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Development of Sea Lettuce (*Ulva lactuca*) and Catla (*Catla catla*) Incorporated Protein and Fiber Rich Fish Burger

N. C. Kumarathunge, J.M.P. Jayasinghe, E.D.N.S. Abeyrathne

***Abstract* – Catla fish have limited scope for consumption due to presence of intermuscular bones. This study was carried out to develop high protein and fiber containing ready to eat catla fish burger, incorporating seaweed (*Ulva lactuca*). Four different seaweeds powder incorporation level (0.5%, 1.0%, 1.5%, 2.0%) were tested to identify the best sensory properties of the sea weed incorporated fish burger and finally, seaweed incorporated fish burger recipe was developed with 64% Catla fish flesh, 17% binder (bread crumble), 12% ice, 4% vegetable oil, 1.5% salt, spices 1% (chili, black pepper, cardamom). Control fish burger was prepared without adding seaweed. Prepared samples were cooked at 80 °C to a core temperature of 65°C. Microbiological, chemical and sensory changes of prepared fish burgers were determined during storage at -18 °C for 14 days. On the basis of sensory evaluation 0.5% (w/w) incorporation level was found to be suitable from the tested four levels of *Ulva lactuca* powder (0.5%, 1%, 1.5% and 2%). Proximate analysis revealed that seaweed incorporated fish burger contains higher amount of protein, fiber and also it contains 62.83% moisture, 7.21% ash, 12.43% fat as the other nutrients. Microbiological studies were not positive for both *E.coli* and *Salmonella* in the both seaweed incorporated and control burger samples and showed reduced total plate count for sea weed incorporated fish burger. During storage at -18°C, the seaweed added fish burger was found to be microbiologically safe and organoleptically acceptable up to 14 days. It can be concluded that 0.5% (w/w) *Ulva lactuca* incorporated fish burger increases not only the protein and fiber content but also it fulfills the nutritional requirement of consumer.**

***Keywords* – Fish Burger, *Catla Catla*, Seaweed, *Ulva Lactuca*, Protein, Fiber.**

I. INTRODUCTION

In recent years, the increasing numbers of working women in the country have led to direct consumer's preference for ready-to-eat fish products like fish cakes, fish crackers, fish fingers, fish patties marinated products and fish burgers [1]. Fish and fishery products contain high quality protein and other necessary nutrients. They are low in saturated fatty acids and contain high amount of unsaturated fatty acids [2]. Even among those fish products fish burgers are acceptable fast food products by the consumers in the world which is one of the secondary minced fish based product and are popularly distributed frozen for longer shelf life [3]. Burgers, often circular in shape and it may also contain filler and spices. A major type of comminuted raw meat product is the burger-type product made from beef but also from poultry meats and fish flesh [3]. Minced catla fish is considered as a meat ingredient for the preparation of fish burgers. However the catla fish has limited scope for consumption in the fresh form due to presence of intramuscular bones. Sea food products, such as fish fingers, fish culets and fish burgers could supply a variety of healthy foods to increase the per capita consumption of this fish. Similarly catla fish flesh can also be converted into value added consumer acceptable products. Value addition means any addition activity that in one way or the other change the nature of a product thus adding to its value at the time of sale [4]. On the other hand burgers are prepared incorporating various non-meat ingredients [5].

Most of people in the population have trend to eat nutrient rich products. Due to that Seaweeds are attracting increasing attention as a valuable food source in the world [6]. Actually seaweeds have become a major food ingredient in products especially in Japan, Korea and China. It is reported that most Europeans and Americans use processed seaweeds as additives in their food preparation [7]. In Asia, seaweeds have been used for centuries in the preparation of salads, soups and also as low-calorie foods [8]. Most of the seaweeds constitute with amino acids. Aspartic and glutamic acids represent the large part of the amino acid profile [9]. Minerals such as iron and copper are present in seaweeds at higher levels than in many well-known terrestrial sources of minerals, such as meats and spinach. The amount of copper and iron content of ulva *sp*. respectively 0.25mg/8g , 1.3mg/8g [6]. Seaweeds contain Iodine which is an important nutrient for metabolic regulation and growth pattern and abundant in most seaweeds [10]. In Asia, seaweeds have been consumed as a vegetable since the beginning of time and represent an important dietary source of iodine. According to worldwide study in 1990 the population had iodine deficiency, goiter, cretinism and more than 43 million people had some degree of mental impairment due to insufficient iodine [10].

