INCORPORATION OF SALT EXTRACTED COMPOUNDS FROM YELLOWFIN TUNA (*Thunnus Albacares*) TO PRODUCE A FISH FLAVORED VEGETABLE BURGER

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Abstract

Nitrogenous compounds present in the flesh, play an important role in flavor and taste of fish. The objectives of this study were to extract flavor compounds from Yellowfin tuna (Thunnus albacares) and incorporate it in to a vegetable burger to maximize the utilization of fish waste generates in fish processing industry. Nitrogenous compounds from fish waste were extracted with different concentrations of NaCl solutions with heat treatment of 60°C for 15 minutes. Tuna fish nitrogenous compounds incorporated vegetable burger was developed after several organoleptic sensory evaluation tests. The nutrient content of the developed fish flavored vegetable burger was analyzed and self-life was determined by pH value, lipid oxidation and microbiological tests. The sensory evaluation results revealed that the best dilution series for flavor extraction was fish: NaCl = 1: 1 and the best NaCl concentration was 01% (w/v). The pH value of the product was not significantly increased (P>0.05) and there was a significant increase of the oxidation after 30 days of storage at 4°C. Including the microbiological results, all the test results were confirmed that the finalized fish flavored vegetable burger was in acceptable level in the storage time period. The protein content, fat, moisture, and ash of the finalized burger were 31.65%, 23.04%, 27.47% 6.93% respectively.

Keywords: Fish, Flavor, Vegetable Burger, Nitrogenous Compounds

INTRODUCTION

Non protein nitrogen (NPN) compounds are the responsible for the flavor of meat. Fish flesh also containing NPN compounds such as ammonia, mono methylamines, di methylamines, tri methilamines, tri methilamonia bases (tri methylamine oxide and betaines) and guanidine derivatives (creatine and argenine) (Borgstrom, 1961; Teerasuntonwat and Raksakulthai, 1995). Fish flavors can be synthetized by chemically but they are not perfectly replacing the natural flavors (Teerasuntonwat and Raksakulthai, 1995). These flavor components can be extracted from fish flesh and can be incorporated in food industry as a method of value addition to the fish waste.

Fish wastes are generated due to the processing of fish (Arvanitoyannis and Kassaveti, 2008). Among the fishes Tuna is a major fish which process by many processing companies. Value added products are produced using tuna which include tuna steaks, tuna loin, tuna fillets, tuna blocks and canned tuna (Hamilton et al., 2011). In processing 40%- 60% of waste are discarded in the industry due to the gutting, deheading, skinning, deboning and loining processes (Kim, 2015). Collected wastes are used to produce fish meal, fish oil, fertilizer and hydrolysates in some countries (Sharp and Mariojouls, 2012). However usually companies remove waste without any utilization (FAO, 2014). Maximum utilization must be needed as a strategy of waste management and maximum generation of revenue by adding value to the natural resources.

There are several chemicals to separate NPN compounds such as use of trichloroacetic acid (TCA), 70% ethanol, dilute NaOH (Bhatty et al., 1973b) and use of tangstic acid (Soest, 1996), sulfosalicylic acid, acetic acid (Bhatty and Finlayson, 1973a). However these methods could not be applied in food

industry as they are toxic compounds. Chemicals mentioned above are expensive, and difficult to scale up. Chemicals used for the extraction should be non-toxic, low cost, easily separable, and available and could be produce large scale production. A study has shown that there are some enzymes such as bromelain can be used to isolate the flavor components and NaCl is one of the best flavor binders used as a non-enzymatic method (Teerasuntonwat and Raksakulthai, 1995). Therefore NaCl was selected as the extraction solution. Soya bean and chick pea were selected to produce vegetable burger because both soya bean and chick pea containing high amount of protein. Soya bean contains 38% to 42% protein (Balasubramaniyan and Palaniappan, 2004). Soya beans equal to animal protein and also a good source of poly unsaturated fatty acids, partially linoleic acid which account for about the half the fatty acid content. It is a good source of iron, calcium and B vitamins (Lean, 2006). Soya protein is extractable and extracted protein can be textured and flavored to resemble meat. This is known as Textured Vegetable Protein (TVP) (Tull, 1996). 100 g of soy flour containing 1871 kJ of energy, 36.8 g of protein, 23.5 g fat, 13.3 g of poly unsaturated fatty acids, 23.5 g of carbohydrates, 210 mg of calcium, 6.9 mg of iron, 0.75 mg of thiamine, 0.31 mg of riboflavin (Lean, 2006). Soya burger is meat alternative which known as a non-meat food (Descheemaeker and Debruyne, 2002). Chick pea has significant amounts of all the essential amino acids and rich in nutritionally important unsaturated fatty acids like linoleic and oleic acid (Jukanti et al., 2012). It is a good source of carbohydrates, protein and minerals such as calcium, phosphorus, iron, magnesium, potassium and zinc. Calcium content of the chick pea is high. Because of the outer coating consist with calcium. 100 g of chick pea containing (20.1-24.6) g protein, (4.5-5.6) g of fat, (2.67-3.14) g of ash (1.7-10.3) g of fiber (Wal and Mishra, 2000). The objective of this study was to use yellowfin tuna fish waste to produce fish flavored vegetable burger for maximum utilization of fish waste by extracting flavors and incorporate to food industry and produce nutrition rich vegetable burger.

