Natural antioxidants from clove for protecting omega-3 fatty acids in sardines (*Rastrineobola argentea*) during deep frying process.

[*]Davis Chaula[1], Charlotte Jacobsen[2], Henry Laswai[1], Bernard Chove[1], Anders Dalsgaard[3], Robinson Mdegela[4] and Grethe Hyldig[2]

[1] Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P.O.Box 3006, Chuo kikuu, Morogoro, Tanzania

[2]National Food Institute, Technical University of Denmark, Kgs. Lynby, Denmark

[3]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

[4]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, Tanzania

1.0 Abstract

Sardines (*Rastrineobola argentea*), popularly known as "*dagaa"* is one of the leading commercial fish species of Lake Victoria. The fatty fish species are attracting great attention because they are good source of omega-3 polyunsaturated fatty acids which are vital for a wide range of biological functions and are implicated in the prevention of numerous diseases. While nutritionally valued omega-3 fatty acids are highly susceptible to oxidation during fish processing due to their unsaturated nature. Oxidation reactions result in loss of omega-3 fatty acids and production of undesired off-flavours which discourage consumption and limit diversification of sardine products.Synthetic antioxidants may be used to prevent lipid oxidation but have been claimed to be carcinogenic at higher levels. The replacement of synthetic antioxidants with ones of natural origin is now in demand.

In this study, natural antioxidants rich extracts from clove buds were applied on sardines in a bid to impede lipid oxidation during deep frying process. Lipid oxidation was assessed by peroxide value (PV), volatile compounds and fatty acid profiles using Gas chromatograph (GC-MS and GC-FID).The results showed that natural antioxidants from clove buds reduced peroxidation and protected highly unsaturated omega-3 fatty acids from oxidation during deep frying process. Total polyunsaturated fatty acids amounted 7.30 % in pre-treated deep fried sardines.Retention of omega-3 fatty acids was 0.70 % more in pre-treated than untreated fish. Significantly lower amounts of representative volatile compounds were produced in sardines pre-treated with clove extracts. The study demonstrated feasibility to pre-treat sardines with natural antioxidants for protecting omega-3 fatty acids against oxidation during deep frying.

Key words: Omega-3 fatty acids, natural antioxidants, lipid oxidation, *dagaa*, Lake Victoria

*Corresponding author: chaula@sua.ac.tz

2.0 Introduction

Sardines (*Rastrieobola argentea*), popularly *dagaa* in Tanzania, are tiny, fatty freshwater fish species of commercial importance in Lake Victoria. The species provide 72.30 % of the total landings by weight on the Tanzanian side of the Lake (URT, 2015). Their proximate composition varies due to environmental factors including the change of seasons and the resultant change of food supply in the Lake (Kilema-Mukasa, 2012; Abdulkarim *et al*., 2016). Sardines are attracting great attention because they are good source of polyunsaturated fatty acids (PUFAs) including omega-3 which are vital for a wide range of biological functions. Omega-3 fatty acids are implicated in the prevention of numerous diseases such as cardiovascular diseases, inflammation, high blood pressure, atherosclerosis, thrombogenesis, cancer, skin diseases and are necessary for the brain development in fetuses (Finley *et al*., 2001;Sidhu, 2003; Minhane *et al*., 2008; Gladyshev *et al.*, 2012).

Sardines are perceived negatively and considered as an inferior food for poor and pro-poor communities despite its economic and nutritional values. This may be attributed to poor handling and processing technologies along the sardine value chain. Roberts *et al*., (2014) found that *dagaa* is richer in omega-3 fatty acids than *Oreochromis niloticus*, *Tillapia zilliii* and *Lates niloticus* of Lake Victoria. Sun dried and fresh *dagaa* are reported to contain 18.50 to 20.88 % and 13.5 to 21.2 % omega-3 fatty acids respectively (Mwanja *et al.,* 2010; Masa *et al*., 2011; Chaula *et al*., 2019).

