Development of a Fish Paste with *Oreochromis mossambicus* and *Coccina grandis* as a Natural Blood Glucose Reduction Food

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***Abstract*—** Tilapia (*Oreochromis mossambicus*) and carp (*Catla catla*) fishes are one of the excellent sources of protein, fat, polyunsaturated fatty acids, vitamins and minerals. Fishes are useful for obtaining many health benefits. Consumption of tilapia and carp is low due to low quality color, odour and flavor of these fishes. According to resent studies, kovakka leaves possess hypoglycaemic properties which are useful for managing the type-2 diabetes mellitus. Present study is to develop a value added product from these fishes by incorporating kovakka leaves which can be used for diabetes patients. Paste was prepared after boiling the fish until internal temperature become 70 °C for 15 minutes and mixing with minced fish with relevant amount of spices. After preparation of the paste it was pasteurized at 85 °C for 15 minutes in a water bath. Preliminary investigations were conducted to determine the suitable levels of spices with different levels of ginger and garlic and 1.5% (w/w) ginger and 1.5% (w/w) garlic containing treatment was selected as the best ($P<0.05$). Tilapia fish was selected as the best fish type for the preparation of the fish paste comparing with carp by using sensory evaluation ($P<0.05$). 1% (w/w) of kovakka leaves was the best amount according to the sensory evaluation ($P<0.05$). The proximate analysis revealed that fish paste contains higher amount of protein showing that it is a better source of protein and also it contains 71.77 ± 1.32% of moisture, 2.60 ± 0.20% of fat and 0.93 ± 0.25% of fiber as the other nutrients. Shelf life studies were carried out by using microbiological tests, pH test and lipid oxidation analysis. The anaerobic micro- organisms like *Escherichia coli*, *Salmonella* and total Coliform did not present in the sample cultured in day 0 but aerobic micro-organisms could be observed in samples from day 0 to day 10. pH test results and the lipid oxidation test results have revealed that the fish paste can be kept without spoilage for 30 days under 4 °C. Therefore it can be determined that the kovakka leaves incorporated fish paste can be used as a ready to eat fish product for the fulfillment of nutritional requirements of the consumers and also it can be used by the diabetic patients because of the paste contains kovakka leaves for maintaining their health.

***Keywords*—** Fish paste, Kovakka leaves, Sensory evaluations, Tilapia,

I. Introduction

IT is well known factor that 8.3% of people are suffering from the type-2 diabetes mellitus and its different complications around the world and also it is estimated that this percentage will increased up to 10.2% by 2030 (Katulanda, 2009). According to the studies of Diabetes Association in Sri Lanka, it is also spreading widely throughout the country and 10.3% of people are suffering from the type-2 diabetes mellitus (Mahesh *et al*., 2009) and the changes of insulin disturb the carbohydrate, fat and protein metabolism and also increase the oxidative stress that plays a major role in the development and progression of diabetes mellitus (Oboh, 2013). It is one of the major requirements for maintenance of healthy diet to control the blood glucose level of the type-2 diabetes mellitus (Srinivasan, 2014) and different food products have been developed especially for the consumption of type-2 diabetes patients (Vann, 2014).

Normally, fish paste is prepared from raw fish, in the countries like China, Japan, Philippines, Thailand and Malaysia for making fish balls that one of the staple foods in these countries (Terri, 2008). Developing a fish paste from fresh water fish is quite useful to increase the consumption of the fresh water fish, since many people do not like to eat these fishes due to their unpleasant color, odour and taste (Josupeit, 2012). Tilapia (*Oreochromis mossambicus*) and carp (*Catla catla*) are readily available fresh water fishes in most of the inland water bodies in Sri Lanka (Silva and Kurukulasuriya, 2010) and these fish are one of the most important sources of protein with lean meat which are healthful to the diabetic patients (Bauer, 2014). 15-20% of digestible protein, 1-10% of fat, 0.02-1% of polyunsaturated fatty acids and 1-2% of vitamins and minerals can be obtained by consuming these fishes (FAO, 2014).