MATERIALS AND METHOD

This study was conducted in Animal Product Processing and Research Laboratory in Uva Wellassa University of Sri Lanka. Frozen vacuum packed yellowfin tuna trimmings were purchased from Jay Sea Food Processing (Pvt) Ltd, Pamunugama, Sri Lanka. The trimmings were kept in below +4°C (around 0°C) to eliminate histamine formation during the whole process. Soya bean, chick pea, garlic powder, chili powder, black pepper powder, salt, oil and wheat flour were purchased from local market in Badulla, Sri Lanka. The proximate analysis was done in Veterinary Research Institute in Peradeniya, Sri Lanka.

Preparation of Vegetable Burger

Vegetable burger was prepared using soya and chick pea as the main ingredients. Soya bean were sun dried using a solar drier. Dried soya bean was steamed at 80°C for 20 minutes and ground using a grinder (IS 4250, Jaipan family mate, India). Weighed ingredients were mixed and the pulp was made. Prepared mixture was then cooked in a steamer (WP5525, WIPRO, China) at 80°C for 20 minutes. Ingredients level of the burger was determined by preliminary sensory evaluations keeping soy and chick pea flour levels constant.

Determination of best combination of soya and chick pea flour

Determination of best combination of soya and chick pea flour, 5 recipes of vegetable burger were prepared by decreasing the soya level from 100% to 0% and increasing the chick pea level from 0% to 100% while keeping the other ingredients constant as shown in table 1. Thirty untrained panelists were used to select the best formula for the burger. Then the recipe of the burger was finalized.

Table 1: Percentages of Soya and Chick pea in the 5 recipes

Ingredients	Recipe 1	Recipe 2	Recipe 3	Recipe 4	Recipe 5
Soya powder	55.00	41.25	27.5	13.75	0.00
Chick pea	0.00	13.75	27.5	41.25	55.00
Wheat flour	6.00	6.00	6.00	6.00	6.00
Garlic	2.00	2.00	2.00	2.00	2.00
Chili powder	1.00	1.00	1.00	1.00	1.00
Pepper	1.00	1.00	1.00	1.00	1.00
Salt	1.50	1.50	1.50	1.50	1.50
Oil	5.50	5.50	5.50	5.50	5.50
Water	28.00	28.00	28.00	28.00	28.00

Extraction of fish flavor from yellowfin tuna

Fish flavor was extracted according to the method of Teerasuntonwat and Raksakulthai (1995) with some modifications. Yellowfin tuna trimmings were cut in to small pieces and ground using the grinder (IS 4250, Jaipan family mate, India) with 1% (w/v) NaCl (Fluka, England). Blended samples were transferred in to a 1000 mL beaker and heated at 60°C for 15 minutes using a stirrer (SR No:67702, VELP® SCINTIFICA, Europe). Heated samples were filtered using Whatman No: 4 filter papers. Filtration was dialyzed to remove salt. Extracted fish flavor was kept under freezing condition (below -20°C) and used instead of water (28%) in vegetable burger preparation.

Determination of best dilution series for extraction of fish flavor

Yellowfin tuna trimmings were ground by changing the ratio of fish muscle to extraction solution (NaCl) as shown in table 2. The concentration of NaCl was constant at 1% (w/v). Four different recipes were used to prepare four samples of burger and best dilution series was selected by a sensory evaluation. Thirty untrained panelists were used to evaluate formula.

