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ORIGINAL ARTICLE

Improving the Physicochemical and Antioxidant Properties of Fish Floss Incorporated With Waste Cassava Leaves

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KEYWORDS

Fish floss;
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ABSTRACT: Fish is one of the main sources of protein and important nutrients which help promote health. This study was an attempt to determine the impacts of using different levels of cassava leaves on antioxidant features, nutritional health values, and the quality of fish floss. Cut cassava leaves in different percentage (5%, 10% and 15%) were added to chili paste and tuna fish to prepare fish floss. The proximate analysis, antioxidant activities, color, and total cyanide content of the fish floss were then examined. The present findings suggest that the antioxidant activity of fish floss was likely to increase as there was an increase in the percentage addition of cassava leave compared with the control fish floss. The fiber content of fish floss increased by adding cassava leaves because cassava leaves are rich in fiber. Furthermore, cyanide content dropped to a lower level during pounding and boiling in water. It could be implied that cassava leaves with a lot of advantageous components could be a great ingredient to enhance antioxidant features and nutritional values (protein and fiber) of fish floss.

INTRODUCTION

In recent years, fish has become a favorite foodstuff because it is considered safer and healthier for consuming purposes than goat meat or chevon, mutton, buffalo meat and chicken. Fish is one of the main sources of protein in the developing countries. Meanwhile, fish have the high potential as a raw material to produce snack food. Shaviklo reported that using low-value fish can be converted into various value-added products such as fish floss, which is produced or locally known as "serunding" in Malaysia [1]. It is made of fish and it is processed by frying with adding sugar, salt and spices in order to obtain a delicious taste. Fish floss contains high protein, fat, and essential amino acid lysine but it still lacks of fiber content that helps promote the digestion system [1].

Todays, there has been a worldwide awareness of the use of industrial wastes in order to increase the important nutritional elements including vitamins, dietary fiber, and protein. Nevertheless, cassava leaves have been used as a raw material, therefore, it is due time to incorporate the cassava leaves as a source nutrient for human nutrition as well as to encourage their value as an addition to food product. Cassava (Manihot esculenta, Crantz) is abundantly found in subtropical and tropical regions such as Latin America, Asia, and Africa. It is estimated that

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nearly 276.7 million tons of cassava are produced [2].

The cassava plant is most commonly used for its starchy tubers; its leaves are regarded as a by-product which is still underutilized. Large tonnages of these leaves are being currently discarded as wastes after harvesting the roots [3]. On the one hand, they are popular fertilizers and animal food [4]; on the other hand, cassava leaves abound 25% protein and provide a great amount of calcium, iron, and vitamins C and A on a dry matter basis. They are also known for their antioxidant function that helps lower the process of aging and promotes the endurance of body against diseases. Cassava leaf protein has important amino acid which is similar to amino acid in the eggs of a hen [5].

Concerning accessibility, expense, and fiber, it seems that cassava leaves are more likely to be used as a great source of dietary fiber, protein, minerals and vitamins.

However, there is no literature indicating the consumption of cassava leaves dietary fiber in the fish floss products. Therefore, this study aimed to add waste cassava leaves into fish floss to increase the essential nutrition and elucidate the effects of cassava leaves on the fish floss' qualities such as chemical, physical, nutrition and antioxidant properties.

MATERIALS AND METHODS

Raw Material

Cassava leaves collected from Kulim, Kedah, Malaysia. Modified cassava flour (MOCAF) is prepared by fermenting lactic acid bacteria from Penang Malaysia. Salt, sugar, chili paste and oil were purchased from a local market in Penang Malaysia.

The chemicals used in this research were Gallic acid, Folin-Ciocalteu reagent, 1,1-diphenyl- 2-picrylhydrazyl (DPPH), ferric chloride hexahydrate, 2,4,6-tripyridyl-striazine, ammonia solution, potassium iodide, silver nitrate (AgNO3) solution, Kjedahl catalyst tablets and Petroleum ether which were purchased from Sigma Chemical Co. All other reagents and solvents were of analytical grade.