Plants possess excellent bioactive molecules that have various medicinal properties and many people in the developing countries depend on these plants to maintain their diseases through folklore medicine (Tamilselvan *et al*, 2011). Among these plants, different herbs containing hypoglycemic properties that can be used for the management and control of the type-2 diabetes and recent studies on kovakka leaves (*Coccinia grandis*) found to have blood glucose lowering effects on human (Munasinghe *et al*, 2011). These leaves possess hypoglycaemic, hypolipidemic, anti-inflammatory, anti-microbial and anti-oxidant properties and inhibit the enzyme glucose-6-pospatase, one of the key liver enzymes involved in sugar metabolism and also it has the ability to inhibit α-amylase and α-glucosidase enzymes to regulate blood glucose level of type-2 diabetic patients (Tamilselvan *et al*, 2011). Therefore, it is recommended that consume less than

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4% of leaves per day because due to its anti-nutritive effects (Attanayake *et al*, 2013).

There is poor use of fishes for the development of food products which can be consumed by the diabetic patients to maintain their blood glucose levels than many other agricultural out puts. Therefore it is quite beneficial to develop a fish paste with kovakka leaves (*Coccinia grandis*) to fulfill that requirement and undesirable organoleptic characteristics of the fresh water fish can be eliminated by improving the culinary properties of the paste through incorporating the spices. Through this improvement the consumption of the fresh water fish can also be increased. The anti-diabetic properties which possess by kovakka leaves, black pepper, ginger, cardamom, clove, turmeric and garlic are also useful for controlling the action of insulin and physiological effects of the diabetic patients. Since the objective of this study is to develop a value added product with fresh water fish and kovakka leaves that is suitable for diabetic patients to maintain their blood glucose levels.

II. Materials and Methods

A. Materials

Fresh tilapia and carp fishes were purchased from the local fish mart. The dried and powdered spices were purchased from the local market. Fresh kovakka leaves were collected from the local areas. Chemicals used for analysis were purchased from Sigma-Aldrich Co. Eosin Methylene Blue Agar powder, Xylose Lysine Desoxycholate powder were bought from Oxid Ltd. Violet Red Bile Agar powder, Baird Parker Agar powder and Peptone Water was bought from Himedia Laboratories Pvt. Ltd. Ethyl alcohol was purchased from Hayman Ltd.

B. Methodology

1. Recipe Development with Different Levels of Spices

Tilapia fish was used for the development of recipe and the suitable amounts of spices were determined by preliminary trials. For that, the paste was prepared by boiling the cleaned fish steaks without water until the internal temperature become 70 °C for 15 minutes (Witt, 2014). After boiling, all the bones were removed and mixed with spices and other ingredients. All ingredients were mixed well and the mixture was ground by using a grinder (Jaipan family mate, IS 4250, India) until it get the texture of a paste. Just after the preparation of the paste, pasteurization was done at 85 °C for 15 minutes in a water bath (Witt, 2014). According to the results of those trials, necessary changes were done to the selected recipe and by changing the amounts of ginger and garlic in the selected recipe (TABLE I) and necessary changes were done according to the results and then a sensory evaluation was done to 30 members of untrained panel by using acceptance test with five point hedonic scale. Results were analyzed with MINITAB 16statistical software.

TABLE I
DIFFERENT AMOUNTS OF GINGER AND GARLIC IN THE RECIPE

Spice	T_1	T_2	T_3	T_4	T_5
Ginger	0%	2.3%	1.5%	0.7%	3%
Garlic	3%	0.7%	1.5%	2.3%	0%

Different treatments which used in the sensory evaluation are mentioned in the table

2. Selecting the Best Fish from Tilapia and Carp

According to finally developed recipe, the paste was developed by changing the fish type including tilapia and carp. Sensory evaluation was done by using 30 members of untrained panel to compare the two different samples.

3. Determination of Drying Conditions of Kovakka Leaves

Solar drier was used to wilt the kovakka leaves. Before wilting, the weight of the cleaned kovakka leaves was measured by using top loading balance (MXX-5001, Electronic Precision Balance, Denver Instruments) and those leaves were wilted for a week at average temperature of 27 °C. The wilting period was 6 hours per day. The weight of the leaves was measured each day until it became constant. The properly wilted leaves were ground by using a grinder (Jaipan family mate, IS 4250, India) until those leaves become powder. Then it was sieved to separate the fine particles.

4. Determination of Suitable Level of Kovakka Leaves

4% of powdered kovakka leaves can be used for a product according to the Ayurvedhic studies (Attanayake *et al* 2013). Therefore to determine the suitable amount of kovakka leaves for the fish paste, the amount of onion in the developed recipe was replaced with kovakka leaves (TABLE II). Preliminary trials were done with 15 members to narrow down the optimum range and then carried out a sensory evaluation with 30 untrained panelists to finalize the best level of leaves to be incorporated. Data were analyzed with MINITAB 16 statistical software.