Among seaweeds, sea lettuce is one of the commonly consumed marine vegetable which contains 16•5% of water-soluble and 13•3% insoluble dietary fibers [11]. Sea lettuce uniquely rich in essential vitamins, especially rich in vitamin B12, water soluble vitamins, such as vitamin C. Sea lettuce are still defined as the unexploited plant resources which is the most and widely distributed along the coasts of the world's oceans [8]. It is consumed different ways in different countries due to its nutrient value and health benefit. For instances, in Cuba, sea lettuce are boiled and drunk as a juice. In Philippines most of people are commonly prepared salad or soup. In British Isles, sea lettuces are commonly toasted to prepare toasted sea lettuce [8].

This study focused on developing seaweed incorporated

fish burger by using underutilized fiber rich sea lettuce (*Ulva lactuca*) and protein rich Catla (*Catla catla*) flesh. Further product may serve as a nutrient rich comminuted ready to cook products for its intended uses.

II. Materials and Methodology

A. Sample Preparation

Sea lettuce (*Ulva lactuca*) was collected from the Galle, Sri Lanka. Collected plants thoroughly removed of their epiphytes, rinsed on the spot with seawater, and then placed in plastic bags. On their arrival at the laboratory, these samples were washed again with distilled water and dried in the air. Dried seaweeds were again solar dried. Then, they were milled in a mechanical grinder (Jaipan, IS 4250, India) for 5 min, to obtain a fine and homogeneous powder and then were stored in hermetic bags at room temperature (25°C). Meantime, Fresh Catla (*Catla catla*) purchased from local market at Badulla, Sri Lanka were washed, de headed and eviscerated. The skinned fishes were filleted and deboned manually. Then minced mixture was prepared by a mechanical grinder (Jaipan family mate: IS 4250, India).

Three preliminary studies were run and sensory properties were analyzed to identify the best spices incorporation level for the final fish burger. Four different seaweeds powder incorporation level (0.5%, 1.0%, 1.5%, 2.0%) were tested to identify the best sensory properties of the sea weed incorporated fish burger and finally, seaweed incorporated fish burger recipe was developed with 64% Catla fish flesh, 17% binder (bread crumble), 12% ice, 4% vegetable oil, 1.5% salt, spices 1% (chili, black pepper, cardamom). Control fish burger was prepared without adding seaweed. Prepared samples were cooked at 80 °C to a core temperature of 65 °C. Samples were subsequently packed in polyethylene bags and stored in a freezer (-18 °C) for further analyses.

B. Sample Analysis

Proximate composition of fish burgers was determined as crude protein by the Kjeldahl method AOAC, moisture content by direct method [12], crude ash content by ISO 936 and crud fat by gravimetric method [13] respectively. The burger color was measured by using color meter (L. a. b system). Furthermore, the L* (Lightness), a* (Redness), and b* (Yellowness) values were determined using a color meter (CR 410, Konica Minolta Inc., Japan), which was calibrated against a white reference tile. The final values were obtained using the average value of three repeated measurements taken from different locations on each sample. The microbial test was done to determine the presence of *Escherichia coli* and *Salmonella*. *E.coli* test were conducted according to SLSI 516: Part (111). *E.coli* was isolated using Eosine Methylene Blue Agar. *Salmonella* test was conducted according to SLSI 516: part (v) method for salmonella which was performed using Xylose Lysine Deoxycholate agar (Oxoid Ltd.,UK, 53g L-1). Total plate count was determined by using plate count agar according to ISO 8443.

Representative samples of fish burger were placed in odorless, disposable paper plates. Each sample was coded separately with a three-digit number and served for the sensory evaluation. Clean water was provided to clean the mouth between each sample. Thirty panelists (Age group 21-26 years /male & female) assessed the appearance, texture, color, mouth feel and overall acceptability scores of the samples at Day 0 using a 5-point hedonic scale.

C. Statistical Analysis

All measurements were carried out in triplicate (n=3), and differences between means were determined by the least significant difference test at $P < 0.05$ and non-parametric data (only sensory data) were analyzed using Friedman test.