Determination of best NaCl concentration for extraction of fish flavor

Different concentrations of NaCl were prepared to isolate the nitrogenous compounds. Vegetable burger was prepared using the recipe developed and separated dialysis solutions were used in the recipe according to table 3.

Table 2: **Dilution ratios of Fish muscle: NaCl 1 % (w/v)**

Recipe Number	Fish Muscle	1 % NaCl	Ratio
01	200 g	200 mL	1:1
02	200 g	400 mL	1:2
03	200 g	600 mL	1:3
04	200 g	800 mL	1:4

Table 3: **NaCl concentrations to extract fish flavor**

Recipe Number	NaCl concentration (%)
01	0.1
02	1.0
03	5.0
04	10.0

The best NaCl concentration was determined by a sensory evaluation test. Thirty untrained panelists were used to evaluate four formulas.

Sensory evaluation

The sensory evaluation tests were held in Sensory Evaluation Laboratory in Uva Wellassa University. The appearance, texture, color, aroma and overall acceptability scores of the samples were determined by 30 untrained panelists using five point hedonic scale.

Quality evaluation of fish concentrate

Lipid oxidation, pH value and proximate composition of fish concentrate were determined in triplicate during 30 days of storage at 4°C. Microbiology tests were done for *Escherichia coli*, *Salmonella*, *Staphylococcus* and total plate counts. Crude protein, crude fat, moisture and ash contents of fish flavored vegetable burger were analyzed using AOAC standard methods (2002).

Statistical analysis

Sensory evaluation data was statistically analyzed using non parametric Friedman test in Minitab 16 software.

RESULTS AND DISCUSSION

Determination of best combination of chick pea and soya flour

There was no significant different between the color, aroma, texture, flavor and mouth feel of the five recipes ($P>0.05$) and overall acceptability of the recipes were significantly different ($P<0.05$). The highest score had given to the 100% of soya flour containing recipe (Figure 1). Therefore after analyzing the cost of production 100 % soya incorporated recipe was selected. Finalized vegetable burger recipe containing 55.0% (w/w) of soya flour, 6.0% (w/w) of wheat flour, 1.0% (w/w) of chili powder, 1.0% (w/w) of pepper, 2.0% (w/w) of garlic, 1.5% (w/w) of salt, 5.5% (w/w) of vegetable oil, and 28.0% (w/w) of water.

Soya protein is the only plant protein equal in quality to animal protein. Thus animal proteins can substitute from soy protein (McMann, 2000). A serving of 113 g containing 14 g of protein and same amount of Tofu (curd of soya milk) has 13 g. It is approximately equal to protein content of two large eggs or one glass of skimmed milk or two-thirds of the protein in the same amount lean minced beef. Soya beans have complete protein with sufficient amount of all amino acids and equal to egg white and casein which are known as the easily absorbable proteins (Nigel et al., 2011).

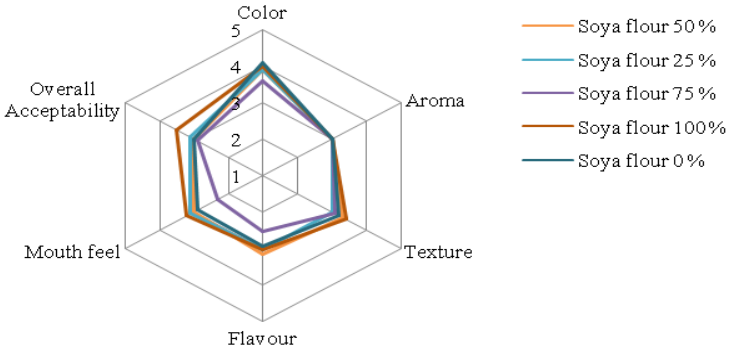

Figure 1: Web diagram of sensory evaluation for determination of best combination of chick pea and soya flour

Soya milk which containing same amount of protein content as cow milk, is used to make a form of cheese or Tofu (Lean, 2006). The protein content of the soya is 38% to 42%. The fat content of the soya bean is 17% to 22% and among the fat, linoleic acid (poly-unsaturated fatty acid) contains 54% (Balasubramaniyan and Palaniappan, 2004). These poly-unsaturated fatty acids reduce the cardio vascular diseases and reduce blood cholesterol levels (McMann, 2000). Soya containing dietary fibers which allows move foods along the digestive tract (Nigel et al., 2011). Adding soya to wheat flour is improved color, texture and nutty flavor in the baking of bread (Lean, 2006). Dried soya bean toasted and used as a coffee substitute which known as a caffeine free coffee (Lean, 2006). Although soya bean a good source of nutrition, it must be well cooked to destroy the toxin soyin (Tull, 1996). Soya is low cost and easily available in local market. According to the above benefits soya plays important role in the human foods. Hence, nutrition value of the vegetable burger is also increased.