TABLE II
DETERMINATION OF SUITABLE LEVEL OF KOVAKKA LEAVES IN FISH PASTE

Ingredient	*Treatment 1*	*Treatment 2*	*Treatment 3*	*Treatment 4*
Kovakka	0.5%	1.0%	1.5%	2.0%

5. Proximate Analysis for the Developed Fish Paste

Proximate analysis were carried out for the determination of crud protein, crude fat, crude fiber, moisture and ash content by following standard AOAC (2000) methods.

6. Determination of Keeping Quality of the Fish Paste

Microbiological Tests:

The microbiological tests were done to determine the presence of Aerobic microorganisms, total Coliform, *Escherichia coli* and *Salmonella* in the fish paste by following standard AOAC methods (2000) with some modifications. Specific culture media were used to detect them as Violet Red

Bile Agar (41.53 g L^{-1}), (Himedia Laboratories Pvt. Ltd, India) for total Coliform, Eosin Methylene Blue Agar (37.5 g L^{-1}), (Oxoid Ltd., England) for *Escherichia coli*, Xylose Lysine Deoxycholate medium (53 g L^{-1}), (Oxoid Ltd., UK) for *Salmonella* and Total Plate Count Agar (17.5 g L^{-1}), (Himedia Laboratories Pvt. Ltd, India) for total aerobic micro-organisms.

Chemical Analysis:

Testing pH of the Product:

The pH test was carried out by using the procedure followed by Dhanapal *et al* 2012 for fish samples with some modifications. pH was measured in three days interval for 36 days at room temperature.

Lipid Oxidation Test:

Oxidation of the lipid present in the fish paste was tasted by using 2-thiobarbituric acid assay according to the Schmedes and Holmer method with some modifications. Three samples were prepared from the fish paste and were pasteurized at 85 °C for 15 minutes in a water bath. One gram from each sample was dissolved in 8 ml of distilled water and it was homogenized properly by using vortex machine (Jeiotec: VM 96B, Korea) for 30 s in 3000 rpm. Then 0.5 ml of 0.2% L-Ascorbic acid (99%) (Sigma-Aldrich, USA) and 0.5 ml of 200 ppm Fe^{2+} solution were mixed with each sample. The prepared samples were incubated at 37 °C for 16 hours in an incubator (YCO-010, Gemmy 888). Then 1 ml from each incubated sample was transferred to 15 ml falcon tubes. 2 ml of 2-thiobarbaturic acid (98%), (Sigma-Aldrich, Germany) and trichloro acetic acid solution (99%), (Sigma-Aldrich, USA) and 50 µl of 10% butylated hydroxyanisole (98%), (Lab Fine Chemical Industries, India) in 90% ethanol (99.5%), (Hayman, England) were added to those samples. Then those samples were mixed by using vortex machine (Jeiotec: VM 96B, Korea) for 30 s in 3000 rpm. Those were incubated in a water bath (Daihan Lab Tech, LWB-111D, Korea) at 90 °C for 15 minutes and then samples were cooled in ice water bath for 10 minutes. Those samples were centrifuged 3000 rpm for 15 minutes by using a centrifuge machine (Gemmy Industrial Corporation, Model PLC-0120, Taiwan). Absorbance was measured at 532 nm against a blank prepared with 1 ml of distilled water and 2 ml of 2-Thiobarbaturic acid (98%), (Sigma-Aldrich, Germany) and trichloroacetic acid (99%), (Sigma-Aldrich, USA) solution by using UV visible spectrophotometer (Thermo Electron Corporation, Geaesys 6, UV-1200). Thiobarbaturic acid value was expressed as milligrams of malondialdehyde per kilogram of fish paste. Lipid oxidation test was continued in 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 days by using 1 g portions from the same 3 samples.

7. Statistical Analysis

All the proximate analysis, microbiological analysis, pH and lipid oxidation analysis were conducted by using triplicates (n=3). The means and standard deviations of these tests except microbiological analysis were analyzed by using Basic statistics and One-way Analysis of Variance in Minitab 16 statistical software (LEAD Technologies Ink, 1991-2014). All sensory evaluations were analyzed by using Friedman's test in Minitab 15statistical software (LEAD Technologies Ink, 1991-2014) and all the significant differences were determined at $P<0.05$.