Dagaa can be preserved by open sun drying, smoking and deep frying processes. The traditional open sun drying of *dagaa* has significant effect on the composition and hence quality of the dried product. Owaga *et al.* (2010) reported a significant decrease in total fat content (from 14.8 to 13.9 %) of *dagaa* after sun drying. Open sun drying process promotes lipid oxidation and in some instances the production of secondary lipid oxidation products in sun dried sardines

exceeds acceptable levels with regard to development of off-flavour (Chaula *et al*., 2019). Off-flavours emanating from lipid oxidation discourage consumption and limit diversification of sun dried *dagaa* products. Deep frying has emerged as an important sardine value addition process. Deep frying involves immersion of sardines in hot oil, typically at temperatures ranging from 165 to 195 °C. At such high temperatures, frying oils and lipids in fish undergo chemical reactions including oxidation, polymerization and decomposition, resulting in off-flavours, nutritional loss and other deteriorative changes (Naz *et al*., 2004; Secci *et al*., 2016). Lipid fraction of deep fried sardines contains significantly lower amounts (16.56 and 8.46 %) than sun dried (29.29 and 20.88 %) of PUFAs and omega-3 fatty acids respectively indicative of oxidative damage of PUFAs during deep frying process (Chaula *et al*. 2019).

Commercially available synthetic compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ) are known to be strong antioxidants. However, different regulatory authorities such as the United States Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and the World Food and Agricultural organization (FAO) have placed limits on the amount of synthetic antioxidants allowed for use in foods typically to levels at or below 200 ppm, due to their potential toxicity (Ito *et al*., 1986; Zheng and Wang, 2001). Such relatively low concentrations allowed do not provide sufficient protection against oxidative damage of PUFAs under frying conditions. Due to safety concerns and increased consumer interest in natural products, nontoxic natural antioxidants of plant origin could potentially be used at higher concentrations than 200 ppm for better protection of PUFAs during frying process. Therefore, the development of strong antioxidants that suppress oxidation and protect the nutritional quality of highly reactive PUFAs is now in demand. In this study, natural antioxidants rich clove (*Szygium aromaticum)* extracts were applied on sardines in a bid to impede lipid oxidation during deep frying process.

3.0 Materials and methods

3.1 Materials

Fresh whole *dagaa* (25Kg) were collected directly from fishermen at Kijiweni landing site at the shore of Lake Victoria, Tanzania placed in ice in insulated boxes and transported to the National Fish Quality Control Laboratory, Nyegezi, Mwanza for experiment. Dry clove *(Szygium aromaticum*) buds were obtained from a local market in Zanzibar, transported at ambient temperature to Mwanza and kept at 5 to 10°C in a refrigerator.

3.1.1 Preparation of clove water extracts

For water extraction, 5, 10 and 20 g grounded powder(to pass through a 250µm sieve) of clove buds were mixed with 1 L boiling water with continuous stirring to make 5, 10 and 20 g L^{-1} concentrations of extracts. The mixtures were boiled for 15 min and subsequently cooled to 0-5 °C in a refrigerator thereafter gravity filtered to remove the particles present.

3.1.2 Preparation of deep fried *dagaa*

Fresh *dagaa* intended for deep frying were washed with portable water then soaked in clove extracts (1:1 *w/w*) for 40 min and spread on wire mesh to drip dry in open sun for three hours, thereafter deep fried in hot sunflower oil at 135-180 °C for 5 minutes. Fish samples without clove pre-treatment were prepared in similar way and used as control. Each treatment experiment consisted of four replicates. For each treatment experiment 100 g portion of whole fish was made into mince using a mixer (Moulinex Moulinette S type 643 02 210, Hamburg, Germany).The fish mince was then stored at -40°C awaiting analysis.