Preparation of cassava leaves

Cassava leaves were pounded into small pieces and boiled in boiling water at 100◦C for 5 minutes. After that, cassava leaves were removed from boiling water and drained for 5 minutes. Then, they were cut (2.5 mm) by using knife and cassava leaves were obtained and used as an ingredient for fish floss.

Fish floss processing

Chili paste was cooked in hot oil (110°C) and added with sugar and salt, then, tuna fish were weighed and mixed with them. After that, cut cassava leaves were divided into three percentages namely 5%, 10% and 15% in the mixer. Then, the mixer was cooked on low heat with constant stirring) ~90 ºC, 30 min) and poured onto a plate layered with napkin, next, cooled down and kept on shelf (~27◦C). A control film was also provided under the similar condition. However, no cut cassava leaves were added to the control film. Figure 1 shows the prepared fish floss with different percentages of cassava leaves.

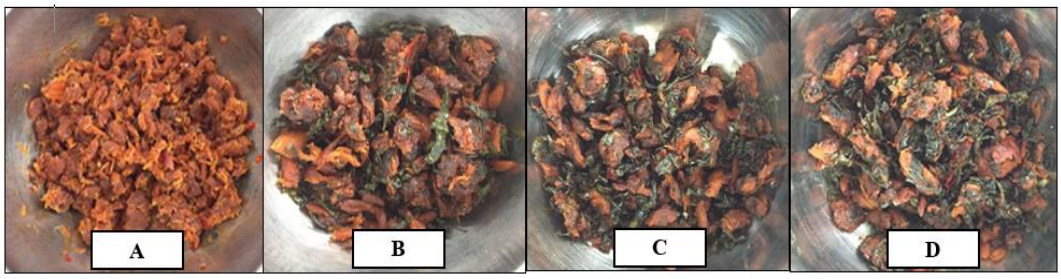

Figure 1. The characteristic of fish floss with different percentages of cassava leaves. A= control, B=5% addition of cassava flour, C = 10 % addition of cassava leaves, D= 15% addition of cassava leaves.

The preparation of fish floss extract

Extraction method was conducted employing the method modified by Suhyun kong et al [6]. Fish floss (10 g) was ground using a mortar. Then it was homogenized with 70% methanol by being shacked in incubate shaking at 37 ºC 1 hr. Then the mixture was centrifuged at 3200 rpm for 10 min. The supernatant was collected and used for analysis.

Proximate analysis

On the basis of AOAC (2000), the Kjeldahl method was used to measure nitrogen; it was also used to convert crude protein (N ×5.7). Being extracted with petroleum ether in a Soxhlet apparatus, crude fat was evaluated by a gravimetric method [7]. Having being incinerated at 550 ° C for 24 h in a furnace, ash content was measured by a gravimetric method. Drying the samples at 105 º C overnight helped measure the moisture content. A TDF assay kit (Megazyme International Ireland, Wicklow, Ireland) was employed to measure total dietary fibre (TDF) content. Carbohydrates were determined by enzymatic kits of analysis [8]. The analysis of composition was done in triplicate.

Cyanide content

The alkaline titration method was used to determine the content of cyanide. 200 cm^3 distilled water was mixed with 20 grams of the fish floss in a liter round bottom flask. The flask mixture was kept for 3 hours. It was, then, steamed so that 150 cm^3 of distillate could be gained. After that, the distillated received twenty-centimeter cube of 0.02 M sodium hydroxide solution and the volume had some changes in cyanide of cassava soak forming up to 250 cm^3 in a volumetric flask. Two aliquots (each 100 cm^3) were gained from the distillate. 2 cm^3 of 5% potassium iodide solution and 8 cm^3 of 6 M ammonia solution were added to each of the aliquots. 0.02 M silver nitrate solution (AgNO3) was used to titrate the final mixture . As soon as there is a change from clear to a faint turbid solution, it could be implied that the titration has reached an end point . Instead of cassava distillate, 150 cm^3 of distillate water was used to prepare a blank sample 5.0 g . The following relation was used to determine the content of cyanide in the sample :1cm^3 0.02M AgNO3 = 1.08 mg HCN.

