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## TABLE OF CONTENTS

## PAGE

• <b>Bioactivity evaluation and freshness identification of cantaloupe fruit (<i>Cucumis melo L. var. cantaloupensis</i>) using biospeckle method</b> Auttapon Sripradit	1 - 11
• <b>Preference mapping of pineapples grown in Chiang Rai with different cultivars and cultivation methods for Thai consumers living in Chiang Rai</b> Chirat Sirimuangmoon1	12 - 26
• <b>Effect of mince washing and packaging on physicochemical quality changes of fish burger made from African catfish (<i>Clarias gariepinus</i>) during frozen storage</b> Wichittra Daengprok, Chananpat Radniyom, Thanes Keokamnerd and Watinee Intharapongnuwat	27 - 43
• <b>The effect of high fiber beverages consumption on anthropometric, defecation and quality of life changes in overweight / obese female volunteers</b> Netnapa Ounti, Nathaphorn Phromphaet, Sarunya Maichan, Sirikarn Naree, Promluck Sanporkar, Chatrapa Hudthagosol	44 - 52

## Effect of mince washing and packaging on physicochemical quality changes of fish burger made from African catfish (*Clarias gariepinus*) during frozen storage

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### Abstract

African catfish (*Clarias gariepinus*) is a fatty fish classified as a dark muscle fish with a strong muddy odor. All these characteristics have limited its utilization in the food industry. However, washing the minced fish meat can help eliminate lipids and undesirable materials including blood, enzymes, and odorous substances such as trimethylamine oxide and formaldehyde. Effect of washing and packaging conditions on physicochemical changes of burgers made from minced African catfish was investigated during frozen storage (-18°C) for 5 months in this study. The fish burgers from unwashed mince and twice washed mince were prepared, cooked, and packed in aerobic or vacuum conditions. They were then kept at -18°C and analyzed for quality changes at 0, 1, 2, 3, 4 and 5 months. In comparison to the cooked burgers derived from unwashed and once washed mince, the cooked burgers from twice washed mince had lower moisture, fat, protein, ash contents, TBARS, total soluble proteins and cooking yield ( $P \leq 0.05$ ). After cooked, the products from twice washed mince also had a lighter color, higher shear force, lower moisture retention and lower fat retention ( $P \leq 0.05$ ). While freezing in vacuum packaging, moisture, fat and protein contents in the cooked burgers from twice washed mince decreased. It was concluded that washing mince twice and packing the cooked burger products in a vacuum condition had potential to preserve the physicochemical quality of fish burgers in frozen storage for 5 months.

**Keywords:** African catfish; fish burger; washing process; packaging condition; frozen storage

## 1. Introduction

Thailand is one of the top six fish exporters in the world. During 2015 to 2018, Thai fish and fishery products had grown from 5.68 billion USD to 6.05 billion USD (FAO, 2018; FAO, 2020). On top of live African catfish (*Clarias gariepinus*), chilled and frozen fillets are ones of Thailand's exported fishery commodities. The export value of catfish products has increased 47.34%, from 1.78 million USD (752.50 tons) in 2015 to 3.76 million USD (2,527.94 tons) in 2020 (MOAC, 2020). African catfish (*C. gariepinus*), a commercially important freshwater fish species, has been widely cultivated all over Thailand due to its easy farming which results in rapid growth, large body size, endurance and resistance to diseases. African catfish has more survival rate in poorly oxygenated water than the indigenous catfish (*C. Batrachus*). Moreover, male African catfish could be easily crossbred with female broadhead catfish (*C. macrocephalus*) to produce a hybrid walking catfish (*C. macrocephalus* × *C. gariepinus*) with better characteristics. However, African catfish meat has a pale color, mushy texture and a strong fishy odor (MOAC, 2007) affecting consumer acceptance. In African catfish, oxidation of fat is significantly higher and often causes rancid and fishy odor as well as undesirable taste. Furthermore, surimi products made from dark muscle fish often turn lighter in color (lower L\* value) and have poor gelation characteristics. Fish meat turns even darker when its pH drops to 5.8. This is a result of muscle degradation and the process which transforms glycogen to lactic acid. All of this relates to the denaturation of myofibrillar proteins (Nowsad, 2007). Washing the mince would eliminate undesirable water-soluble substances including fat, blood, pigment and odorous substances such as trimethylamine oxide, formaldehyde and fishy odor. The washed mince then has potential to make fish burger with better characteristics, namely, whiter color, blander taste and smell as well as better texture in accordance with better gel forming ability.

Production and uses of fish burgers from African catfish are expected to expand, due to the washing process and packing condition of this fish. Therefore, this study was designed to compare the qualities of unwashed, once washed, and twice washed mince, raw and cooked fish burgers, under certain conditions. The quality changes of cooked fish burgers derived from unwashed and twice washed mince during five months of frozen storage period between aerobic and vacuum conditions were also investigated.

## 2. Materials and Methods

### 2.1 Fish burger preparation and analysis

Fresh African catfishes (*C. gariepinus*) with an average weight of 300-350 g were purchased from a local market (Sansai district, Chiang Mai, Thailand). The fishes were manually beheaded, gutted, skinned, washed and filleted. The fillets with approximately 30% yield were kept frozen at -18°C until used. Before the day of producing the fish burger, the frozen fillets were taken out of the refrigerator and kept at 4-5°C to defrost for 24 h. The defrosted fish fillets were cut into 1"×1"×1" pieces and minced with a meat grinder (Talsa, W32L, Germany) with a 5 mm-hole plate. The minced fish were divided into three groups, the unwashed, the once washed and the twice washed groups. The unwashed minced fish were immediately processed to fish burger.