3.2 Methods

3.2.1 Dry matter content and lipid extraction

The dry matter content for fish samples was determined by weighing after drying a sample of approximately 2 g of homogeneous fish mince at 105 °C for 18 h according to the AOAC (2012) and results expressed as a percentage dry matter.

Lipids were extracted following the Bligh and Dyer method (1959) with modifications according to Iverson *et al*., 2001. The sample (5 g of fish mince) was homogenized in chloroform, methanol, and water mixture (1:1:0.8 v/v) at the speed of 15,000 rpm for 90 sec using an Ultra Turrax homogenizer (T25 Homogenizer, Staufen, German).The homogenate was centrifuged at 2,800 rpm at 18°C for 10 min using a centrifuge (Sigma 4K15, Osterode am Harz, German) to obtain the extract (Chloroform phase).The lipid content was determined by gravimetry after evaporation of chloroform and expressed as percentage of dried fish sample

3.2.2 Primary and secondary lipid oxidation products

Peroxide values (PV) of the lipid extracts were determined according to the method of Shantha and Decker (1994) based on the formation of an iron−thiocyanate complex. The colored complex was measured by spectrophotometer (Shimadzu UV1800, Shimadzu Scientific Instruments, Columbia, MD) at 500 nm. The analysis was done in duplicate, and the results were expressed in millequivalent peroxides/Kg oil (meq O_2/Kg oil).

The secondary oxidation products were determined as volatile compounds from fish mince collected using the dynamic headspace technique. The procedure was carried out using 1 g of fish mince in which 30 mg of internal standard, 4-methyl-1-pentanol were added and mixed with 15 mL of distilled water. The volatiles were collected in Tenax GR tubes at 37 °C by purging with nitrogen for 30 min at 150 mL/min. The tubes were flushed with nitrogen at 50 mL/min for

20 min to remove water. The trapped volatiles were desorbed from the Tenax tubes by heat (200 °C) using an automatic thermal desorber (ATD-400, PerkinElmer, Norwalk, CT), cryofocused on a cold trap (−30 °C), released again at 220 °C, and led to a GC an Agilent 5890IIA model (Palo Alto, CA, USA) equipped with a HP 5972 mass selective detector. Separation was done on a DB1701 column (30 m × ID 0.25 mm × 0.5µm film thickness, (J&W Scientific, Folsom, CA).The carrier gas used was helium at flow rate of 1.3 mL/min. The oven temperature was rising by 2.0 °C/min from initial temperature of 45 °C to 80 °C followed by an increase of 3.0 °C/min to 150 °C and finally increased by 12.0 °C/min to 240 °C. The individual compounds were identified by MS-library searches and addition of the internal standard. Quantification was done through calibration curve made by adding the standard directly on the Tenax tubes as described by Nielsen *et al*. (2007). For the quantification, a stock solution of 19 volatiles was prepared and a calibration curve was conducted in a range from 0 to 1.2 mg/g. The analysis was carried out in triplicate.

3.2.3 Free fatty acids and fatty acid profiles

Free fatty acids (FFAs) content was determined by acidometric titration of the lipid extract using NaOH (0.1 M). The FFAs content was calculated as oleic acid according to the AOCS (1998) and results were reported as % oleic acid.

The fatty acid profiles of deep fried sardines were determined as fatty acid methyl esters (FAMEs) according to the American Oil Chemists' Society (AOCS) official method; Ce 1i-07 (AOCS, 2009). 1g of oil extract was evaporated to dryness under nitrogen. Thereafter, 100µL of internal standard solution (2% w/v C23:0 in heptane), 200 µL of heptanes, 100 µL of toluene and 1 mL of boron trifluoride in methanol (BF3-MeOH) were added. Methylation was done in microwave oven (Microwave 3000 SOLV, Anton Paar) for 10 min at 100°C and 500W and cooled down for 5 min. 1 mL of saturated salt water (NaCl) and 0.7 mL of heptane with BHT