III. Results and Discussion

A. Recipe Development with Different Levels of Spices

1. Results of the Sensory Evaluation with Different Levels of Spices

The sensory evaluation test results have obtained significant differences for flavor, mouth feel and overall acceptability ($P<0.05$) except color, aroma, texture and spreading ability of the 1.5% (w/w) ginger and 1.5% (w/w) garlic containing treatment (data not showen). Therefore that treatment was determined as the best among other treatments. Ginger and garlic are widely using spices for the preparations of different dishes because of these spices possess excellent culinary properties (Auta, 2011). According to different studies ginger and garlic have the hypoglycaemic properties which are useful for the management of type-2 diabetes mellitus (Jafri *et al*, 2011). Zingiberene and allicin are the active chemical agents of ginger and garlic and the antioxidant and antimicrobial properties of these compounds can retard the rate of food deterioration. Ginger and garlic also possess anti-inflammatory, anti-tumor, increased appetite, anti-cancerous, anti-mutagenic, anti-vomiting, anti-edemas, anti-platelet, calming and hypolipidaemic calming like medicinal properties which are useful to manage human health (Tilakarathne, 2013).

B. Determination of the Best Fish Type from Tilapia and Carp for Fish Paste

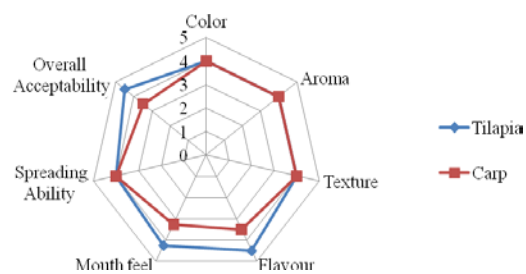

Fig. 1 Results of the sensory evaluation for the determination of suitable fish type from tilapia and carp for making fish paste

According to fig. 1, fish paste produced with tilapia have obtained significant differences for flavor, mouth feel and overall acceptability ($P<0.05$) while color, aroma, texture and spreading ability had no any significant difference ($P>0.05$). Therefore the treatment made from tilapia was determined as the best. Tilapia (*Oreochromis mossambicus*) possesses slightly pinkish color flesh with fine texture and also they have slightly fatty flavor (FAO, 2014). Carp (*Catla catla*) also contains white color flesh with fine texture. Normally, the taste of carp flesh is slightly bitter or off-taste and the taste of carps depend more on the species. Other reasons for this undesirable taste of the carps growing in the wild may be due to the poor water quality and eating blue-green algae (Dey *et al*., 2010). Therefore it can be determined that tilapia flesh is useful for

increasing the flavor of the fish paste comparing with carp flesh. So selection of the treatment made with tilapia may be due to the desirable flavor and other desirable organoleptic properties of tilapia than carp.

C. Determination of Suitable Amount of Kovakka Leaves

1. Results of Sensory Evaluation with Different Levels of Kovakka Leaves

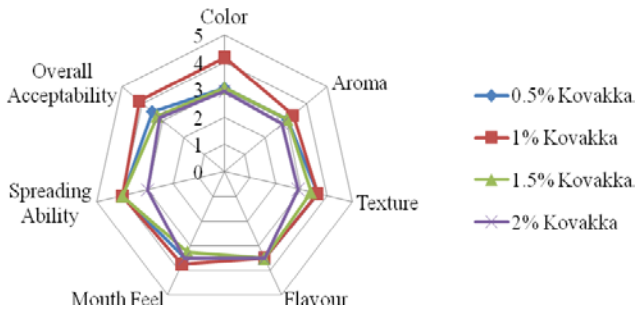

Fig. 2 Results of the sensory evaluation for the determination of suitable amount of kovakka leaves for the fish paste

1% (w/w) kovakka leaves containing treatment has obtained significant difference for texture, mouth feel, color, spreading ability and overall acceptability ($P<0.05$) (Fig. 2). Therefore 1% (w/w) kovakka leaves incorporated treatment was determined as the best. Kovakka leaves play a vital role in hypoglycaemic effects on the type-2 diabetic patients and these leaves possess compounds that can inhibit the enzymes involving sugar metabolism (Tamilselvan *et al*, 2011). In the indigenous system of medicine, 4% of maximum kovakka leaves are recommended for the consumption of diabetic patients per day because that amount is toxicologically safe (Attanayake *et al* 2013). Kovakka leaves also contain phenols, acidic compounds, alkaloids, sterols and tannins (Devid *et al*., 2011). Antioxidant and antimicrobial properties of these compounds are useful for retarding the spoilage of fish paste.