The washing process was done according to Elyasi *et al.* (2010) by washing the mince under cold water (10°C) at a mince-to-water ratio of 3:4 (w/v) for 10 min, which incorporated with slow stirring and followed by dewatering manually with a 120-mesh sieve and a thin cloth. The unwashed, once washed and twice washed mince obtained samples were analyzed for proximate composition (moisture, fat, protein and ash content), pH, thiobarbituric acid reactive substances (TBARS), soluble proteins and color values (L\*, a\* and b\*).

The African catfish burger from the unwashed, once and twice washed mince were produced according to Bainy *et al.* (2015a). The burger formulation consisted of 81.83% mince, 1.23% iodized table salt (Prung Thip®, Thailand), 0.08% onion powder (Nguan Soon®, Thailand), 0.08% garlic powder (Nguan Soon®, Thailand), 0.16% ground coriander seed (Nguan Soon®, Thailand), 0.08% black pepper powder (Nguan Soon®, Thailand), 0.16% monosodium glutamate (Ajinomoto®, Thailand), 4.09% all-purpose wheat flour (Kite®, Thailand), 4.09% rice bran oil (King®, Thailand) and 8.18% cold water. All ingredients were thoroughly mixed by a kitchen blender (Sharp, EM-14, Thailand), weighed (80 g each piece) and formed using a conventional burger mould (8.5 cm diameter and 1 cm thickness). Raw fish burgers were determined for proximate composition (moisture, fat, protein and ash content), pH, thiobarbituric acid reactive substances (TBARS) and soluble proteins.

The fish burgers were fully cooked on an electric griller (Tefal, Versalio deluxe, China) at 150°C and then cooled down to room temperature. The cooked burgers were sampled and determined for reduction of diameter, cooking yield, moisture retention, fat retentions, shear force, proximate composition (moisture, fat, protein and ash content), pH, TBARS, soluble proteins and color values (L\*, a\* and b\*).

The cooked fish burgers made from unwashed, once and twice washed mince were packed in high density polyethylene bags under aerobic and vacuum conditions before kept at -18°C in the freezer (Panasonic, SF-PC900, Thailand). The products were observed for physicochemical changes within 1, 2, 3, 4 and 5 months. The frozen African catfish burgers stored were analyzed for moisture, fat, protein, TBARS, soluble proteins and shear force.

## 2.2 Cooking characterization and shear force

Cooking characterization of African catfish burgers was measured for cooking yield, diameter reduction, moisture and fat retentions. The raw and grilled fish burgers were determined for cooking yield following Bainy *et al.* (2015a) method. The burgers were weighed on a semi-analytical balance before and after cooking. The results were calculated and expressed as % cooking yield.

$$\% \text{ cooking yield} = \frac{\text{g cooked burgers} \times 100}{\text{g raw burgers}}$$

The samples were determined for diameter reduction following Bainy *et al.* (2015b) method. The diameters of raw and cooked fish burgers were measured by using a digital vernier caliper. The results were calculated and expressed as % diameter reduction as follows:

$$\% \text{ dia. reduction} = \frac{[\text{dia. of raw burgers (mm)} - \text{dia. of raw burgers (mm)}] \times 100}{\text{dia. of raw burgers (mm)}}$$

The moisture and fat contents of raw and cooked fish burgers were determined after cooking according to Bainy *et al.* (2015a) method as follows:

$$\text{moisture retention} = \frac{(g \text{ cooked burgers} \times \% \text{ moisture in cooked burgers}) \times 100}{(g \text{ raw burgers} \times \% \text{ moisture in raw burger})}$$

and

$$\% \text{ fat retention} = \frac{(g \text{ cooked burgers} \times \% \text{ fat in cooked burgers}) \times 100}{(g \text{ raw burgers} \times \% \text{ fat in raw burgers})}$$

The shear force (maximum force required to cut the sample) was determined by using a Texture analyzer (TA-XT2i, Stable Micro Systems Ltd., USA) with a Warner-Bratzler blade. The crosshead speed was 2 mm/min and the diameter of the cylindrical sample was 2.5 cm (Das *et al.*, 2008).

### 2.3 Proximate composition

The three fish burgers were sampled from each batch and analyzed in triplicates for moisture, ash, total nitrogen, fat and carbohydrate in accordance with the AOAC (2003) method. The moisture content was determined by drying samples in a hot air oven at 105°C until having a constant weight. The ash was determined as the remnant weight after the incineration of samples in a muffle furnace at 550°C for 3 h. The total nitrogen was determined using Kjeldahl method with a 6.25 nitrogen-to-protein conversion factor. The crude fat was measured by Soxhlet extraction method using hexane as a solvent. The results were expressed as g/100 g product. The remaining percentages of the total proximate analyses were considered carbohydrates.

### 2.4 pH, TBARS, and soluble proteins

#### 2.4.1 pH

The unwashed, once washed and twice washed mince, raw and cooked fish burgers were weighed 1 g and mixed with 10 mL distilled water. The blended samples were filtered with Whatman® filter paper No.1 then the filtrate was measured for pH with a pH meter (Metrohm, 744, Switzerland).

#### 2.4.2 TBARS

The secondary oxidative products were analysed as TBARS by the method of Erkan and Ozden (2008) with a slight modification. Briefly, a 1-g muscle sample was extracted with 5 mL of 50% (v/v) acetic acid solution containing 0.01% BHT. The sample was then added with 4 mM thiobarbituric acid (TBA) and heated in 95°C water bath for 60 min to form the pinkish colored complex. The absorbance was measured at 532 nm and a calibration curve was used to quantitate the concentration

of TEP (1,1,3,3-tetra-ethoxypropane). The TBARS content, expressed as mg malonaldehyde per kg muscle sample, was calculated with the following equation:

$$\text{TBARS (mg MDA/kg)} = \frac{\text{Ac} \times \text{V}}{\text{W}}$$

Where Ac is the TEP concentration from the calibration curve, W is the sample weight (g) and V is the sample volume (mL).