III. Results and Discussion

A. Proximate Results of Seaweed Incorporated Fish Burger

Sea weed incorporated fish burger was determined for crude protein, crude fiber, crude fat, moisture (Tab 01).

Table 01. Proximate composition of developed seaweed incorporated fish burger

Constituents	Percentage
Crude protein	16.25 ± 0.26
Fat	12.43 ± 0.15
Moisture	62.83 ± 0.58
Ash	07.21 ± 0.017
Crude fiber	02.00 ± 0.5

**All proximate values are shown in their mean with standard deviation*

According to the proximate analysis results, seaweed incorporated fish burger contained high protein content (16.25 ± 0.26). High amount of protein is required especially for athletics, sportspersons and underweight individuals. Ravichandran *et., al* [14] has well explained the importance of fish as source of high quality, balanced and easily digestible protein, vitamins and polyunsaturated fatty acids. Hence the product serve higher amount of protein per serve to the its intended users. Additionally sea lettuce (*Ulva lactuca*) incorporated fish burger has recorded 02.00 ± 0.5 of fiber value and its comparatively significant figure. Seaweeds basically contain high proportions of polysaccharides along with various other potentially beneficial compounds such as good-quality protein and essential fatty acids, particularly long-chain n-3 polyunsaturated fatty acids [15]. So the seaweed incorporated fish burger may serve as a protein and fiber rich healthier food regarding the nutritional value of it. According to Babji, *et., a*l [16] the proximate composition of chicken burger patty recorded these values 53.0%, 22.0%, 2.0%, 12.6% respectively for moisture, fat, ash and protein. Beef burger patties which were formulated incorporating soy granules contained 61.80% moisture, 14.90% fat, 3.50% ash and 15.73% protein [17]. Results of the proximate composition of seaweed incorporated fish burger revealed that it contains high amount of protein and also contain ash, moisture, fat and fiber.

B. Microbiological Results of Seaweed Incorporate Fish Burger

Microbial count of seaweed incorporated fish burger was lower than the value of control burger which was formulated without sea lettuce (*Ulva lactuca*). Considering the results, *E. coli* was negative in samples because of the hygienic preparation, handling of product and it is not subjected to cross contamination. Fresh meat and meat products can be easily contaminated with microorganisms and, if these are not properly handled and preserved, they support growth of spoilage and pathogenic bacteria, leading to loss of their quality and constitute potential health problems. According to Total Plate Count results seaweed incorporated fish burgers obtained desired keeping quality and also it revealed that it contains antimicrobial activities which helpful to increase the shelf life of the product. It sustained by maintaining lower TPC value for sea weed incorporated fish burger during storage time.

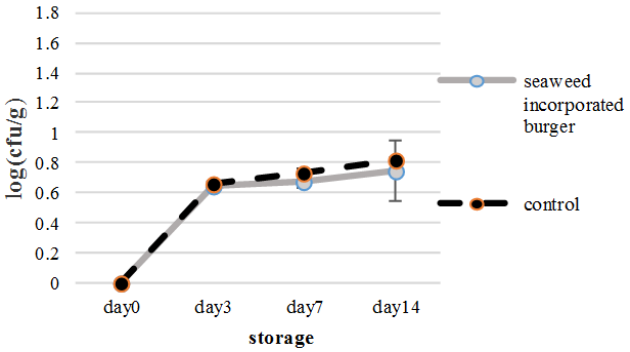

Fig. 1. changes of total plate count with storage period

Further, the seaweed incorporated fish burger was shown negative results for the *salmonella* results. It further sustained the antimicrobial properties which seaweed incorporated fish burger has.

C. Sensory Results of Seaweed Incorporated Fish Burger

Color, taste, texture, flavor, overall acceptability and appearance had significance difference ($p<0.05$) in 0.5% (w/w) incorporated seaweed samples (Fig 02). While increasing the amount of seaweed powder, level of preferences decreased. Considering sensory data analysis 0.5% (w/w) seaweed powder incorporation level selected as the best incorporation level of seaweed for seaweed incorporated fish burger. In most of the studies found in the references, Dietary fiber was added to seafood products for technological purposes and therefore the amount added was small [18]. According to studies, nutrition value of 100g of edible seaweed (*Ulva lacuta*) contains 3.8g of total dietary fiber [6]. Seaweeds are also a source of other bioactive compounds, such as phytochemicals, sterols, tocopherols and phycocyanins, that are recognized to possess health benefits for humans, such as antitumoural, anticholesterolaemic, antiviral and antioxidant activities [18]. Therefore, sea weeds incorporated fish burger may serve as a healthy product which gives more nutritional benefits to the consumers and its intended users beyond the traditional nutritional profile of a burger.