D. Proximate Analysis

TABLE V
PROXIMATE COMPOSITION OF THE DEVELOPED FISH PASTE

Nutrient Compound	*Amount (%)*
Crude Protein	16.90 ± 0.30
Crude Fat	2.60 ± 0.20
Crude Fiber	0.93 ± 0.25
Ash	4.70 ± 0.36
Moisture	71.77 ± 1.32

The mean values ± standard deviations are mentioned in the table for each parameter.

Fishes are a major source of high quality protein, and fish occupies an important place in human nutrition (Nargis, 2006; Obemeata and Christoper, 2012). Fish paste contains tilapia fish flesh as the major ingredient and it has greater contribution to the nutritional composition. Typically fish meat contains 19% of protein similar in amino acid composition and this value varies from 1%-20% depending on the fish species (Ndome *et al*. 2010; Obemeata and Christoper, 2012). According to different studies, it is found that tilapia possesses 17.8% protein content (Wimalasena and Jayasuriya, 2009) and also the fish paste contains higher crude protein content (TABLE V). Therefore the fish paste can be considered as a better source of protein with healthier white meat. Normally tilapia is considered as semi-fatty fish which contains 1%-10% of fat. Some studies revealed that fat content of tilapia is around 2.3% (Wimalasena and Jayasuriya, 2009) and this level is healthier for human consumption and our results are similer to their findings.

E. Determination of Keeping Quality of the Fish Paste

1. Microbiological Tests

When preparing ready to eat food products like fish paste, many cooked and un-cooked ingredients are used (Maturin, 2010). Therefore it is important to check the presence of microorganisms which create hygienic, toxic or health problems. According to the results all three replicates of fish paste were within the acceptable level in all microbiological tests carried out.

2. Chemical Analysis pH of the Product:

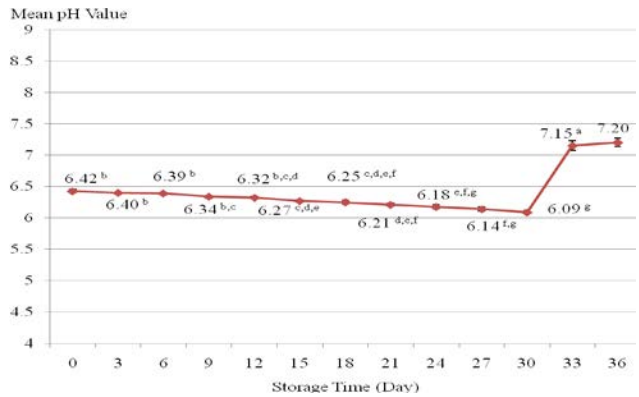

Fig.3 Graphical expression of pH of fish paste with increasing storage time

The mean values with standard deviations are mentioned in the graph. The values with different superscript simple letters are significantly different at $P<0.05$.

pH test is one of the commonly using method for the determination of the deterioration conditions of the muscles of the fishes (Howgate, 2009; Susanto *et al.*, 2011) and typically the post mortem pH value of the tilapia fish is around 6.0 which means slightly acidic (Huss 1998; Susanto *et al.*, 2011). pH test results for the fish paste has obtained 6.42 pH value for day 0 and it has been sequentially reduced with extending storage time (Fig. 3). Fish paste contains fish flesh as the major ingredient and the initial slightly acidic pH value of the fish paste may be due to the tilapia fish flesh possess white muscles with low amount of glycogen than red muscle (Huss, 1995; Tejada, 2009; Susanto *et al.*, 2011). One of the reasons for the pH reduction of the fish paste with increasing storage time at 4 °C may be due to the accumulation of lactic acid from the decomposition of glycogen in fish muscles after the rigor mortis condition (Ikeda, 1979; Susanto *et al.*, 2011) and these lowering pH conditions promote the bacterial spoilage and lipid oxidation of the product (Ozogul, 2010). Normally, tilapia fish muscles deteriorate within 9 days under freezing conditions and the pH values become around 7.5 (Susanto *et al.*, 2011) and the slow rate of pH reduction of the fish paste may be due to the antioxidant properties of the

major active chemical compounds of the spices and kovakka leaves. Black pepper, ginger, cardamom, clove, turmeric and garlic contain pipperene, zingiberene, cineol, eugenol, curcumin and allicin as the active chemical compounds and also these spices possess many other bio-reactive compounds which are useful for retarding spoilage of food (Shanmugavelu *et al*., 2005). Typically, the reducing pH values promote the lipid oxidation values of the food (Ozogul, 2010). pH of the fish muscles can be increased due to the accumulation of alkaline compounds such as ammonia, hypoxanthine, dimethylamine, esters and trimethylamine with increasing storage time (Özyurt *et al.*, 2009; Susanto *et al.*, 2011) and these compounds give bad color and odour to fish flesh. The increasing pH values at higher rate comparing with initial pH values show that fish flesh has spoiled and it is not suitable for the consumption (Susanto *et al.*, 2011). Therefore according to the pH test results (Fig.3), it can be determined that fish paste can be kept 30 days at 4 °C without spoilage.