were added. The upper phase of the sample (around 0.7 mL) was transferred into vials. Samples were analyzed by gas chromatography system (HP-5890 A, Agilent Technologies, Santa Clara, CA, USA). FAMEs were separated and detected by the GC column Agilent DB-wax (10 m x100µ m x 0.1µm), from Agilent Technologies (CA, USA). The carrier gas was helium with a flow rate of 0.38 mL/min and an inlet pressure of 51psi. The oven temperature program for separation was from 160 to 200°C, then from 200 to 220°C and from 220 to 240°C at 10.6°C /min. All analyses were done in duplicate. The result of each fatty acid was expressed as g fatty acid/100 g lipid.

3.2.4 Antioxidant activity of clove water extracts

3.2.4.1 Total phenolic content

The total phenolic compounds of the extracts were determined using Folin–Ciocalteu reagent by a procedure described by Farvin and Jacobsen (2013) in which gallic acid was used as a standard. The standard curve was prepared in distilled water at a concentration range of 0–125 µg/mL. The original extracts were diluted with water as necessary to fit within the standard curve. The absorbance was read at 725 nm using UV-vis spectrophotometer and results reported in µg gallic acid equivalent (µg GAE)/mL of clove water extracts. All measurements were performed in duplicate.

3.2.4.2 Free radical scavenging ability

The free radical scavenging activities of clove water extracts were measured by utilizing the stable radical, 1,1-diphenyl-2-picryl-hydrazil (DPPH) as described by Yang *et al*., 2008. The solutions of prepared extracts were diluted with water *(*1:1 *v/v)*.Diluted solutions (100µL) were added to the microplate and mixed with 100µL of 0.1 mM DPPH in ethanol (96%). The mixtures were shaken vigorously and maintained for 30 min at ambient temperature in the dark. The

absorbance of mixtures and the control (100µL DPPH solution + 100µL BHT) was measured at 517 nm against a reagent blank by using a UV–Vis spectrophotometer. The scavenging activity was calculated as inhibition percent by using the following equation:

$$\text{Inhibition } (\%) = \left(1 - \frac{A_s - A_0}{A_b}\right) \times 100$$

Where A_s is the absorbance of DPPH after reaction with antioxidant, A_0 is the absorbance of antioxidant and ethanol (blank) and A_b is the absorbance of water and DPPH (blind).

3.2.4.3 Iron (Fe^{2+}) chelating ability

The ferrous ion chelating activity of clove extracts was measured as described by Farvin *et al*. (2010) with 20 µL of 0.5 mM ferrous chloride and 20µL of 2.5 mM ferrozin being mixed with 100 µL of clove extracts. The mixture was allowed to equilibrate in the darkness at room temperature for 10 min before measuring the absorbance. The decrease in the absorbance at 562 nm of the iron (II)-ferrozin complex was measured. EDTA was used as the positive control and the ability of the extracts to chelate $Fe^{2+}$was calculated using the equation:

$$Fe^{2+}chelatingactivity = \left(\frac{A_{blank} - (A_{sample} - A_{blind})}{A_{blank}}\right) \times 100$$

A_{blank} is the absorbance of blank (only iron chloride and Ferrozin), A_{sample} is the absorbance of sample and A_{blind} is the absorbance of blind (only antioxidant).

4.0 Statistical analysis

Data were analyzed using IBM SPSS (SPSS for Windows Version 20.0, 2013, IBM, Bethesda, MD, USA). Data were reported as mean ± standard deviation. Differences between means were determined using one-way analysis of variance (one-way ANOVA) with Tukey's HSD post hoc test, according to the equal variance of different groups. The correlations among variables were determined using a two tailed Pearson correlation coefficient. A p-value <0.05 was considered statistically significant.