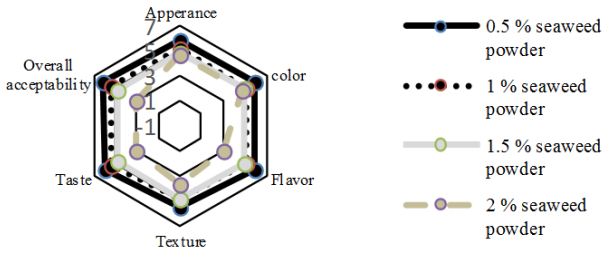

Fig. 2. Sensory analysis for best seaweed powder (w/w) for fish burger
0.5% seaweed powder incorporation level has shown significant difference.

D. Color Analysis of Seaweed Incorporated Fish Burger

Color of seaweed incorporated fish burger and control one was determined and burgers treated with seaweed (*Ulva lactuca*) showed significant difference for a* value compared with control burger (Tab 02). The typical red brownish color of cooked burger is mainly determined by the presence of denatured globin hemochromes formed as a result of high temperature and other meat components of the burger [19].

Table 02. Color of Seaweed incorporated fish burger and control sample

Type	L*	a*	b*
Control	$51.84^{a}\pm0.41$	$8.39^{a}\pm0.06$	$22.65^{a}\pm0.26$
Seaweed incorporated fish burger	$51.94^{a}\pm0.21$	$6.01^{b}\pm0.05$	$22.52^{a}\pm0.18$

All values are given in their means with standard deviations. ^{ab} *Means that do not share a letter are significantly different.*

Redness value may reduce due to adding of seaweed to the burger. According to [20] pigments present in seaweed can influence meat product depending on the seaweed type and concentration added. Therefore, seaweed (*Ulva lactuca*) will alter the burger redness value in terms of reduction of a* value significantly. This reduction could minimize with lower concentration of sea weeds added with fish. However sea weed incorporated fish burger has recorded highest acceptability in sensory analysis. It further recommended the lower redness did not impact negatively on the consumer preference whereas yellowness and lightness have not shown significant alteration by incorporation of seaweed (*Ulva lactuca*).

IV. Conclusions

Sensory evaluation strongly recommended 0.5% (w/w) seaweed (*Ulva lactuca*) as the best level and comparatively higher amount of protein (16.25%) and fiber contain in seaweed incorporated fish burger appeared

to be having more health value and nutritional quality. Moreover microbiological tests results disclosed the antimicrobial properties of seaweed (*Ulva lactuca*) and it is microbiologically safe for 14 days of storage (-18 °C). It further recorded satisfied values for color which is appreciated by the consumers during purchasing.

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AUTHORS' PROFILES

Dr. E.D.N.S. Abeyrathne, corresponding author and the supervisor of this study is an eminent academia and a researcher who has completed Bachelor of Science in Agriculture in University of Peradeniya, Master in Science in Dairy and Meat Product Technology in Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka and subsequently his PhD in Biomodulation in Seoul National University, Seoul, South Korea (2010-2013). His major field of study includes isolation and separation of bio active compounds from poultry eggs and fresh water fish.

Currently he has been serving as a Senior Lecturer attached to the Department of Animal Science, Uva Wellassa University of Sri Lanka, Badulla since 2007. Further he has contributed his valuable service as a Research Assistant, Protein chemistry Lab, Seoul National University, Seoul, Korea (09-2010to08-2013) and Research Assistant, Poultry Meat Lab, Iowa state University, Ames, IA, USA(11-2012 to 02-2013).