Lipid Oxidation:

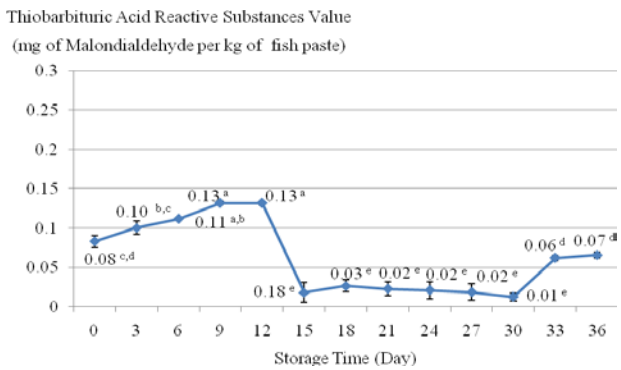

Fig.4 Graphical expression of thiobarbituric acid reactive substances of the fish paste with increasing storage time

Mean values with standard deviations are mentioned in the graph. The values with different superscript simple letters are significantly different at P<0.05.

Thiobarbituric acid reactive substances have sequentially increased from day 0 to day 12 and these values have decreased with a sharp drop from day 12 to day 15. Then the values have decreased sequentially up to the day 30 and these values have increased from day 33 to day 36 as the lipid oxidation test results (Fig. 4). Fish lipid contains large amount of polyunsaturated fatty acid moieties which make the fish products more susceptible to lipid oxidation, through autocatalytic mechanism. During the auto-oxidation reaction hydroperoxides are broken down in to secondary products of shorter carbon chain-length like aldehydes, ketones, alcohols, small carboxylic acids and alkanes which give rancid odour and yellowish discoloration to the product (FAO, 2010). These compounds are considered as thiobarbituric acid reactive substances and typically these values have been increased with increasing storage time indicating the deterioration of the product (Barriuso Astiasaran and Ansorena, 2011). Through the thiobarbituric acid assay the absorbance of the malondialdehyde-thiobarbituric chromophore is measured by using the ultra-violet spectrophotometric method and interference of the thiobarbituric acid reactive substances to the thiobarbituric acid assay increases lipid oxidation and creates different changes in the reaction due to thiobarbituric acid is not selective to the malondialdehyde (Susanto *et al*., 2011). Malondialdehyde also can form linear or cyclical Schiff bases, or even cross-linked bonds, with lysine and arginine from proteins (Barriuso *et al*, 2011). Therefore poor quantification selectivity, poor molecular specificity and selectivity of malondialdehyde can be occur with thiobarbituric acid assay resulting low absorbance value for spectrophotometric test (Barriuso *et al*, 2011). Antioxidant properties of the active chemical compounds of the spices like pipperene:-black pepper, zingiberene:-ginger, cineol:-cardamom, eugenol:-clove, curcumin:-turmeric, allicin:-garlic and compounds in Kovakka leaves may be retard the lipid oxidation. The high temperature values (95 °C - 100 °C), extended incubation times and strong acidic conditions also can cause an artifactual peroxidation of sample constituents even in the presence of added antioxidants like butylated hydroxyanisole in ethanol though these conditions required for the formation of malondialdehyde-thiobarbituric complex (Susanto *et al*., 2011).

Therefore it can be supposed that the reduction of the thiobarbituric acid reactive substances values of the fish paste with increasing storage time due to these reasons. Increasing thiobarbituric acid reactive substances values after the reduction indicate the deterioration of the fish paste with rancid odour and discoloration. Therefore it can be determined that fish paste can be kept 30 days at 4 °C without spoilage according to the lipid oxidation test results (Fig. 7).

IV. Conclusion

The fish paste can be used as a ready to eat fish product which has high protein content and can reduce the blood glucose level. The organoleptic properties of the product are acceptable and it can be stored in refrigerated condition for 30 days. The level of blood glucose reduction will be carried out with mice trial followed with a clinical trial.

Acknowledgment

This research was foundered by uva wellassa university, badulla sri lanka.

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