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Research Paper

Development of a bread-spread using *Catla catla* and mature flower buds of *Rhizophora apiculata*

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Abstract: Indian carps (*Catla catla*) are considered as an excellent source of protein, vitamins and minerals while they are low in saturated fat. However, carps have limited consumer acceptability due to the presence of intramuscular bones. Further, mangrove plants such as *Rhizophora* spp. are considered as valuable therapeutic agents in both modern and traditional medicines. The present study was focused on developing a value added product from *C. catla* by incorporating mature flower buds of *R. apiculata* (Mahakadol), which is a nutritional and medicinal supplement. Bread-spread was prepared by mixing boiled and minced fish with other ingredients and pasteurizing at 85 °C for 15 min. The suitable levels of ingredients were determined using 30 untrained panelists ($p<0.05$). The final levels included 89% (w/w) of *C. catla*, 6% (w/w) of mature flower buds of *R. apiculata*, 1% (w/w) chili, 1% (w/w) salt, 1.5% (w/w) white pepper and 1.5% (w/w) lime. Proximate analysis of the final product showed that moisture is at 72.50±0.03%, protein 20.82±1.49%, fat 2.81±0.02%, fiber 2.10±0.11% and ash 1.94±0.01% in the final product. The shelf life of the bread-spread did not change significantly within the storage period of 30 days under refrigerated condition ($p>0.05$), the total microbial plate counts were within the acceptable level for 28 days, and the coliform and Salmonella were absent. Therefore, the developed bread-spread can be considered as a healthy food having high nutrient and medicinal benefits and with a shelf life up to 28 days.

Keywords: Bread-spread, *Catla catla*, Flower, *Rhizophora apiculate*

Introduction

There is a growing demand for ready-to-eat and ready-to-cook convenience products due to social and cultural changes of the society in the recent past (Sehgal and Sehgal, 2002). Thus, the present trends in the market reflect a rapid growth in the demand for processed foods that are more convenient to handle, store and prepare (Pagarkar *et al*., 2011). Nevertheless, fish and fish-based products have received wider attention due to increase in urbanization, education, awareness and consciousness towards quality, freshness, nutrition, hygiene and health.

Currently the consumers have shown more interest in food products, which are available in ready-to-eat or ready-to-cook form such as fish fillet, finger, cutlet, patties, burger, sausages and fish balls. This has led to the development of several fish-based products varied in taste, texture and appearance (Pagarkar *et al*., 2011). Fresh water Indian major carps Catla (*Catla catla*), Rohu (*Lebio rohita*) and Mrigal

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(*Cirrhinus mrigal*) are considered as the high-protein table fish species, where carps have limited consumer acceptability due to the presence of intramuscular bones (Sehgal and Sehgal, 2002). Processing of carp for value-addition would enhance their acceptability and market value (Pawar *et al*., 2012).

Developing a fish-based product from fresh water carp is useful in promoting the consumption of fresh water fish by overcoming its limitations such as the unpleasant color, odor and taste. Fish provides not only high-value protein, but also a wide range of essential micronutrients, including various vitamins (A, B and D), minerals (including Ca, I, Zn, Fe and Se) and polyunsaturated omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) (Pauly and Froese, 2012). The total lipid content of *Catla* spp. has been reported to be high, ranging from 1.2% to 7.9%, saturated fatty acids (SFA) were the most abundant (60.92%), and polyunsaturated fatty acids (PUFA) was 12.5% (Jakhar *et al*., 2012). Recent studies identified the initial moisture content, protein, fat and ash content of Catla mince to be 78.65±0.72%, 16.64 ± 0.34%, 2.23 ± 0.08% and 1.05 ± 0.03%, respectively (Vanitha *et al*., 2013).

The medicinal value of mangroves provide priceless therapeutic agents, both in modern and traditional systems of medicine (Revathi *et al*., 2014). *Rhizophora apiculata* (*Mahakadol*; family Rhizophoracea), is an important mangrove plant used in traditional medicines in Asia and Africa, The most commonly used species of family *Rhizophoraceae,* are *R. apiculata, R. mucronata* and *R. mangle* (Abidin *et al*., 2013). *Rhizophora apiculata* contains an abundance of biologically active compounds due to its special salt-tolerant living surroundings (Rahim *et al.,* 2008). They are rich sources of secondary metabolites such as steroid, triterpens, saponnins, flavonoids, alkaloids and tannins (Jadhav and Jadhav, 2012). It is reported that R. *apiculata* contains flavanoids, tannin, catechin, anthroquinone, and phenolic groups. Many flavonoids specifically affect the function of enzymes involved in inflammatory processes. Tannins have strong effective antioxidant activity. Flavonoids have important effects in plant biochemistry and physiology, and are known to possess anti-inflammatory, anti-oxidant, anti-allergic, and anti-carcinogenic properties (Rahim *et al*., 2008).