Total polyphenol content

Based on a method developed by the International Organization for Standardization (ISO) 14502-1, spectrophotometry was used to determine the total polyphenol content (TPC) employing gallic acid as a standard. Tubes which contained 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water were used to hold 1.0 mL of the diluted sample extract. After that, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added to the extract. Then, the tubes were kept at room temperature for one hour. Then, UV-vis spectrophotometer, (Shimadzu, UV mini-1240, Kyoto, Japan) was used to determine absorbance at 765 nm. The TPC was considered to be gallic acid equivalents (GAE) in g/100 g sample. The polyphenol concentration in samples comes from a standard curve of gallic acid.

DPPH Free Radical Scavenging Assay

The bleaching of the purple colored DPPH methanol solution was used to measure the electron donation function and the hydrogen atom of the given extracts [9]. Some pure compounds were also measured in the same way. In light of a method developed by Braca et al. (2001), the extracts' antioxidant activity was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [10]. The extract (0.1ml) was added to 2.9 ml pf a 0.004% DPPH solution in methanol. The samples were first kept in a dark place for 30 min. Then, absorbance was measured at 517 nm using UV-vis spectrophotometer, (Shimadzu, UV mini-1240, Kyoto, Japan) and the percent inhibition (I%) of activity was calculated as:

DPPH-RSA(%)

$$=\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power Assay (FRAP)

The determination of the total antioxidant activity in the extract using the FRAP assay followed a modified method from Nur Faezah Omar et al [11]. Extract (200 µL) was

added to FRAP reagent]3 mL, 10 parts 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tri)2-pyridyl-(s-triazine)TPTZ (solution and 20 mM FeCl·6H2O solution)and the reaction mixture was incubated in a water bath at 37 °C for 30 min . The increase in absorbance was measured at 593 nm using UV-vis spectrophotometer, (Shimadzu, UV mini-1240, Kyoto, Japan) . The percent inhibition was calculated as:

$$I\ (\%)=\frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

Color

The color factor of fish floss was determined using colorimeter (Minolta, CM-3500d, Tokyo, Japan). Greenness or redness was represented b-y –a/+a represents, the lightness of color (0 = black; 100 = white) was represented by coordinates 'L', and blueness or yellowness was represented by –b/+b. Assessments were done in triplicate.

Statistical analysis

One-way statistical analysis (ANOVA) was used to analyze the data based on Duncan test in the experiments. All data were processed using SPSS package (SPSS 22.0 for Windows, SPSS Inc, Chicago, Illinois, U.S.A) and expressed as mean value ± standard deviation. The significant level was set at P>0.05.

RESULTS AND DISCUSSIONS

Proximate Composition

The proximate composition of fish floss with different percentage of cassava leaves are shown in Table 1. As expected, the fiber content tended to increase as there was an increase in the percentage of adding cassava leaves in fish floss compared with control [12]. However, fat, carbohydrate, moisture, and protein altered but not significantly as there was an increase in the percentage of adding cassava leaves.

Table 1 display that as there was an increase in the percentage of cassava leaves, ash content significantly decreased compared to the control sample because fish have higher mineral than cassava leaves.

Table 1. Proximate composition of fish floss.

Sample	Moisture	Ash	Protein	Fat	Fiber	Carbo
Control	6.32±0.17a	8.40±0.11a	20.17±0.48a	27.11±0.27a	0.35±0.03c	37.65±0.96a
5% cassava leaves	6.52±0.93a	7.43±0.10b	20.51±0.64a	24.77±0.66a	2.22±1.05b	38.55±0.69a
10% cassava leaves	7.54±1.15a	7.43±0.09b	20.57±0.53a	25.91±2.45a	4.26±0.52a	34.30±1.89b
15% cassava leaves	7.59±1.11a	7.21±0.20b	20.73±0.61a	24.64±0.43a	5.74±1.35a	34.09±2.33b

Values are expressed as means ± SD (n=3). Means followed by the same letters within the same column are not significant at P>0.05.

