, bael pulp, or dragon fruit peel in fish or fish products. Hence, the aim of this study was to determine the antioxidant and antimicrobial properties of fruit powder incorporation in improving quality and oxidative stability of fish nuggets stored at 4±1°C, as well as to determine the best powder-product combination.

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MATERIALS AND METHODS

Raw material and chemicals

The experiment was conducted at the Department of Livestock Products Technology, West Bengal University of Animal and Fishery Sciences, Kolkata, during 2018-2019. Catfish, procured from local market of Belgachia, Kolkata, were beheaded, eviscerated and cleaned laboratory. The fillet obtained was washed with cold water and then kept in

deep freezer (-18 ± 2°C) till product preparation and further analysis. For the present study, whole guava fruit, bael pulp and peel of dragon fruits were separated and kept in the hot air oven (50±1°C) for drying. Dried guava, bael pulp and dragon fruit peel were milled into a fine powder and sieved through a fine mesh with an average particle size of <1.0 mm. The dried samples were aerobically packed in UV sterilised low density polyethylene containers and held at 4+1°C until further utilization. All the spice ingredients were cleaned, dried in the hot air oven at 60°C for 2 h and then ground using proportionate quantity to obtain dry spices mix for preparation of nuggets. All the chemicals of analytical grade were procured from standard firms *viz.*, Himedia, Qualigens and Loba Chemie along with food grade commercial salt.

Preparations of fish nuggets and analysis

One kg formulation of emulsion was made for each treatment. The control formulation consisted of 70% minced fish, 10% refined mustard oil, 10% ice flakes, 1.6% salt, 0.3% sodium tripolyphosphate, 0.3% sugar, 1.8% dry spices powder, 3% condiments (onion and garlic 3:1) and 0.3% refined wheat flour. Sodium nitrite at 150 ppm was also added to the above formulations. In treated formulations, guava powder (1.5%), bael pulp powder (1.5%), dragon fruit peel powder (1.5%) powder were incorporated by replacing an equal amount minced fish in the control formulation. The emulsions were prepared in a bowl chopper (Talsa Ltd, Germany). Fish emulsion (approx.750 g) was placed into stainless steel moulds (18×12×4 cm), tightly packed and cooked in a steam oven (100°C) at atmospheric pressure for 35 min. The meat blocks were cooled to room temperature and cut into cubes of suitable sizes. About 200 g nuggets were packed in separate polyethylene pouches and stored at 4±1°C till further analysis.

Analytical procedures

The cooking yield per cent of the fish meat blocks was calculated as the difference in weight before and after cooking. The Emulsion stability of fish nuggets was determined as per procedure of Baliga and Madaiah (1971). The moisture, protein, fat and ash content of fruit powder and fish nuggets were determined by the methods of AOAC (1995). The pH of the emulsion and the nuggets were determined by the method suggested by Troutt *et al*. (1992). Thiobarbituric acid number of samples was measured according to method described by Witte *et al.* (1970) and Strange *et al.* (1977). Total plate count (TPC) and psychrophilic count were determined by APHA (1992) using pour plate method.

Texture Profile Analysis of the fish nuggets was determined (Bourne, 1978) on day 0 by TA-HDi Texture Analyzer (Stable Micro Systems,UK). Samples were thawed overnight and were allowed to attain a room temperature (25°C). Central core of five pieces of each sample, each measuring 1.5cm x 1.5cm x 1.5cm, from the middle portion of each nuggets were used as the test samples. These were then placed on platform fixtures and compressed twice to 80% of the original height at a crosshead speed of 2mm/s, through two cycle sequence at pre-test speed of 2mm/s, post-test speed of 2mm/s, keeping a distance of 8.5 mm and a trigger of 0.15N using 50kg load cell and 75 mm compression platen probe (P75).

The sensory parameters of the fish nuggets were evaluated by mean descriptive analysis method using 8 points hedonic scale (Keeton *et al*.1984) where 8 denoted extremely desirable and 1 unacceptable. A sensory panel of seven semi trained panelists was set up to evaluate the product for different quality attributes *viz.* color, flavor, tenderness, juiciness and overall acceptability.