5.0 Results and Discussion

5.1 Antioxidant activity of clove water extracts

The clove water extracts analyzed in this study had total phenolic content levels in the range from 18.18 -28.75 µgGAE/mL (Table 1). As expected the 20 g L^{-1} extracts had significantly higher total phenolic content than that of 5 and 10 g L^{-1}. The total phenolic content did not increase linearly with the amount of dry clove extracted in 1 L of water. This suggests that longer time periods might be needed for efficient extraction of phenolic compounds when larger amounts of clove powder are used. The recovery of phenolic compounds from plant matrices during aqueous extraction is known to depend on factors such as temperature, extraction time and solvent to solid ratio (Çam and Aaby, 2010). The ability of clove extracts to donate hydrogen to the DPPH radical, ranged from 93 to 95 % .This could be due to higher phenolic content in clove extracts. There was no linear relationship between total phenolic content and DPPH suggesting presence of compounds other than phenolics (e.g flavonoids) that contributed to the antioxidant activity of clove extract.

Table 1: Antioxidant capacity of clove water extracts

Extracts (g/L)	Total phenolic content (µgGAE/mL)	DPPH scavenging (% inhibition)	Fe^{2+} chelating activity (%)
CL 5	$18.18^{a} \pm 1.29$	$93.33^{g} \pm 0.21$	$14.74^{p} \pm 0.21$
CL 10	$25.94^{b} \pm 2.62$	$95.59^{h} \pm 1.44$	$20.87^{q} \pm 0.43$
CL 20	$28.75^{c} \pm 1.35$	$94.34^{i} \pm 0.38$	$22.24^{r} \pm 0.32$

CL: Clove, GAE: Gallic acid,5,10 and 20:Grams of clove extracted in 1 L water. Means marked with different letters in a column are statistically significant.

The DPPH decreased from 95.59 to 94.34 % when the amounts of clove extracted in one litre of hot water was increased from 10 to 20 g .This could be due to decrease in extraction efficiency of phenolics in boiling water at concentration above 10 g/L (Slavin *et al*., 2016). Clove water extract has been found to contain substantial amounts of phenolic compounds and powerful antioxidant activity in linoleic acid emulsion with its iron chelating capacity being dependant on concentration and type of solvent used (Gülçin *et al.*, 2004). Essential oils of clove have been tested in omega-6 and omega-3 fatty acids enriched food supplements and found to have high radical scavenging activity, iron-chelating properties and higher hydrogen donating power than the standard antioxidants BHT and α-tocopherol (Bag & Chattopadhyay, 2017).

5.2 Fat, free fatty acids and dry matter content

The dry matter content of clove was 86.40 % and there was no significant difference in mean dry matter content of treated and untreated sardines (Table 2). Fat content in the samples ranged from 39.42 to 41.69 %. Such high fat content in deep fried sardines is because during the process oils tend to replace water in the product and thus, there is a correlation between initial water

content and oil uptake (Dana and Saguy, 2006). Free fatty acids in all samples were less than 1% suggesting limited lipolysis because Lipolytic enzymes might have been inactivated at high temperatures during deep frying process.

Table 2: Fat, free fatty acids and dry matter content in deep fried (DCL) sardines pre-treated with clove water extracts

Sample	Fat content (%)	Free fatty acids (%)	Dry matter (%)
DCL 0	$39.99^{e} \pm 0.36$	$0.48^{f} \pm 0.09$	$92.33^{h} \pm 1.13$
DCL 5	$41.69^{e} \pm 0.89$	$0.87^{g} \pm 0.06$	$89.78^{h} \pm 4.90$
DCL 10	$39.42^{e} \pm 0.04$	$0.15^{i} \pm 0.01$	$90.93^{h} \pm 0.10$
DCL 20	$39.95^{e} \pm 0.15$	$0.18^{i} \pm 0.02$	$90.68^{h} \pm 1.60$

5, 10 and 20: Grams of clove extracted in 1 L water. Means marked with different letters in a column are statistically significant