#### 2.4.3 Soluble proteins

The samples were solubilized with a mixed solution (pH 9) of 0.02% sodium carbonate solution containing 5% sodium chloride solution with the ratio of sample-to-solution of 1:10. The mixture was mixed at 150 rpm for 1 h using an orbital shaker and then centrifuged with a centrifuge at 9,000 rpm for 5 min. The supernatant was taken and analysed for soluble protein content using Dc protein assay kit (BioRad, Life Science AP Co. Ltd., Thailand) with bovine serum albumin (BSA, Himedia, India) as the standard. The absorbance was observed at a wavelength of 750 nm (Lowry *et al.*, 1951). The soluble protein contents were expressed as % soluble proteins.

#### 2.5 Color

The color values of mince, raw and cooked fish burgers were measured with a colorimeter and reported in the CIELab Scale (CIE, 1976) as lightness (L\*), redness (a\*) and yellowness (b\*).

#### 2.6 Statistical analysis

The results of the analysis were presented as means value  $\pm$  standard deviation (SD). Data were analysed by analysis of variance (ANOVA) (SPSS version 16.0 for Windows; SPSS Chicago, IL., USA), and the means were separated by Duncan's multiple range test. The significance was defined at P<0.05.

### 3. Results and Discussion

#### 3.1 Cooking characteristics and shear force of African catfish burgers

The dimensional changes and cooking characteristics are important for monitoring the quality of fish burgers. Table 1 showed that cooking yield and moisture retention of cooked fish burgers made from once and twice washed mince were lower than those made from unwashed mince (P<0.05) because of lipid and water loss from fish meat during washing and leaching process. In addition, the water/fat binding capacity of proteins was also important in retaining water/fat after cooking, increased cooking yield and decreased diameter reduction. Okuskhanova *et al.* (2017) demonstrated that protein and ion had a role in water binding capacity of meat.

However, there was evidence that the ion associated with protein did not affect water binding (Karmas and Turk, 1976). The moisture and fat retention of the cooked fish burgers made from twice washed mince were the lowest while the shear force was the highest ( $P<0.05$ ). The highest cooking yield of the cooked fish burgers made from unwashed fish mince were noticed ( $87.32 \pm 1.21\%$ ). The results were in concordance with Bainy *et al.* (2015c), which reported on the cooking yield and diameter reduction of cooked tilapia burgers as 88.40% and 6.08%, respectively. The high diameter reduction, low cooking yield, low moisture retention and low fat retention were high correlated with high shear force (Table 1). The shear force, an instrumental tenderness value, reflects the water/fat binding capacity of the products, i.e., the higher the shear force the lower water/fat binding capacity (Berry and Abraham, 1996).

**Table 1** Cooking characteristics and shear force of African catfish (*C. gariepinus*) cooked burgers made from unwashed, once washed and twice washed mince.

	Cooked burgers		
	Unwashed	Once washed	Twice washed
Dia. reduction <sup>ns</sup> (%)	$7.27 \pm 1.15$	$7.13 \pm 1.86$	$7.25 \pm 1.34$
Cooking yield (%)	$87.32 \pm 1.21^a$	$85.69 \pm 0.49^b$	$84.72 \pm 0.11^b$
Moisture retention (%)	$83.88 \pm 0.72^a$	$80.37 \pm 0.29^b$	$76.24 \pm 0.43^c$
Fat retention (%)	$92.80 \pm 4.66^a$	$91.07 \pm 1.72^a$	$82.52 \pm 1.72^b$
Shear force (g)	$751.56 \pm 10.33^c$	$859.59 \pm 4.47^b$	$962.13 \pm 13.60^a$

Data are expressed as mean values  $\pm$  standard deviation (n=6). Means in the same row with different letters are significantly different ( $P<0.05$ ).

### 3.2 Quality of African catfish mince (unwashed, once washed and twice washed) and raw and cooked burgers made from unwashed, once washed and twice washed mince

#### 3.2.1 Proximate composition of African catfish mince, raw and cooked burgers

Results of the proximate composition of African catfish mince (unwashed, once washed and twice washed), raw and cooked burgers made from unwashed, once washed and twice washed mince were shown in the Table 2, 3 and 4, respectively. The mean percentages (%) of the chemical composition of fish muscle (unwashed mince) were  $75.35 \pm 0.51$  for moisture,  $5.60 \pm 0.14$  for fat,  $15.94 \pm 0.18$  for protein, and  $0.93 \pm 0.03$  for ash and were similar to those studied by Mahboob *et al.* (2019). The proximate composition in fish meat was affected by species, age, and aquacultural environment. A significant decrease in moisture, fat, protein and ash contents of fish mince was observed after the washing process (Table 2). Consequently, the moisture, fat, protein and ash contents of fish burgers made from both once and twice washed fish mince also significantly decreased ( $P<0.05$ ) (Table 3-4).

**Table 2** Proximate composition of African catfish (*C. gariepinus*) mince.

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Unwashed	75.35±0.51 <sup>a</sup>	5.60±0.14 <sup>a</sup>	15.94±0.18 <sup>a</sup>	0.93±0.03 <sup>a</sup>
Once washed	70.32±0.50 <sup>b</sup>	5.11±0.06 <sup>b</sup>	14.65±0.14 <sup>b</sup>	0.51±0.05 <sup>b</sup>
Twice washed	68.82±1.55 <sup>c</sup>	4.82±0.02 <sup>c</sup>	13.68±0.15 <sup>c</sup>	0.31±0.03 <sup>c</sup>

Data are expressed as mean values ± standard deviation (n=3). Means in the same row with different letters are significantly different (P<0.05).