Cyanide content

Cassava leaves contain 20–1860 ppm of total cyanide (fresh weight basis) and cassava leaves are reported to be extremely poisonous [13]. Cassava has three forms of free cyanide, cyanohydrins, and cyanogens viz. cyanogenic glucoside (95% linamarin and 5% lotaustralin) [14]. There are several factors that affect the amount of cyanogenic including environmental conditions like drought, which bring causes changes in the level of cyanogenic, cultivars, the status of soil nutrient, and locations [15]. World Health Organization suggests 10 ppm of cyanide in food [16]. In this experiment, cyanide demonstrated a lower level of content than the level recommended by FAO, implying its safety for consumption. As Figure 2 displays, there was no significant change in the level of cyanide as there was an increase in cassava leaves compared with the control samples. As Shih Peng Wonge et al found, some amounts

of cyanide content could be lost during the soaking and cooking processes of cassava [17]. Pounding and boiling helped prepare the cassava leaves in this study. It seems that these two processes led into losing some amounts of content in cassava leaves. Bradbury and Denton confirmed that boiling the pounded cassava leaves in water (a traditional method) can cause all cyanogens to be removed even at the expense of losing nutritious ingredients [12]. Having being chopped, as soon as cassava leaves are boiled, nearly 85% of cyanogenic glucosides are lost in presence of water [17].

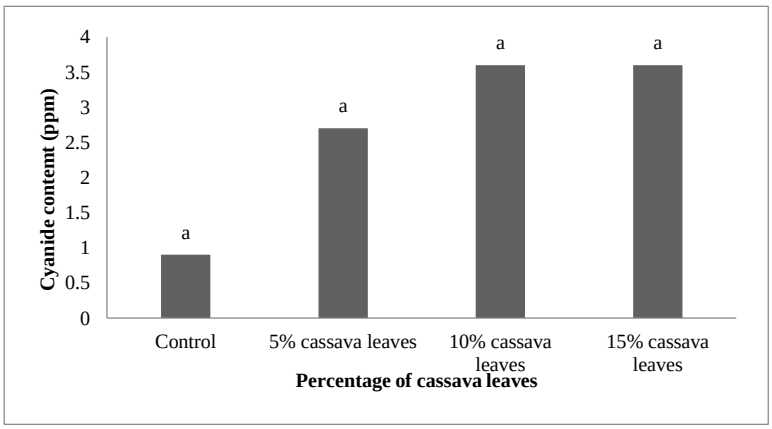

Figure 2. The cyanide content of fish floss with different concentrations of cassava leaves. Values are expressed as mean ± standard deviation (n=3). Means with same letter are not significant at P>0.05.

Total polyphenol content

The total polyphenol content (TPC) of the fish floss was measured in terms of gallic acid equivalent using the Folin Ciocalteu reagent and can be seen in Figure 3. The results showed that compared with the control sample, the total polyphenol content tended to increase as there was an increase in the percentage of cassava leaves added to fish floss. The total polyphenol of control was found to be the lowest amounts of polyphenols whereas the highest polyphenol was observed in the fish floss that contained 15% of cassava leaves. Generally, the sample that contained a high amount of polyphenols also exhibited a high antioxidant activity. Catechin and its derivatives were reported to be the polyphenols found in cassava [18]. The polyphenols are regarded to be in the same class of compounds which are related with cardiovascular health advantage popular in green tea [19]. Mostly the leaves' polyphenolic compounds are considered to be tannin equivalents and shown in a non-specific way [17]. Although polyphenols are reported to be great antioxidants, they contain essential minerals in their compounds which hamper their beneficial absorption.