Statistical analysis

All the data obtained during the present investigation were analyzed statistically to draw valid conclusion by using SPSS (Version 24.0) software. The data obtained were analyzed by randomized block design (4 treatments×6 no of samples) and subsequent one-way ANOVA analysis except storage study parameters were analyzed by complete randomized design for (3 treatment×6 no of samples ×5 storage days) by two-way ANOVA. Further the significance between the data was compared by Tukey's Post Hoc Test by SPSS-24® software package. A probability value of $p<0.05$ was described as significant and $p<0.01$ was noted as highly significant.

RESULTS AND DISCUSSION

Proximate composition

The pH and proximate composition of control as well as treated fish nuggets are presented in Table 1. Addition of fruit powder significantly ($p<0.05$) lowered the pH of fish nuggets compared to control samples. Fish nuggets treated with DFPP showed the lowest (6.16) pH value among all different fruit fibre treated nuggets and control possess highest (6.19) pH value. This could be due to the low pH values of fruit powder for example GP is a good source of ascorbic acid, which might have lowered the pH of the products. Addition of higher level of dragon fruit peel extract also lowered the pH of beef sausage (Manihuruk *et al.*, 2017).

Incorporation of fruit powder significantly ($p<0.05$) affected the moisture percentage of fish nuggets. The highest value (68.13%) was recorded with fish nuggets treated with DFPP, followed by BPP and GP and control nuggets showed the lowest moisture percentage. The highest moisture percentage in fruit powder treated nuggets could be due to absorption of added water by the addition of powder during product preparation. Similar results were recorded in sheep meat emulsion with guava powder (Verma *et al*., 2013) and goat meat nuggets prepared with bael pulp residue (Das *et al.*, 2015).

Addition of fruit powder significantly ($p<0.05$) affected the protein percentage of fish nuggets. Fish nuggets added with DFPP possess highest (12.46) protein content and control showed lowest protein content as compared with different fruit fibre treated nuggets. According to Manihuruk *et al.* (2017), protein content of beef sausages was unaltered due to incorporation of red dragon fruit peel extract.

Incorporation of fruit powder significantly ($p<0.05$) affected the fat percentage of nuggets. Fish nuggets treated with DFPP possessed the highest value of fat percentage (13.41) and nuggets treated with BPP recorded lowest value of (12.95) fat percentage. The high fat percentage of DFPP might have increased the fat content of nuggets in the present study. Beef sausages incorporated with red dragon fruit peel extract did not affect its fat percentage (Manihuruk *et al.,* 2017).

Thiobarbituric acid (TBA) values

The TBA values (mg malonaldehyde per kg) of the treated fish nuggets were recorded on day 0, 5, 10, 15 and 20 day (Table 2). Incorporation of fruit powder significantly ($p<0.05$) lowered the TBA value of fish nuggets. Although, the TBA values were increased in all the groups significantly ($p<0.05$) throughout the storage period irrespective of any treatment, fish nuggets treated with GP had the lowest TBA value followed by DFPP, BPP and control. Polyphenolic extracts produced from a variety of plant-based products have been successfully used as natural antioxidant additives in the prevention of lipid oxidation in fish. Quince extracts (8.9±0.4 mg phenolics/mL) reduced peroxide levels and inhibited the formation of TBARS in the fat fraction of mackerel (*Scombrus scombrus*) fillets during refrigeration (4°C) (Fattouch *et al.*, 2008).

Microbiological parameters

The total plate count, psychotropic count and coliform count (log_{10} cfu/gm) of the treated fish nuggets were presented in the Table 3. The TPC of fish nuggets incorporated with different fruit fibre was significantly ($p<0.05$) lower than control. The lowest total plate count was observed in DFPP (4.75) followed by GP and BPP. Psychotropic Count of different antioxidant fruit fibre treated fish nuggets were significantly ($p<0.05$) lower than the control. No growth was recorded in treated nuggets till 5th day of storage study. Fish nuggets treated with DFPP (3.43) showed lowest psychotropic count followed by GP and BPP. Incorporation of different fruit powder significantly ($p<0.05$) lowered the total coliform count of fish nuggets. No growth was observed in treated nuggets till 7th day. The lowest value of total coliform was observed in DFPP (1.32) treated fish nuggets followed by GP and BPP. Similar results were observed by Lin and Chou, (2004) who opined that combined effects of oregano and cranberry extracts in control *Listeria* growth in refrigerated fish system as compared to individual extract when the fish slices were kept at 4°C in refrigerated systems.