**Table 3** Proximate composition of African catfish (*C. gariepinus*) raw burgers.

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Unwashed	67.91±0.45 <sup>a</sup>	12.40±0.16 <sup>a</sup>	15.64±0.19 <sup>a</sup>	1.84±0.04 <sup>a</sup>
Once washed	65.78±0.06 <sup>b</sup>	11.92±0.15 <sup>b</sup>	14.33±0.34 <sup>b</sup>	1.65±0.01 <sup>b</sup>
Twice washed	65.00±0.31 <sup>c</sup>	11.62±0.03 <sup>c</sup>	14.23±0.39 <sup>b</sup>	1.55±0.01 <sup>c</sup>

Data are expressed as mean values ± standard deviation (n=3). Means in the same row with different letters are significantly different (P<0.05).

**Table 4** Proximate composition of African catfish (*C. gariepinus*) cooked burgers.

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Unwashed	65.24±0.15 <sup>a</sup>	13.17±0.32 <sup>a</sup>	14.37±0.18 <sup>a</sup>	2.48±0.06 <sup>a</sup>
Once washed	61.69±0.19 <sup>b</sup>	12.66±0.13 <sup>b</sup>	13.75±0.10 <sup>b</sup>	2.15±0.05 <sup>b</sup>
Twice washed	58.49±0.05 <sup>c</sup>	11.31±0.04 <sup>c</sup>	13.41±0.15 <sup>c</sup>	1.95±0.05 <sup>c</sup>

Data are expressed as mean values ± standard deviation (n=3). Means in the same row with different letters are significantly different (P<0.05).

The highest moisture content was found in the cooked burgers made from unwashed mince (65.24 ± 0.15%) and the lowest in those made from twice washed mince (58.49 ± 0.05%). Similarly, the fat content of the cooked burgers from unwashed mince was the highest (13.17 ± 0.32%) and those made from twice washed mince was the lowest (11.31 ± 0.04%). The moisture and fat contents in the fish burgers from twice washed mince were the lowest because of the mince washing process which leached out the fat component and reduced the water in the dewatering process thereafter.

Once the fish burgers had been cooked, their moisture and protein contents decreased, while their fat and ash contents increased (Table 3-4). The moisture content of raw and cooked fish burgers compared to that of the mince were reduced because water was adsorbed by the added thickener in the formulation. The wheat flour along with some other ingredients used to make the burgers were also dehydrated during grilling. In addition, the rice bran oil in the fish burger formulation caused a higher fat content in the products. Furthermore, moisture, protein and ash contents of the cooked African catfish burgers made from unwashed mince (65.24 ± 0.15%, 14.37 ± 0.18%, and 2.48 ± 0.06% respectively) were similar to the report of moisture, protein and ash contents in the cooked tilapia burgers (68.40%, 16.63%, and 2.60%, respectively) (Bainy *et al.*, 2015c). While the fat content of cooked tilapia burgers (6.62%) (Bainy *et al.*, 2015c) was lower than the cooked African catfish burgers (13.17%) in this study, which implied that the tilapia is classified as a lean fish. In addition, the fat content in of raw tilapia fillets were also low (2.56%) (Bainy *et al.*, 2015c).

### 3.2.2 pH, TBARS and soluble proteins of African catfish mince, raw and cooked burgers

pH: The pH of fresh fish meat after postmortem varies from 6.0–6.8 (Khalafalla *et al.*, 2015). Table 5 shows the pH of mince, raw and cooked fish burgers. The pH of unwashed mince was  $6.24 \pm 0.04$ , which was closed to the pH of the unwashed mince from African catfish (6.25) reported by Durães *et al.* (2012). The pH of the once and twice washed mince were significantly higher when compared to that of the unwashed one. Particularly, the washing step gave the highest pH in the mince because the large amounts of water-soluble substances as free amino acids, lactic acid, free fatty acids and other soluble compounds were removed (Asgharzadeh *et al.*, 2010). Furthermore, the pH is one of the factors that influences fish protein gel formation. If the pH value of a product is lower than 6.0, the gel will not be formed. The characteristics of a good quality surimi had the pH values of 6.5–7.0, which is similar to the pH of the once and twice washed mince (as 6.59 and 6.84, respectively) in this study.

**Table 5** pH of African catfish (*C. gariepinus*) mince (unwashed, once washed and twice washed) and raw and cooked burgers from unwashed, once washed and twice washed mince.

Treatment	Mince	Raw burgers <sup>ns</sup>	Cooked burgers
Unwashed	$6.24 \pm 0.04^c$	$6.30 \pm 0.03$	$6.45 \pm 0.02^b$
Once washed	$6.59 \pm 0.02^b$	$6.30 \pm 0.05$	$6.54 \pm 0.03^a$
Twice washed	$6.84 \pm 0.03^a$	$6.30 \pm 0.00$	$6.54 \pm 0.06^a$

Data are expressed as mean values  $\pm$  standard deviation (n=3). Means in the same row with different letters are significantly different (P<0.05).

**Table 6** TBARS (mg MDA/kg) of African catfish (*C. gariepinus*) mince (unwashed, once washed and twice washed) and raw and cooked burgers from unwashed, once washed and twice washed mince.

Treatment	Mince	Raw burgers	Cooked burgers
Unwashed	$0.06 \pm 0.00^a$	$0.16 \pm 0.01^a$	$0.38 \pm 0.02^a$
Once washed	$0.04 \pm 0.00^b$	$0.10 \pm 0.01^b$	$0.25 \pm 0.01^b$
Twice washed	$0.02 \pm 0.00^c$	$0.06 \pm 0.01^c$	$0.16 \pm 0.01^c$

Data are expressed as mean values  $\pm$  standard deviation (n=3). Means in the same row with different letters are significantly different (P<0.05).