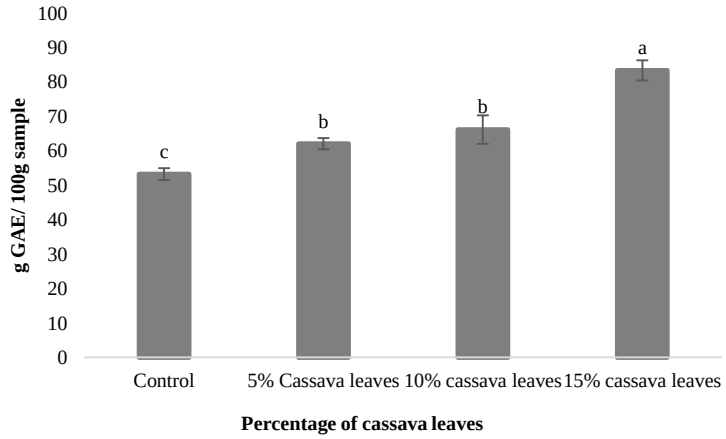

Figure 3. The total polyphenol content of fish floss with different concentrations of cassava leaves. Values are expressed as mean ± standard deviation (n=3). Means with same letter are not significant at P>0.05.

Antioxidant activity

DPPH free radical scavenging activity

The primary antioxidant activities were measured using the stable radical DPPH. These activities are commonly concerned with the free radical scavenging activities of food materials, extracts of fruit and plant, and pure antioxidant compounds. The main benefit of cassava leaves for the long term is that it can be a healthy natural antioxidant. One leaf of cassava contains enough vitamin C to prevent the negative effects of free radicals on the health. In this study, the antioxidant activity of fish floss was expressed as the percentage of inhibition (%). The DPPH free radical scavenging activity of fish floss is shown in Figure 4. Concerning the antioxidation activity, the percentage of inhibition tended to increase when there was an increase in the percentage addition of cassava leaves in fish floss. The highest percentage of DPPH inhibition was observed in fish floss containing 15% of cassava leaves, which was not significantly different from fish floss containing 10% of cassava leaves. The present result is in agreement with what found by Novelina, who reported an increase in the proportion of cassava leaf extract can cause an increase in the wet noodles' antioxidant activity [20,21]. Novelina also found that cassava leaf's chlorophyll increases the level of the wet noodles' antioxidant. According to present findings, it could be implied that the antioxidation activity was greatly influenced by cassava leaves which could naturally generate an antioxidation protection [20].

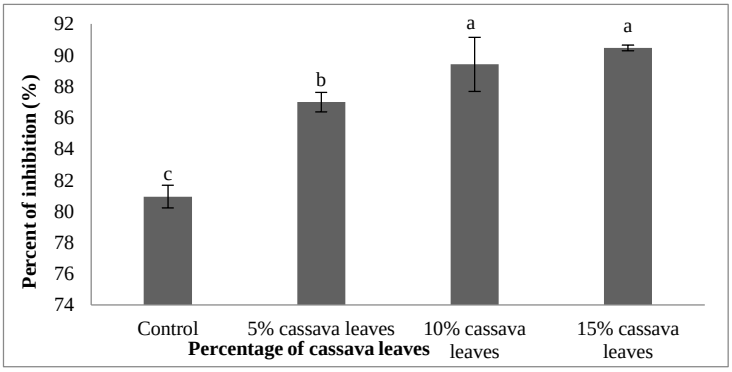

Figure 4. The DPPH scavenging activities of fish floss with different concentrations of cassava leaves**.** Values are expressed as mean ± standard deviation (n=3). Means with same letter are not significant at P>0.05.