Texture profile analysis

Texture profile parameters of fish nuggets treated with different fruit powder were presented in Table 4. Addition of GP, BPP and DFPP to fish nuggets resulted in significant ($p<0.05$) changes in hardness, springiness, cohesiveness, gumminess and chewiness values. Hardness and

Table 1: Physiochemical properties and proximate composition of fish nuggets.

Parameters	Fish nuggets			
	Control	T1	T2	T3
Moisture	65.80±0.02^{c}	66.00±0.07^{b}	65.99±0.07^{b}	68.13±0.02^{a}
Protein	12.34±0.02^{c}	12.39±0.01^{b}	12.37±0.01^{bc}	12.46±0.03^{a}
Fat	13.35±0.01^{b}	13.38±0.02^{ab}	12.95±0.03^{c}	13.41±0.02^{a}
Ash	2.30±0.01^{c}	2.51±0.02^{a}	2.36±0.02^{b}	2.56±0.04^{a}
pH	6.19±0.01^{a}	6.17±0.01^{b}	6.18±0.01^{bc}	6.16±0.01^{c}

Mean ± S.E. values bearing different superscripts in rows differ significantly ($p<0.05$).

Control = Fish nuggets without phytochemicals.

T1 = Fish nuggets with 1.5% GP.

T2 = Fish nuggets with 1.5% BPP.

T3 = Fish nuggets with 1.5% DFPP.

Table 2: TBA values (mg malonaldehyde/kg) fish nuggets incorporated with fruit powder during refrigerated storage (4±1°C).

Treatments	Storage day				
	0 Day	5th day	10th day	15th day	20th day
Control	0.33±0.02^{fA}	0.52±0.01^{dA}	0.65±0.01^{cA}	1.08±0.01^{bA}	1.31±0.02^{aA}
GP	0.30±0.01^{fB}	0.42±0.01^{dB}	0.49±0.01^{cC}	0.66±0.01^{bC}	1.13±0.01^{aC}
BPP	0.30±0.01^{fB}	0.42±0.01^{dB}	0.52±0.01^{cB}	0.81±0.01^{bB}	1.15±0.01^{aB}
DFPP	0.29±0.01^{fB}	0.41±0.02^{dC}	0.51±0.01^{cB}	0.63±0.03^{bD}	1.14±0.01^{aC}

Mean±S.E. values for all data (both rows and columns) differ significantly ($p<0.05$).

cohesiveness were significantly ($p<0.05$) decreased except springiness, gumminess and chewiness which increased significantly ($p<0.05$) in treatment groups. The lowest values for the hardness, gumminess and chewiness were recorded in nuggets treated with GP. When white grape dietary fibre concentrate was added to minced horse mackerel muscle, samples became smoother, less springy and cohesive (Sanchez-Alonso *et al.,* 2007). The nature of dietary fiber ingredients in the present study and extent of their distribution in the meat batter could also have influenced the uniformity of three-dimensional protein network, thus the texture of the nuggets.

Sensory properties

The fish nuggets treated with GP, BPP and DFPP had significant ($p<0.05$) differences in sensory properties *viz.* appearance, flavor, texture, tenderness and juiciness and overall acceptability scores. All the results observed during the present study were presented in Table 5. Fish nuggets treated with different fruit powder had significantly ($p<0.05$) lower appearance, flavour, texture, tenderness and juiciness scores in comparison to control. Overall, the acceptability of control nuggets was significantly ($p<0.05$) higher than other treatment groups. Elhadi *et al.* (2017) found that moringa leaf powder incorporated chicken patties had

Table 3: Microbiological parameters of fish nuggets incorporated with fruit powder during refrigerated storage (4±1°C).