Rhizophora leaves are used to treat diarrhea traditionally, and the fruits are consumed. The bark, flowers, fruits, leaves of *R. apiculata* are used to treat various types of diseases like diarrhea, hemostatic, hepatitis and typhoid (Bandaranayake, 1998) and also as an antiemetic, antiseptic agent, which can arrest bleeding. Several studies have proven that *R. apiculata* plant has various functional properties including antioxidant activities in its bark extracts (Rahim *et al*., 2008), anti-inflammatory and anti-tumor activity (Prabhu and Guruvayoorappan, 2012), antimicrobial activities of tannins from bark extracts (Lim*et al*., 2006) and anti-viral properties of polysaccharide from leave extracts showing anti-HIV activity (Premanathan *et al*.,1999). In Malaysia *R. apiculata* flower buds are a source of food for the villagers as they are easily available and could consume raw. In addition, it is also known to help improving the appetite of patients recovering from fever (Baba *et al*., 2013). Therefore, development of a fish-based product incorporating flower

buds of *R. apiculata* could provide therapeutic effects as well as help reducing the undesirable organoleptic characteristics of the fresh water fish. The present study was thus attempted to develop ready-to-eat bread-spread using Catla and mature flower buds of *R. apiculata.*

Materials and Methods

Medium size, matured Catla (*Catla catla*) with average weight of 950±4 g and 7 months of age were purchased from the local fish market. Mature flower buds of *Rhizophora apiculata* were collected from Trincomalee in Sri Lanka. The samples were brought to the laboratory of Uva Wellassa University at Badulla and stored under refrigerated condition. Spices for the preparation of bread-spread were purchased from the local market and violet red bile agar (Himedia, India), Eosin methylene blue agar (Oxoid, England), buffered peptone water (Himedia, India) brilliant green agar (Himedia, India), *Salmonella* enrichment broth (Sigma-Aldrich, Switzerland), total plate count agar (Himedia, India) were used to determine the microbiological parameters.

Preparation of bread-spread

The whole fish was washed with running water, degutted and skinned to make fillets. After boiling for 20 min, the fish fillets were minced to make a fine paste. Mature flower buds of *R. apiculata* were also washed with running water and the calyxes of the buds were removed. Cleaned flower buds were ground by using a mechanical grinder (IS 4250, Jaipan family mate, India) until fine powder. Preliminary investigations were conducted to determine the suitable levels of other ingredients (fine powder of mangroves flower buds, salt, chili powder, white pepper and lime juice) to be mixed with minced fish as described below. After mixing all ingredients, the mixture was ground by using a grinder. Finally bread-spread was pasteurized at 85 ℃ for 15 min.

Preliminary investigations

A series of preliminary trails were conducted to determine the best ingredients percentages for the final recipe. Different levels of Catla and mature flower buds of *R. apiculata* were used to prepare the bread-spread samples. While selecting the best combination of Catla and mature flower buds of *R. apiculata*, other ingredients (salt, chili powder, white paper and lime juice) were added at a constant level. After selecting the best percentages of Catla and mature flower buds of *R. apiculata*, the levels of incorporation of other ingredients including salt, chili powder, white pepper and lime juice were finalized with several sensory evaluations. The best sample was selected by sensory evaluation with 30 untrained panelists at the age of 21-25 years in both sexes by using five point hedonic scales. The proximate analysis was carried out for determination of crude protein, crude fat, crude fiber, moisture and ash content by following standard AOAC (2000) methods.

Determination of keeping quality of the bread-spread

The microbiological tests were done to determine for the presence of the total Coliform, *Escherichia coli* and *Salmonella* counts in the bread-spread by following standard AOAC methods (2000) with some modifications, *i.e.* Violet red bile agar (Himedia, India) for total Coliform, Eosin methylene blue agar (Oxoid, England), Buffered peptone water (Himedia, India), Brilliant green agar (Himedia, India) for *Escherichia coli*, *Salmonella* enrichment broth (Sigma-Aldrich, Switzerland) for *Salmonella*, and total plate count agar (Himedia, India) for total aerobic microorganisms.