Ferric ion reducing activity

The comparison between Figures 4 and 5 showed that the FRAP scavenging activities of fish floss were very different from their DPPH scavenging activities. The DPPH scavenging assay of fish floss tended to increase as there was an increase in the percentage addition of cassava leaves that had a positive correlation with the total polyphenol content. While the FRAP scavenging activity was not significantly different when there was an increase in the percentage addition of cassava leaves that had a negative correlation with the total polyphenol. Such results could be explained in light of maillard reaction during cooking fish floss. Yilmaz and Toledo showed that maillard reaction products gained from heated histidine and glucose had peroxyl radical scavenging activity and that it was strongly related with ORAC assay which was the main reason for high antioxidation in fish floss [22]. However, the FRAP scavenging activity values were higher than those obtained for the DPPH scavenging activities. The lower DPPH scavenging activity values could be attributed to the presence of compounds not reactions to DPPH. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals efficiently due to steric hindrance [16].

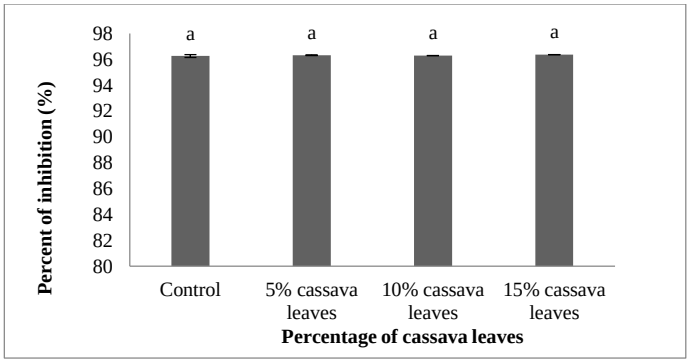

Figure 5. The FRAP scavenging activities of fish floss with different concentrations of cassava leaves**.**
Values are expressed as mean ± standard deviation (n=3). Means with same letter are not significant at P>0.05.

Color

It seems that color plays a significant role in the customers' daily life since they constantly encounter a wide range of colors. This diversity of colors deeply impacts consumers' preferences when they intend to buy an item in a particular environment. Color parameter of fish floss with different percentage of cassava leaves was shown in Table 2. Adding different percentages of cassava leaves changed the color characteristic of fish floss. The lightness (L*) of fish floss ranged from 21.22 to 24.74. An increase in the percentage of cassava leaves significantly decreased the lightness of fish floss.

The a* value color parameter ranged from 7.13-15.32 representing a variation from green to red. The a* value was positive, implying that the color of fish floss became red. The red color of fish floss tended to decrease as there was an increase in the percentage of cassava leaves. The red color of fish floss came from the color of chili paste in fish floss. However, because cassava leaves were dark green, the lightness and red color of fish floss decreased. The b* value of fish floss ranged from 7.31 to 15.66 representing a variation from blue to yellow. The b* value was positive implying that fish floss tended to change to yellow. The incorporation of natural pigment not only promotes the sensory features of food but also functionally enhances the nutrition quality of food [23].

Table 2. Color parameter of fish floss with different percentages of cassava leaves.

Sample	L*	a*	b*
Control	24.74±0.05a	15.32±0.02a	15.66±0.02a
5% cassava leaves	21.78±0.04b	8.12±0.01b	8.42±0.03b
10% cassava leaves	21.40±0.04c	8.09±0.03b	8.19±0.01c
15% cassava leaves	21.22±0.04d	7.13±0.04c	7.31±0.04d

Values are expressed as means ± SD (n=3). Means followed by the same letters within the same column are not significant at P>0.05. *RF=rice flour, CF=cassava flour.

CONCLUSIONS

The present research improved the physico-chemical, nutritional and antioxidant properties of fish floss by adding cassava leaves. Cassava leaves contained beneficial components and high antioxidation. The antioxidant activity of fish floss was likely to increase as there was an increase in the percentage addition of cassava leave compared with the control fish floss. The fiber content of fish floss increased by adding cassava leaves because cassava leaves are rich in fiber. Furthermore, cyanide content dropped to a lower level during pounding and boiling in water and the amount of cyanide content was lower than the FAO maximum recommended level. Its low amount makes cassava leaves safe for consumption. The present findings suggested that cassava leaves could be used as a great ingredient in order to enhance fish floss' antioxidant features and nutritional values.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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