Treatments	Storage day				
	0 Day	3 Day	5 Day	7 Day	10 Day
Total plate count (TPC)					
Control	2.04 ± 0.01^{eA}	2.31 ± 0.01^{dA}	2.75 ± 0.01^{cA}	3.47 ± 0.01^{bA}	4.15 ± 0.01^{aA}
GP	1.92 ± 0.01^{eB}	2.27 ± 0.01^{dB}	2.71 ± 0.01^{cB}	3.20 ± 0.01^{bC}	4.09 ± 0.01^{aAB}
BPP	1.94 ± 0.02^{eAB}	2.18 ± 0.01^{dC}	2.71 ± 0.01^{cB}	3.24 ± 0.01^{bB}	4.15 ± 0.06^{aA}
DFPP	1.86 ± 0.01^{eC}	2.09 ± 0.01^{dD}	2.64 ± 0.01^{cC}	3.17 ± 0.02^{bD}	3.99 ± 0.04^{aB}
Psychrophilic count					
Control	ND	ND	ND	2.05 ± 0.01^{bA}	2.12 ± 0.01^{aA}
GP	ND	ND	ND	1.95 ± 0.03^{bB}	1.98 ± 0.01^{aC}
BPP	ND	ND	ND	1.89 ± 0.02^{bC}	2.05 ± 0.03^{aB}
DFPP	ND	ND	ND	1.79 ± 0.02^{bD}	1.86 ± 0.01^{aD}
Coliform count					
Control	ND	ND	ND	ND	1.65 ± 0.06^{A}
GFP	ND	ND	ND	ND	0.95 ± 0.03^{B}
BPP	ND	ND	ND	ND	0.96 ± 0.01^{B}
DFPP	ND	ND	ND	ND	0.86 ± 0.01^{C}

N.D. - Not Detected.

Mean ± S.E. values for all data (both rows and columns) differ significantly ($p<0.05$).

Table 4: Texture profile analysis of fish nuggets incorporated with fruit powder.

Parameters	Control	GP	BPP	DFPP
Hardness(N/cm^2)	45.29±0.002a	41.61±0.003d	43.39±0.002c	44.48±0.002b
Springiness (cm)	0.65±0.001b	0.66±0.002a	0.64±0.002c	0.64±0.002c
Cohesiveness	0.25±0.002b	0.26±0.001a	0.25±0.001b	0.25±0.002b
Gumminess (N/cm^2)	12.78±0.004b	10.15±0.003d	12.44±0.003c	12.85±0.003a
Chewiness(N/cm)	10.09±0.002c	9.11±0.002d	9.76±0.002a	10.71±0.002b

Mean + S.E. values with different superscripts in rows differ significantly ($p<0.05$).

Table 5: Sensory parameters of fish nuggets incorporated with fruit powder.

Parameters	Control	GP	BPP	DFPP
Color	6.11 ± 0.28^{a}	5.85 ± 0.35^{c}	5.79 ± 0.23^{d}	5.91 ± 0.21^{b}
Flavor	6.08 ± 0.39^{a}	5.91 ± 0.26^{b}	5.94 ± 0.38^{ab}	5.25 ± 0.20^{c}
Texture	$6.52\ \pm0.36^{a}$	6.48 ± 0.37^{ab}	6.37 ± 0.33^{b}	5.98 ± 0.51^{c}
Tenderness	6.15 ± 0.27^{a}	6.04 ± 0.39^{b}	6.14 ± 0.23^{a}	5.69 ± 0.48^{c}
Juiciness	5.51 ± 0.25^{a}	5.10 ± 0.23^{c}	5.41 ± 0.21^{b}	4.96 ± 0.33^{d}
Overall acceptability	5.92 ± 0.38^{a}	5.75 ± 0.25^{b}	5.83 ± 0.38^{ab}	5.77 ± 0.32^{c}

Mean ± S.E. values with different superscripts in rows differ significantly ($p<0.05$).

significantly lower colour, taste, tenderness and juiciness scores than the control.

CONCLUSION

Guava, bael pulp and dragon fruit peel powder are rich sources of phenolic compounds, total carotenoids and vitamin C. Fruit powder can be used as a functional ingredient in emulsion-based fish products to change their physicochemical properties and improve textural parameters without affecting their acceptability. Fruit powder's strong antioxidant and antimicrobial properties can be used in the meat industry to maintain the microbial stability and minimise oxidation of meat and meat products throughout storage upto 10-15 days.

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