Extraction of fish flavor

Protein precipitation was observed when heating (60°C) the mixture. Protein precipitation was increased simultaneously with the increasing of the NaCl concentration. It was due to the salting out reaction which effect to protein in the high NaCl concentrations. Salting out is one of the classical method in the protein biochemistry and widely used salt is ammonium sulfate due to its polyvalent anions (Dennison, 2003). To carry out the salting out process an excess of salt usually sodium chloride, sodium sulfate, and magnesium sulfate is added to the aqueous mixture and vigorously shaken or stirred. Then resulting mixture is separated by filtration (Ledgard, 2007).

Determination of best dilution series for extraction of fish flavor

As per the sensory evaluation results shown in figure 2, 1:1 ratio of NaCl and fish was having high flavor compounds ($P<0.05$). Therefore fish:NaCl 1:1 ratio was selected as the best combination to separate flavor compounds from yellowfin tuna.

Determination of best NaCl concentration for flavor extraction

According to the figure 3 results, 01% (w/v) of NaCl was giving the high organoleptic properties compared to rest ($P<0.05$).

Therefore to separate flavor compounds 01% (w/v) with 1:1 dilution can be considered as the best.

Proximate analysis results of finalized fish flavored vegetable burger

Proximate composition showed that the fish flavored vegetable burger contained 31.63% protein and 23.04% fat (Figure 4). These amounts are similar to the reported value of soya burgers that contain 20% to 30% of soya protein (Shewry and Gutteridge, 1992). Soy bean, nuts and rapeseed containing poly unsaturated (ω 3) fatty acids (Linolenic acids). Soy bean containing 7% of α-linolenic acid and 18 carbon omega-3 fatty acids with 3 double bonds. ω-3 fatty acids prevent from the cardio vascular disease. ω-3 fatty acids such as docosahexaenoic acid are incorporated in to neural tissues, notably brain and retina (Nettleton, 1995). Hence poly unsaturated fatty acids are healthier than saturated fatty acids such as animal fat.

Changes of the pH in storage period

There was no significant increase in pH during storage at 4°C. The pH value of the burger was ranging 6.29 to 6.8 during storage time. Soy protein has a relatively high pH value compared to the meat protein. Lean meat has approximately pH of 6.0. Therefore, increasing the levels of soy protein have a tendency to increase the pH value (Hoogenkamp, 2005).

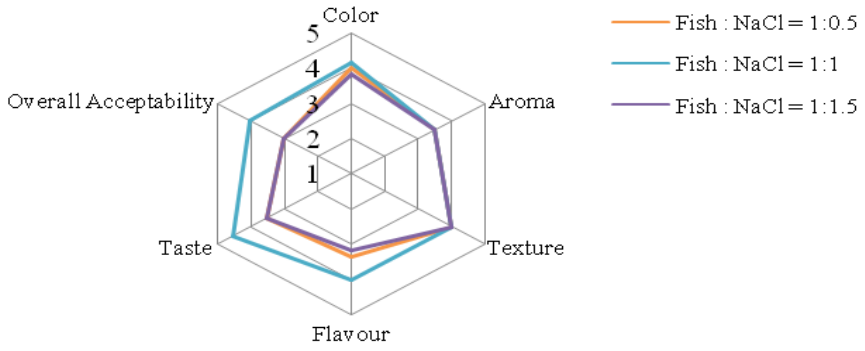

Figure 2: Web diagram of sensory evaluation for determination of best dilution series

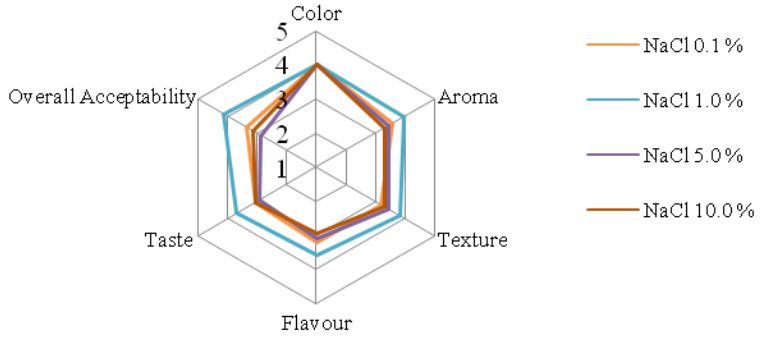

Figure 3: Web diagram of sensory evaluation for determination of best NaCl concentration