5.3 Primary and secondary lipid oxidation products

The peroxide value (PV) and the volatiles analyses were used to determine the primary and secondary lipid oxidation products in pre-treated fish and the control sardine samples. From Figure 1, it can be seen that peroxidation was more pronounced untreated than pre-treated deep fried sardines. The control samples had significantly higher peroxide values and concentrations of most of representative volatile compounds than the clove pre-treated samples (Figure 1&2). The peroxide values and the concentrations of volatile secondary oxidation products among clove treated samples decreased as the amount of clove extracted in 1 L of water increased indicating the effect of extract concentration on lipid oxidation. Soaking sardines in 5, 10 and 20

g L^{-1} clove water extracts for 40 min prior to deep frying resulted in respectively 21.20, 10.70 and 11.20 % reduction of peroxide values in products relative to the control samples.

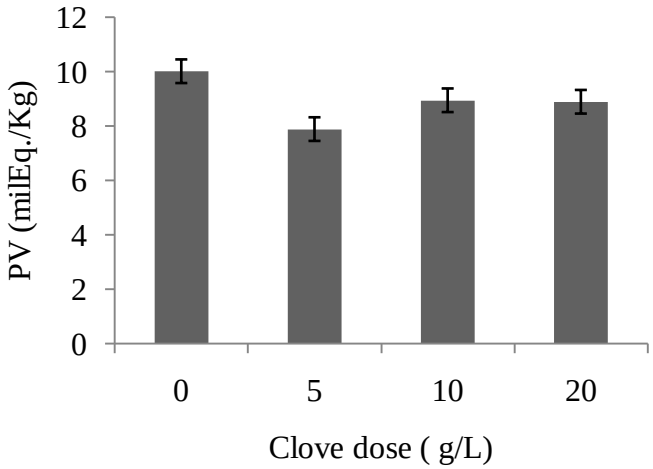

Figure 1: Peroxide value (PV) in deep fried sardines pre treated with different doses of clove extracts.

The pre-treatments resulted into remarkable decrease in concentrations of individual volatile compounds, including 4-heptanal and t, t-2, 4-heptadienal (Figure 2) which are recognized as decomposition products of EPA and DHA (Venkateshwarlu *et al*., 2004).These observations indicate that lipid oxidation reactions were more pronounced in untreated than in clove treated sardines. The peroxide value reduction and lower concentrations of volatile compounds in clove treated samples suggest that phenolic compounds in the extracts played an anti-oxidative role during processing. The anti-oxidative effect of phenolic compounds can be through different mechanisms such as scavenging of free radicals, singlet oxygen quenching, oxygen scavenging, metal chelation and inhibition of oxidizing enzymes (Shobana and Akhilender, 2000; Dudonné *et al*., 2009). The use of whole spices and herbs or their extracts with strong antioxidant activity (Gachkar *et al*. 2007) can control lipid oxidation in muscle food such as mullet fish, frozen chub mackerel and smoked rainbow trout (Emir Çoban *et al*. 2014). Clove essential oils have been

applied in smoked and vacuum packed rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage (at 2° C) resulting in reduction of peroxide values (Emir Çoban and Patir, 2013).

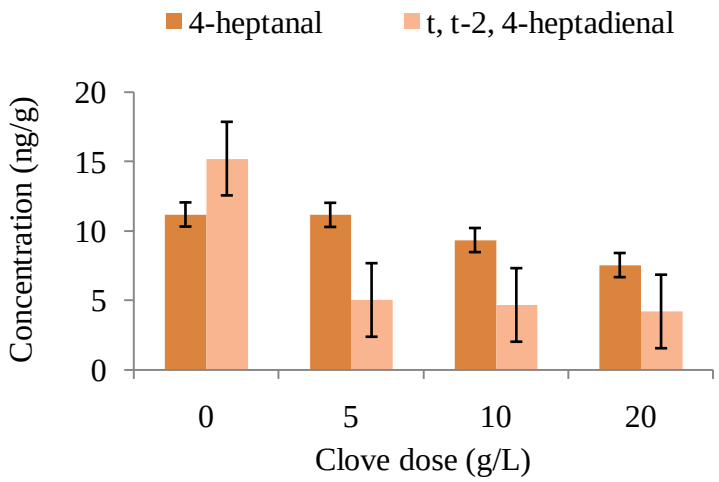

Figure 2: Concentration of representative volatile compounds in deep fried sardines pre-treated with different doses of clove extracts