Meantime he has published many scientific papers in various reputed journals and conferences both locally and internationally. Few important publications are as follows. Abeyrathne E.D.N.S., Lee, H.Y., Jo, C., Suh, J.W. and Ahn, D.U.. Enzymatic hydrolysis of ovomucin and the functional and structural characteristics of peptides in the hydrolysates. *Food Chemistry*. 2016, 192: 107-113.

Ishani A.H.M.E. Herath, Jayasinghe J.M. Priyanath, Dong U Ahn, E.D. Nalaka S. Abeyrathne.. Use of lysozyme from chicken egg white as a nitrite replacer in an Italian-type chicken sausage. *Functional Food in Health and Diseases*. 2015, 5(9): 319-329.

Abeyrathne E.D.N.S., Lee, H.Y. Jo, C., Suh, J. W. and Ahn, D.U.. Enzymatic hydrolysis of ovomucoid and the functional properties of its hydrolysates. 2015,94 (9) :2880-2287. His current research interests basically focus on development of functional food to control type II diabetics and obesity, development of value added products from poultry eggs and fresh water fish, identifying natural antioxidants from livestock products, identifying hazard points in food processing and minimizing the hazards with cost effective methods.

Moreover, Dr. Abeyrathne is a life time member in Sri Lanka Association of Animal Production (SLAAP) and Sri Lanka Institute of Food Science and Technology (IFST-SL). Further he has been awarded with several titles including Young Scientist research award in WCU Biomodulation, 2013, Third place awarded in the poster retreat of WCU Biomodulation Major 2011 under PhD category, 4th International Biomodulation Symposium, Seoul national University, Second place awarded in the poster retreat of WCU Biomodulation Major 2012 under PhD category in WCU Biomodulation Major, Department of Agricultural. Additionally, Dr. Abeyrathne's outstanding findings in the doctoral studies have been rewarded with patents and he has bagged two patents in Separation of ovotransferrin from chicken egg white using environmental friendly techniques (Patent number :10-2012-0143230) and sequential separation of lysozyme and ovalbumin from chicken egg white (Patent number : 10-2013-0003215).

Mr. J.M.P. Jayasinghe who contributed in preparation, writing, formatting of this paper has completed Bachelor of Animal Science (BASc) (2009-2013) in Department of Animal Science, Uva Wellassa University of Sri Lanka, Badulla and subsequently completed Diploma in Quality Management (2013-2014) at Sri Lanka standards Institution (SLSI), Colombo 08. His major area of study is animal science with special emphasis on animal products and nutrition.

He initiated his career as a research student at Global Seafoods (pvt) ltd, badalgama, Sri Lanka and later he has contributed his valuable service as a nutrition consultant at Fonterra Brands Lanka (Pvt) Ltd, Biyagama, Sri Lanka and further served as an assistant lecturer attached to the same department where he graduated (2014-2016). Meantime he published several research publications in reputed journals in both local and overseas.

Few recently published scientific findings are as follows.

Ishani A.H.M.E. Herath, Jayasinghe J.M. Priyanath, Dong U Ahn, E.D. Nalaka S. Abeyrathne.. Use of lysozyme from chicken egg white as a nitrite replacer in an Italian-type chicken sausage. *Functional Food in Health and Diseases*. 2015, 5(9): 319-329.

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Sandalanka, H.G.A.D.K., Jayasinghe. J.M.P. and Abeyrathne E.D.N.S. (2016). Development of a simple food safety model for sustainable food security of university cafeteria. *Journal of Agricultural Engineering and Food Technology*, Krishi Sanskriti Publications. New Delhi. Vol 3 (1): 1-3. His current research studies mainly concentrate on animal originated products development and quality evaluation discipline.
Additionally Mr. Jayasinghe is a member of Alumni association of quality management, Sri Lanka Standards Institution (SLSI), Sri Lanka and the Animal Science Society, Uva Wellassa University of Sri Lanka.

Ms. N. C. Kumarathunge who contributed in investigating, sample preparation for the study and writing the paper as a part of her undergraduate research study completed her (BSc) in Aquatic Resource Technology (2011-2015) in Department of Animal Science, Uva Wellassa University of Sri Lanka.
Further, Ms. Kumaarathunga has shown strong interest on researching the facts on aquatic food products development and commercialization. Further she is a member of aquatic club, Uva Wellassa University of Sri Lanka.