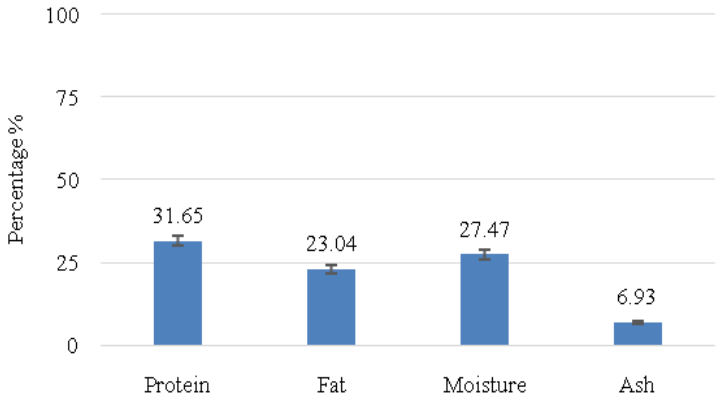

Figure 4: Proximate analysis results of fish flavored vegetable burger

Oxidation test

There was a significant increase in oxidation during storage at 4°C even after 30 days of storage (Figure 5).

Polyunsaturated fatty acids oxidized readily because their double bonds are unstable. Oxidation of unsaturated fats produces a variety of compounds that smell and taste

rancid. Saturated fats are more resistant to oxidation (Whitney et al., 2011).

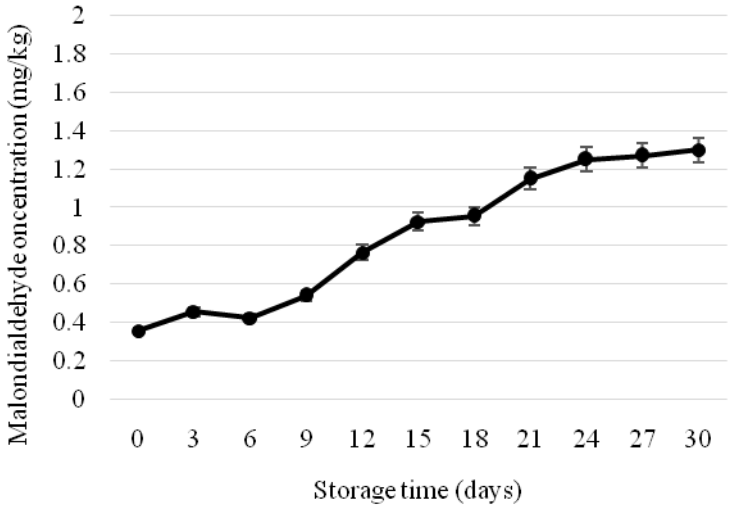

Figure 5: Changes of lipid oxidation with storage time under -20 ºC

Soya bean is rich in lipoxygenase and unsaturated fatty acids. Mechanical destruction and the presence of water or heat can accelerate the rate at which lipoxygenase catalyze unsaturated lipid oxidation (Riaz, 2006). Therefore increase of the oxidation due to the continuous oxidizing process of the poly unsaturated fatty acids of the soya bean. But according to the results, level of oxidation was within the acceptable level. Therefore, the product developed is suitable even after 30 days of storage even though the lipids oxidized during storage.

Microbiological Test

There was no growth of hazardous microorganism in the sample during the time of testing. Microbiology test were done according to SLSI specification for quick frozen whole fish, fish fillets, steaks and minced fish products. There were only positive results in total plate count but levels did not exceed the SLSI recommended limits. Total coliform test was not including the SLSI specification for quick frozen whole fish, fish fillets, steaks and minced fish products. But it also had showed zero level within the storage period. According to the cost analysis, marketable one burger (14.50 g) can be produced with the cost of Rs. 10.00.

CONCLUSION

The best dilution series for fish flavor extraction is Fish: NaCl = 1:1 and the best NaCl concentration for fish flavor extraction is 1% (w/v) NaCl. Fish flavored vegetable burger was a nutrient rich low cost product.

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