The pH of all fish burger samples complied with both Kose *et al.* (2006) and Ozyurt *et al.* (2007) which suggested that acceptable level of pH of fish should be in the ranges of 6.8–7.0. After the fish burger preparation step, the pH of the raw fish burgers made from the unwashed, once washed and twice washed mince was not different (P>0.05). The results indicated that adding a small amount of sodium chloride salt could lower the anion repulsion between the proteins from the connection of the sodium ion and a higher free hydrogen ion. This is because the sodium chloride salt splits into positive and negative charges (Na<sup>+</sup> and Cl<sup>-</sup>, respectively) and merges with the muscle proteins (DeMan, 1999). In contrast, the pH of the cooked fish burgers made from unwashed, once washed and twice washed mince increased to  $6.45 \pm 0.02$ ,  $6.54 \pm 0.03$  and  $6.54 \pm 0.06$ , respectively which is in

agreement with previously reported results of Huang *et al.* (2011) and Dal Bosco *et al.* (2001) which may be attributed to the loss of free acidic group of the proteins (Huang *et al.*, 2011).

**TBARS:** The TBARS values were used as an indicator of the degradation of the fat in fish meat. The TBARS of the unwashed mince was 0.06 mg MDAk/g, which was lower than what had been reported by Durães *et al.* (2012) (0.216 mg MDA/kg). The TBARS of the once and twice washed mince significantly decreased when compared to the unwashed mince ( $P<0.05$ ). The washing step lowered the TBARS in the mince due to the removal of a large amount of fat and blood. Haemoglobin in blood can react with the muscle fat and accelerate fat oxidation, therefore, lowering lipid and blood by washing may delay the onset of lipid oxidation (Richards *et al.*, 1998). After the fish burger preparation and cooking steps were completed, the raw and cooked fish burgers made from the unwashed, once washed and twice washed mince significantly increased in TBARS when compared with the TBARS in the mince ( $P<0.05$ ). The results indicated that the hydrolysis of the triglycerides and the oxidation of fat caused the formation of the hydroperoxides to react further to non-radical products such as aldehydes, ketones, hydrocarbons, furans, acids, alcohols (Gandotra *et al.*, 2012).

**Table 7** Soluble proteins (%) of African catfish (*C. gariepinus*) mince (unwashed, once washed and twice washed) and raw and cooked burgers from unwashed, once washed and twice washed mince.

Treatment	Mince	Raw burgers	Cooked burgers
Unwashed	1.88 $\pm$ 0.06 <sup>a</sup>	1.48 $\pm$ 0.04 <sup>a</sup>	1.48 $\pm$ 0.09 <sup>a</sup>
Once washed	1.47 $\pm$ 0.10 <sup>b</sup>	1.25 $\pm$ 0.04 <sup>b</sup>	1.21 $\pm$ 0.05 <sup>b</sup>
Twice washed	1.28 $\pm$ 0.03 <sup>c</sup>	1.02 $\pm$ 0.05 <sup>c</sup>	0.99 $\pm$ 0.04 <sup>c</sup>

Data are expressed as mean values  $\pm$  standard deviation ( $n=3$ ). Means in the same row with different letters are significantly different ( $P<0.05$ ).

**Soluble proteins:** As shown in Table 7, the unwashed mince had the highest amounts of soluble proteins up to 1.88% compared to other treatments. The soluble proteins of the once and twice washed mince significantly decreased when compared to the unwashed mince. The results indicated that the protein solubility initially decreased at approximately pH 6.5 (Kristinsson *et al.*, 2005) while the pH of the once and twice washed mince were 6.59 and 6.84, respectively. The pI (isoelectric point) of muscle proteins are approximately 5.5 which shows the lowest protein solubility due to the almost zero net charges of the muscle proteins (Damodaran, 1996). Therefore, the more pH of meat closer to the pI, the less meat protein solubility was found. Furthermore, the soluble proteins of raw and cooked fish burgers made from the unwashed, once washed and the twice washed mince significantly decreased because the fat absorption and water loss in preparation and cooking steps directly affected lower protein solubility.

### 3.2.3 Color of African catfish mince and cooked burgers

The color values of the mince and cooked fish burgers made from the unwashed and twice washed mince are shown in Table 8. The L\* and b\* values of the once washed and twice washed mince significantly increased when compared to the unwashed mince. Moreover, the L\* value in the cooked fish burger from the

unwashed mince ( $41.49 \pm 21.34$ ) were similar to the grilled catfish burgers which was reported by HassabaAlla *et al.* (2009). The  $a^*$  value of the once washed and the twice washed mince significantly decreased when compared to the unwashed ones owing to the fact that the red muscle fibres and the pigment concentrations which includes myoglobin, haemoglobin and carotenoids could be removed effectively through the washing process. The additional the ingredients such as wheat flour, etc., used in making the African catfish burgers directly affected the color after cooking, partly as a consequence of the Maillard's reaction.

**Table 8** Color values of African catfish (*C. gariepinus*) mince (unwashed, once washed and twice washed) and cooked burgers from once washed, once washed and twice washed mince.

Color values	Unwashed	Once washed	Twice washed
L*			
Mince	$37.44 \pm 0.51^c$	$49.16 \pm 1.05^a$	$47.16 \pm 1.15^b$
Cooked burgers	$41.49 \pm 1.34^c$	$44.20 \pm 0.18^b$	$46.60 \pm 0.18^a$
$a^*$			
Mince	$1.32 \pm 0.03^a$	$0.43 \pm 0.03^b$	$0.44 \pm 0.04^b$
Cooked burgers	$0.43 \pm 0.07^a$	$0.20 \pm 0.07^b$	$0.22 \pm 0.03^b$
b*			
Mince	$7.19 \pm 0.24^b$	$7.95 \pm 0.07^a$	$7.86 \pm 0.12^a$
Cooked burgers	$11.34 \pm 0.14^b$	$11.71 \pm 0.44^a$	$11.62 \pm 0.07^a$

Data are expressed as mean values  $\pm$  standard deviation (n=3). Means in the same row with different letters are significantly different ( $P<0.05$ ).