5.4 Polyunsaturated fatty acids

Lipid fractions of untreated sardines, contained significantly lower amounts ($P<0.05$) of PUFAs (6.95 %) than those from sardines pre-treated with clove extracts with 7.03- 7.61 % PUFAs (Figure 3). Clove pre-treatment prior to deep frying processes resulted into 0.67 % more retention of total omega-3 fatty acids in the final products relative to untreated fish. With respect to individual omega-3 fatty acids pre-treated samples had significantly higher content of DHA, 2.96 – 3.12 % in pre-treated deep fried than the control (untreated) which had 7 2.77 % of DHA.

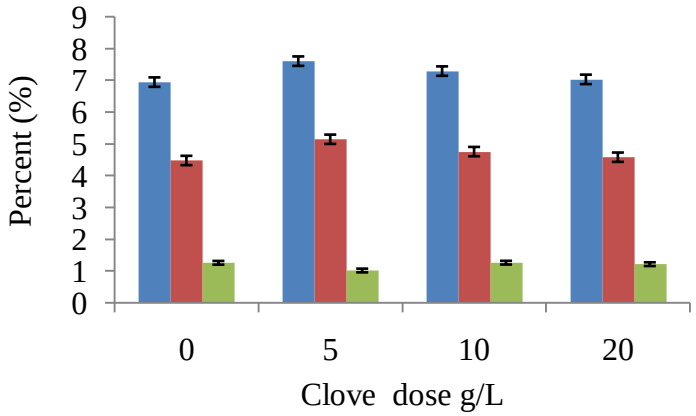

Figure 3: Fatty acid profiles in deep fried sardine pre-treated with different doses of clove extracts. PUFAs; polyunsaturated fatty acids

Higher proportions of DHA and total PUFAs in lipid fractions of treated sardines are evidences that natural antioxidants in clove extracts exert protective effect against lipid oxidation during deep frying process.

Clove has been reported to have high phenolic content and antioxidant components with high thermal stability (Shobana and Akhilender, 2000; Shan *et al*., 2005). The use of spices like clove as natural antioxidant to protect lipids in meat and fish oil has been demonstrated (Falowo *et al*., 2014; Shah *et al*., 2014). Improved retention of long chain polyunsaturated fats and preservation of omega-3 fatty acids in oven dried sardine (*R.argentae*) pre-treated with clove water extracts has also been shown (Slavin *et al.*, 2016).Water extracts of clove are also reported to have as strong peroxidation inhibitory effect as ethanol extract in linoleic acid emulsion (Gülçin *et al.*, 2004).The antioxidant activity of clove extracts may be attributed to strong hydrogen donating ability, metal chelating ability, and effectiveness as free radicals scavenger. The major phenolic compounds in clove are phenolic acids such as flavonol glucosides, phenolic volatile oils and tannins, recovery of which is highly dependent on extraction conditions, differences in solvent and extraction method (Wu *et al*., 2004; Shan *et al*., 2005; Dudonné *et al*., 2009).

6.0 Conclusion and recommendations

The present study evaluated the efficacy of clove water extracts to retard lipid oxidation during deep frying of sardines. Pre-treatment of sardine with clove water extracts resulted in improved retention of nutritionally valued long chain PUFAs, including the omega-3 fatty acids DHA. However, the success of these pre-treatments to impede lipid oxidation may partly be attributed to small size and weight of sardine fish. Further researches on other sources of antioxidants from edible plant sources are needed. The researches should include investigation on the effects of natural antioxidants applications on sensory attributes of pre treated sardines. The information would be of interest during sardine product diversification through its incorporation into other food product formulation at industrial scale.