Measuring pH of the product

The pH of the developed bread-spread was measured using the procedure explained by (Dhanapal *et al.,* 2012) for fish samples with some modifications. The pH of the homogenate sample was measured using a calibrated digital pH meter (DELTA 320 digital pH-meter) at five-day intervals during the 30 days storage period at 4 °C. All measurements were triplicated.

Determination of color

Color of the final product was determined with Chroma meter (CR – 410 Konika Minolta, Japan). The color properties included L***** (lightness), a***** (redness), and b***** (yellowness) whereas whiteness was calculated using the Equation 1

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{\frac{1}{2}} \qquad \text{(Equation 1)}$$

Statistical Analysis

The results were analyzed using descriptive statistics and ANOVA whereas the sensory data were analyzed using Friedman's test in Minitab 17 statistical software.

Results and Discussion

Determination of the best combination of *C. catla* and mature flower buds of *R. apiculata* for the bread-spread

The bread-spread produced with 90% (w/w) of Catla and 6% (w/w) of mature flower buds of *R. apiculata* received significantly high ranks ($p<0.05$) for spreading ability and overall acceptability (Figure 1). The color, taste, appearance and smell received similar ranking to other preparations ($p>0.05$). Therefore, the bread-spread made from 90% (w/w) of Catla and 6% (w/w) of mature flower buds of *R. apiculata* was considered the best among the tested preparations. Catla offers distinct advantages such as white color meat and good taste and thus, good scope for value addition. Value-addition enhances their acceptability and market value as revealed by the sensory evaluation of processed products (Pagarkar *et al.*, 2011). Studies on *R. apiculata have* confirmed the presence of anti-oxidant, anti-diabetic, anti-cancer, anti-

inflammatory, anti-tumor, anti-microbial and anti-viral properties in various parts of the plant (Bandaranayake, 1998)

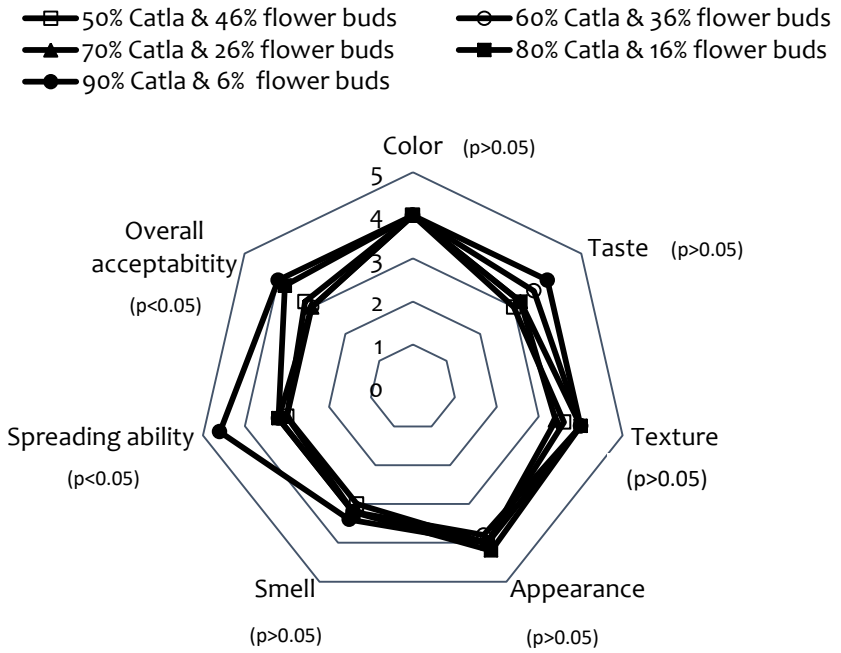

Figure 1. Sensory evaluation for the determination of optimum incorporation levels of *Catla catla* and mature flower buds of *Rhizophora apiculata*

Determination of the best salt, chili, white pepper, and lime juice percentages for the bread-spread