### 3.3 Quality changes of African catfish cooked burgers during frozen storage (-18 °C) with aerobic or vacuum packaging for 5 months

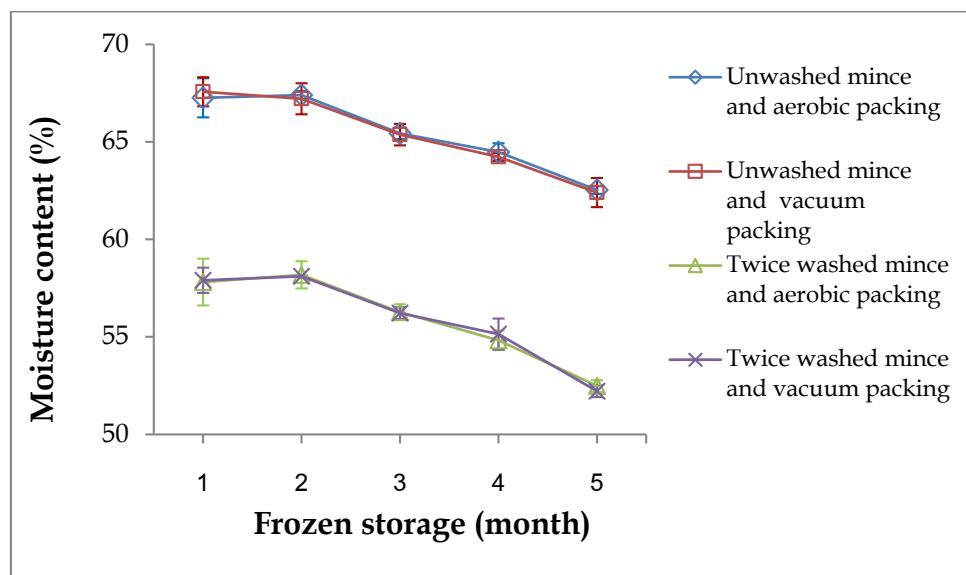
The African catfish burgers made from the twice washed mince was grilled, packaged in either aerobic or vacuum condition and stored in a frozen condition at -18°C for 5 months. The samples were analysed every month for investigating the chemical and physical changes. The African catfish burgers made from the unwashed mince packaged in aerobic or vacuum condition were used as the control.

#### 3.3.1 Moisture, fat and protein contents

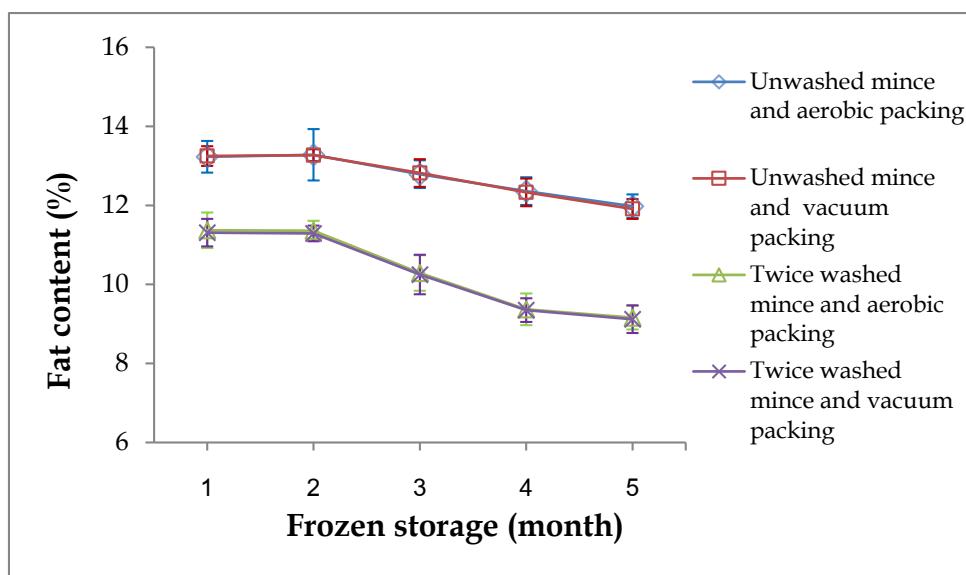
The changes in the moisture, fat and protein content of the cooked fish burgers made from the unwashed and twice washed mince, packed in an aerobic and vacuum packaging, stored at -18°C for 5 months were shown in Fig 1-3. The Moisture content of the all cooked fish burger samples were stable within the second month and likely to decrease at through the third, fourth, and fifth month. These results were similar to the study of El-Lahamy *et al.* (2019) which demonstrated that the moisture content of the frozen sand smelt (*Atherina hepsetus*) burgers decreased from 57.22% to 56.31% during kept for 60 to 90 days.

The fat content of the cooked fish burgers made from the unwashed and twice washed mince packed in the aerobic or vacuum conditions were unchanged throughout the first and second month in refrigerated storage. Moreover, the fat content in all the cooked fish burgers gradually decreased from the third to the fifth month as shown in Fig 2 owing to the oxidation and hydrolysis of the fat converted

the fats to some volatile compounds, such as aldehydes and ketones (Gandotra *et al.*, 2012).