The bread-spread produced with 1% (w/w) of salt have obtained significantly ($p<0.05$) high rank for taste and overall acceptability. The color, texture, appearance, smell and spreading ability had no significant ($p>0.05$) difference to other preparation. Therefore, the treatment made from 1% (w/w) salt was considered the best. According to the recent studies, nearly 1.0 - 1.5% (w/w) salt was used to prepare the value added minced fish based product from Catla (Vanitha *et al*., 2013). The sensory evaluation for the developed bread-spread showed similar results. The bread-spread produced with 1% (w/w) of chili had obtained significantly ($p<0.05$) high ranks for color, taste, appearance and overall acceptability, while texture and smell had no significant ($p>0.05$) difference compared to other preparations. Therefore, the treatment made from 1% (w/w) chili was determined as the best. The bread-spread produced with 1.5% (w/w) of white pepper have obtained significantly ($p<0.05$) high rank for color, taste and overall acceptability while texture, appearance smell and spreading ability showed no significant difference ($p>0.05$). Therefore, the treatment made from 1.5% (w/w) white pepper was determined as the best. There are scientific evidence to prove that pepper have health benefits, particularly in

enhancing digestive tract function (Singletary, 2010). Adding 1.5% (w/w) white pepper gives favorable color and also enhances the health and nutritional values of the final product. The bread-spread produced with 1.5% (w/w) of lime juice have received significantly high acceptability ($p<0.05$) for color, taste and smell while texture, appearance and spreading ability showed no significant ($p>0.05$) difference among the preparations. Therefore, the treatment made from 1.5% (w/w) lime juice was selected as the best.

Determination of keeping quality of the bread-spread

Some ready-to-eat foods are considered as potentially hazardous as these foods can support growth of pathogenic bacteria. Hence, such food items must be stored in recommended temperatures to minimize the growth of food-borne pathogens or to prevent the formation of toxins in the food. Presence of total coliforms (*Enerobacter, Klebsiella, Citrobactor* and *Escherichia)* is considered as a specific bacterial indicator of fecal pollution of water and food. As such, their presence in ready-to-eat foods is an indication of poor hygiene and sanitation or inadequate heat treatments.

Environmental contamination can also result in the presence of *Salmonella* in foods even at lower numbers. Their presence in ready-to-eat foods may be a result of undercooking, poor handling practices and cross-contamination. Sri Lankan Standards (SLS) for mince fish products only allow 11 cfu/g (colony forming units per gram) of *Escherichia coli* and absence of *Salmonella*, and a maximum total plate count (TPC) of 10^5 cfu/g. The TPC indicates the level of microorganisms in a product. The TPC on fish and fishery products generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelf life and post heat-processing contamination. In the present study, the microbiological observation confirmed that the bread-spread developed was free of total coliform and *Salmonella*. However, even the absence of target pathogens in tests specific for them only provides a degree of probability of absence in the whole batch of food (Greenwood and Roberts, 2003). Table 1 shows that the TPC of the samples tested was well below the SLS limit of specification for quick frozen whole fish, fish fillets, steaks and minced fish. Therefore, the product developed was found to be a safe food for the consumers up to 28 days.

Table 1. Changes of Total Plate Count of bread spread prepared with *Catla catla* and mature flower buds of *Rhizophora apiculata* during storage at refrigerated temperature.

Storage Time (days)	Colony count (cfu)
0	3.12 ± 0.26*
7	4.29 ± 0.09
14	4.57 ± 0.01
21	4.9 ± 0.02
28	4.99 ± 0.01

*mean values ± standard deviation

Keeping quality analysis

It is a common practice to measure the pH of the muscle tissue (Howgate, 2009) in order to capture fish deterioration. Directly after death of the fish, a series of biochemical reactions starts, which is important for the quality and shelf life of products (Rehbein and Oehlenschlager, 2009). The pH of the bread-spread developed in the present study was 6.36±0.06 for day 0 and it has been sequentially reduced during storage period (Table 2).

Table 2. Changes of mean pH of bread spread prepared with *Catla catla* and mature flower buds of *Rhizophora apiculata* during storage at refrigerated temperature.

Storage Time (days)	pH
0	6.36 ± 0.06*
5	6.35 ± 0.04
10	6.33 ± 0.05
15	6.29 ± 0.02
20	6.27 ± 0.08
25	6.21 ± 0.03
30	6.19 ± 0.01

*mean values ± standard deviation

The bread-spread contains Catla fish flesh as the major ingredient. The slightly acidic pH value of the bread-spread at the beginning may be due to the presence of white muscles with low amount of glycogen in the flesh of Catla fish compared to red muscle (Susanto *et al*., 2012). When the fish was killed, anaerobic glycolysis continues, increasing the concentration of L-lactate in the fillet with a concomitant decrease in the pH value (Rehbein and Oehlenschlager, 2009). Typically, the post-mortem pH value of the fresh water carps is slightly acidic, around 6.0 (Susanto *et al*., 2012). The pH decrease in post-mortem fish meat depends on the lactic acid produced by the decomposition of glycogen. The rate of change of pH in fish is influenced by glycogen content in fish meat. The pH value of fish with high amounts of glycogen red muscle is lower than that of the fish with high amounts of white muscle (Tejada, 2009). Fish bacteria are sensitive to low pH, which promotes oxidation of myoglobin (Ozogul, 2010). Amount of glycogen in red meat is higher than that in white meat.

One of the reasons for the pH reduction of the bread-spread during the storage at 4 °C may be due to the accumulation of lactic acid from the decomposition of glycogen in fish muscles after the rigor mortis condition (Susanto *et al.,* 2012). The slow rate of pH reduction during storage of the newly developed bread-spread may be due to the antioxidant properties of mature flower buds of *R. apiculata* and other ingredients including white pepper, chili and lime juice. The literature shows that *R. apiculata* contains flavanoids, tannin, catechin, anthroquinone, and phenolic group (Ravikumar *et al*., 2010), and these tannins have strong antioxidant activity (Rahim *et al*., 2008). The increase of pH may be attributed to the accumulation of alkaline compounds such as ammonia and trimethylamine derived from microbial action

during fish muscle spoilage (Ozyurt *et al*., 2009). Nevertheless, increasing pH values at a higher rate compared to initial pH values show that fish flesh has spoiled and it is not suitable for the consumption (Susanto *et al*., 2012). According to a recent study Kovakka (*Coccina grandis*) leaves-incorporated tilapia (*Oreochromis mossambicus*) paste can be kept without spoilage for 30 days under refrigerated condition (Illangakoon and Abeyrathne, 2015) and this observation is comparable to the observation of the present study.

Proximate analysis of bread-spread

Fish has attained a great nutritional significance in recent years for providing best source of protein and lipids (Hussain*et al*., 2011). It has been recognized as an excellent food source for human beings. Fish is preferred as a perfect diet not only due to its excellent taste and high digestibility, but also because of having higher proportions of unsaturated fatty acids, essential amino acids and minerals for the formation of functional and structural proteins (Jakhar *et al.*, 2012). The bread-spread developed in the present study contains Catla fish flesh as the major ingredient and it has a significant contribution to the nutritional composition.

The total lipid content of Catla is generally high, ranging from 1.2 % to 7.9 %. According to Jakhar *et al*. (2012), the saturated fatty acids are abundant in Catla (60.92%) while polyunsaturated fatty acids are present only around 12.5%. Normally Catla contains 77.9% moisture (Shailender, 2013). The proximate analysis of the newly developed bread-spread in this study showed comparable levels as reported by Vanitha *et al*. (2013) (Table 3).

Table 3. Proximate compositions of the bread-spread prepared with *Catla catla* and mature flower buds of *Rhizophora apiculate*

Nutrient Compound	Amount (%)
Crude protein	20.82±1.49*
Crude fat	2.81±0.02
Ash	1.94±0.01
Moisture	72.50±0.03
Crude fiber	2.10±0.11

*mean values ± standard deviation

Determination the color of bread-spread

Table 4 shows that the lightness (L*) of the bread-spread is higher than the carps mince fillets. The high L* value may be due to the incorporation of other ingredients in bread-spread specially chili and white pepper. Both redness (a*) and yellowness (b*) were positive in both bread-spread and minced fish fillets, indicating that there are no greenness and blueness in the final product. Myoglobin plays an essential role in the whiteness of most fish based minced products (Chen, 2002). A recent study reported that the whiteness of carp surimi gel was 61.17±0.49 (Abdel-Aal *et al*., 2014). However, compared to minced fillets, whiteness was high in bread-spread.

Table 4. Color parameters of bread-spread prepared with *Catla catla* and mature flower buds of *Rhizophora apiculate*

Samples	L* (lightness)	a* (redness)	b* (yellowness)
Catla bread-spread	61.22±0.54‡	7.48±0.29	22.79±0.11
Carp minced fillets†	44.13±1.59	8.47±1.60	11.58±1.34

†Source: Abdel-Aal*et al*. (2014); ‡The mean values ± standard deviation

Conclusion

The bread-spread developed with 89% of Catla (w/w), 6% of mature flower buds of *Rhizophora apiculata* (w/w), 1% chili (w/w), 1% salt (w/w), 1.5% white pepper (w/w) and 1.5% lime juice (w/w) provided the best composition. The proximate analysis revealed that the bread-spread contains 72.50±0.03% of moisture, 20.82±1.49% of protein, 2.81±0.02% of fat, 2.10±0.11% of crude fiber and 1.94±0.01% of ash. The product is microbiologically safe up to 28 days under refrigerated condition.

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