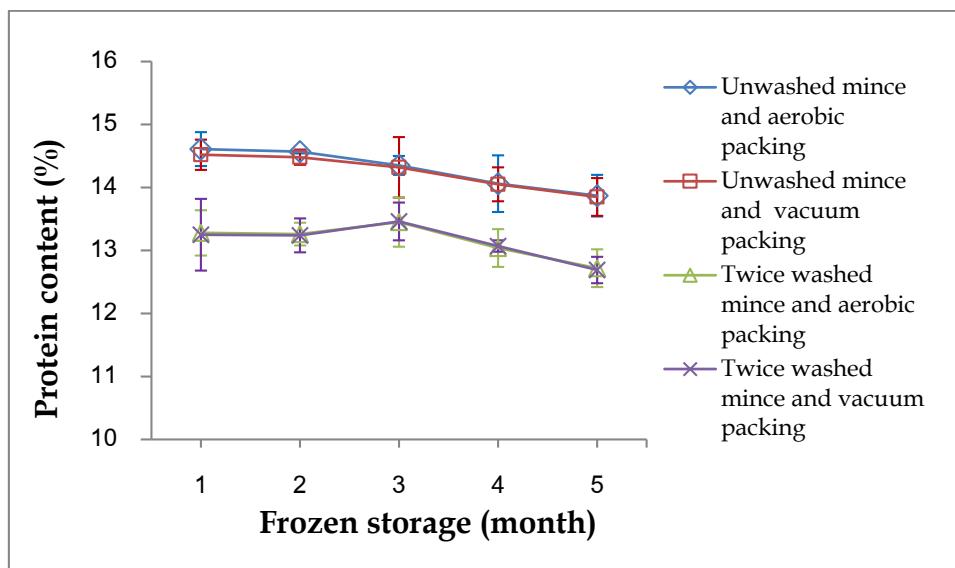
**Fig 1** Moisture content of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values ( $n=3$ ).



**Fig 2** Fat content of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values ( $n=3$ ).

The changes in the protein content of the cooked fish burgers during the frozen storage were shown in Fig 3. The results indicated that the protein content of the unwashed and twice washed fish burgers in the aerobic or vacuum conditions gradually decreased from the three-month storage onwards. Protein loss during frozen storage of fish products had been studied and various mechanisms were suggested to explain this behavior. Abo-Taleb (1997) reported that the decreased protein content during frozen storage may be due to the loss of some volatile

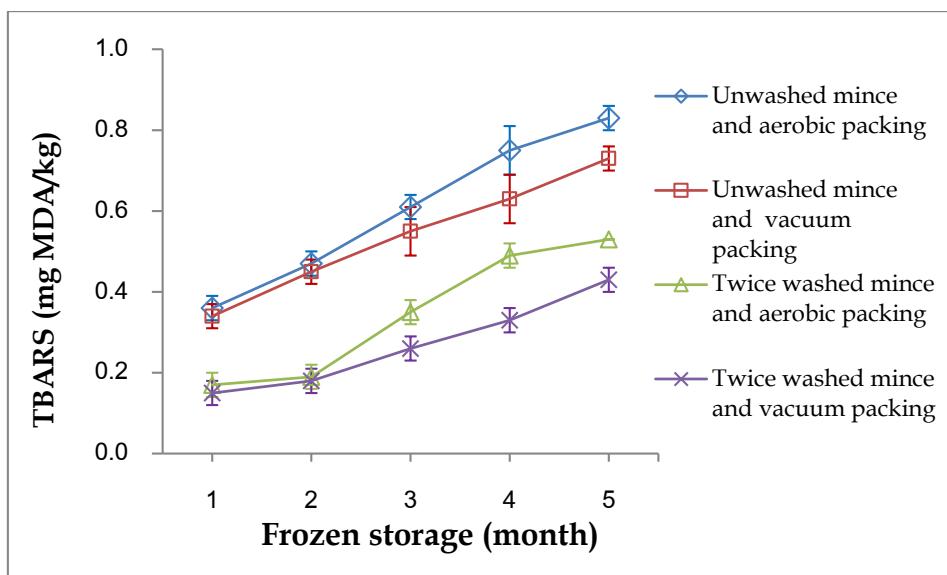
nitrogenous compounds and the endogenous enzymatic protein hydrolysis to small molecules increasing the loss of soluble proteins in the drip during thawing. Gandotra *et al.* (2012) attributed protein loss during frozen storage to drip loss in thawing process making some amino acids and soluble proteins leaked from fish products.



**Fig 3** Protein content of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values ( $n=3$ ).

### 3.3.2 TBARS

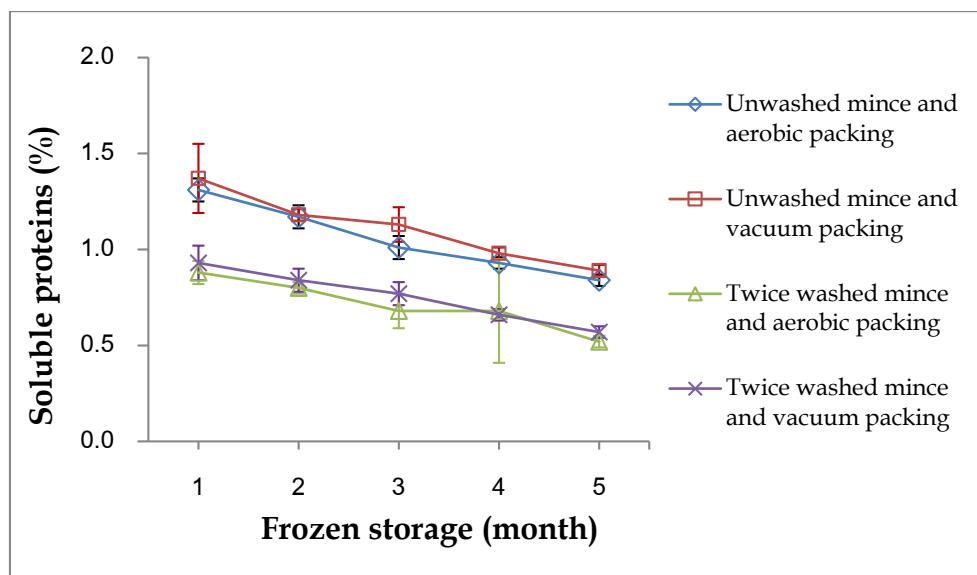
Lipid oxidation was measured by the determination of TBARS which quantified aldehyde compounds, the secondary oxidative products. The TBARS of the cooked fish burgers made from the unwashed and twice washed mince which had been packed in the aerobic or vacuum conditions increased rapidly throughout the 5 months in frozen storage as shown in Fig 4. The samples under aerobic condition were sharply higher at the third, fourth, fifth month as compared to those under vacuum condition. The vacuum packaging excludes the amount of oxygen in the products and retards lipid oxidation and also TBARs values (Fernandez-Espla and O'Neill, 1993). Moreover, these TBARS values were well below the acceptable limits of 1–2 mg malondialdehyde/ kg product in all samples through 5 months storage (Mir and Masoodi, 2017). The cooked fish burgers contained high amount of unsaturated fatty acids to accelerate fat oxidation, which directly affected the changes in color, taste and texture of the products from fat oxidation products such as free radicals, peroxides, alcohols, aldehydes and ketones. The fat oxidation products enhanced protein denaturation and crosslinking resulting in the loss of protein solubility and texture characteristic of product (Li *et al.*, 2016).



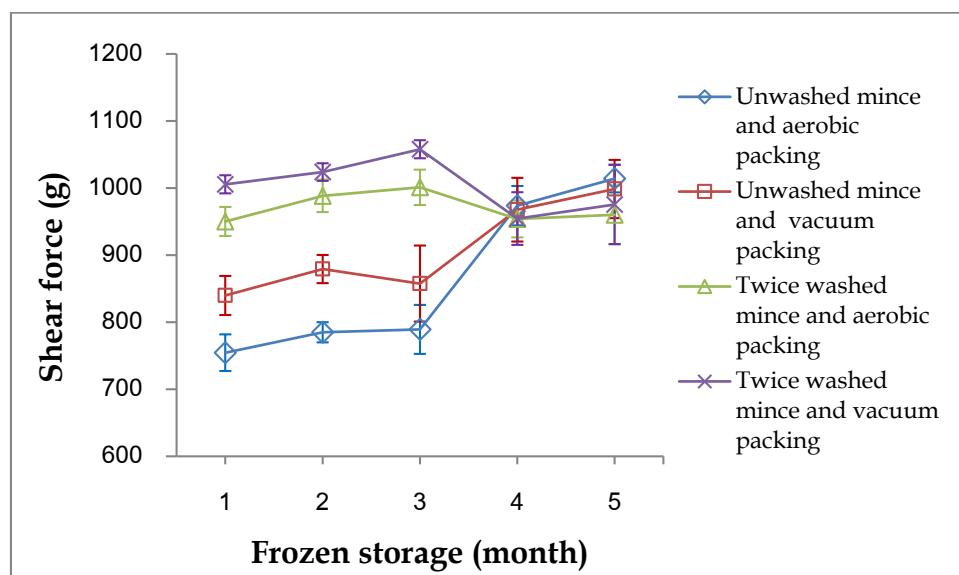
**Fig 4** TBARS of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values ( $n=3$ ).

### 3.3.3 Soluble proteins

The Soluble proteins in relations to an index of the frozen protein denaturation were determined to indicate changes in the texture characteristics of the frozen products (DeKoning and Mol, 1991). The soluble proteins of the cooked fish burgers made from the unwashed and twice washed mince packed in aerobic or vacuum conditions tended to decrease throughout the 5-month frozen storage as shown in Fig 5. The denatured protein affects soluble proteins reduction, due to the aggregation of myofibrillar proteins, thus causing changes in the texture characteristics (Mackie, 1993). Furthermore, the reduction of the soluble proteins directly affects the reduction of the juiciness of the fish burgers because of the loss of moisture content and water holding capacity (Gokoglu *et al.*, 2018).



**Fig 5** Soluble proteins of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values (n=3).



**Fig 6** Shear force of African catfish (*C. gariepinus*) cooked burgers during frozen storage at  $-18^{\circ}\text{C}$  for 5 months. Data are expressed as mean values (n=3).

### 3.3.4 Shear force

The shear force is used to measure the maximum force of a functional blade movement and the compression to cut off a sample. The results of this measurement were able to show the hardness (tenderness and toughness) of the food product. The shear force of the cooked fish burgers made from unwashed mince packed in an aerobic condition, together with the twice washed mince packed in both the aerobic and vacuum condition trended to slightly increase around the second to third month. Whereas the cooked fish burgers made from the unwashed mince packed in the vacuum packaging increased at the second month and decreased at the third month. After the fourth and the fifth month of frozen storage, the shear force of the cooked

fish burgers made from the unwashed mince packed in the aerobic or vacuum packaging decreased and slightly increased, respectively. The cooked fish burgers made from the twice washed mince kept in the aerobic or vacuum packaging constantly increased. The results showed that the texture changes (increased hardness) of the frozen fish burgers were especially caused by not only the denaturation of the myofibrillar proteins but also the decrease of the water holding capacity (Damodaran, 1996).

#### 4. Conclusion

Through this research, the African catfish is believed to be a good meat source for producing fish burgers. This observation concluded that the mince should be washed twice in cold water at a 3:4 ratio of mince to cold water by weight to remove fat as well as odorous and coloring substances. The burgers should be packed in vacuum packaging and kept at frozen conditions at a temperature of  $-18^{\circ}\text{C}$ . This preparation and packaging process could potentially help extend the shelf-life of the African catfish burgers for at least 5 months. This research hopes to encourage the food industries to produce frozen fish burgers from African catfish (*C. gariepinus*) meat at a commercial scale.

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