Acknowledgments

The authors acknowledge for the financial support provided by the DANIDA supported project "Innovations and Markets for Lake Victoria Fisheries (IMLAF) **DFC 14 –P01 –TAN**)". National Food Institute, Technical University of Denmark is acknowledged for granting permission and technical support during laboratory work. The authors acknowledge Inge Holmberg, Rie Sørensen, Lis Berner, Thi Thu Trung Vu for their technical support and day to day assistance during laboratory analyses.

REFERENCES

Abdulkarim, B. Wathondi, P. O. J. and Benno, B. L. (2016). Seasonal variations in the proximate compositions of five economically- important fish species from Lake Victoria and Lake Tanganyika, Tanzania. *Journal of Pure and Applied Sciences,* 9(1): 11 – 18.

AOCS. (1998). AOCS official method Ca 5a-40: free fatty acids. In Official Methods and Recommended Practices of the American Oil Chemists'Society; Champaign, IL, USA.

AOAC International (2012). *Official Methods of Analysis*, (19th ed.).Gaithersburg, MD: AOAC International.

Bag, A. & Chattopadhyay, R. R. (2017). Evaluation of antioxidant potential of essential oils of some commonly used Indian spices in in vitro models and in food supplements enriched with omega-6 and omega-3 fatty acids. *Environmental Science and Pollution Research*, 25(1): 388-398.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8): 911–917.

Çam, M. and Aaby, K. (2010). Optimization of extraction of apple pomace phenolics with water by response surface methodology. *Journal of Agricultural and Food Chemistry*, 58(16): 9103-9111

Chaula, D., Laswai, L., Chove, B., Dalsgaard, A., Mdegela, R., and Hyldig, G. (2019). Fatty acid profiles and lipid oxidation status of sun dried, deep fried and smoked sardine (*Rastrineobola*

argentea) from Lake Victoria, Tanzania, *Journal of Aquatic Food Product Technology*, 28(2): 165-176.

Dana, D., Saguy, I. S. (2006). Review: mechanism of oil uptake during deep-fat frying and the surfactant effect-theory and myth. *Adv. Colloid Interface Sci.*, 128: 267–272.

Dudonné, S., Vitrac, X., Couti_ere, P., Woillez, M. &Merillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 57: 1768–1774.

Emir Çoban, Ö., & Patir, B. (2013). Antimicrobial and antioxidant effects of clove oil on sliced smoked *Oncorhynchus mykiss*. *Journal of Consumer Protection and Food Safety*, 8:195-199.

Emir Çoban, O¨, Patir, B, Yilmaz, O¨. (2014). Protective effect of essential oils on the shelf life of smoked and vacuum packed rainbow trout (*Oncorhynchus mykiss* W.1792) fillets . *J. Food Sci. Technol.*, 51(10):2741-2747

Falowo, A. B., Fayemi, P. O. & Muchenje, V.(2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: a review. *Food Research International*, 64:171–181.

Farvin, K. H. S., Baron, C. P., Nielsen, N. S., & Jacobsen, C. (2010).Antioxidant activity of yoghurt peptides: Part 1 - In vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry*, 123:1081–1089

Farvin, K. S. & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food chemistry*, 138 (2): 1670-1681.

Finley, J. W., Shahidi, F. (2001). The chemistry, processing, and health benefits of highly unsaturated fatty acids. An overview. *ACS Symp. Ser.* 788: 2–11.

Gachkar, L., Yadegari, D., Rezaei, M. B., Taghizadeh, M., Astaneh, S. A., Rasooli, I. (2007) Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils. *Food Chem.*, 102:898–904

Gladyshev, M. I., Lepskaya, E. V. and Sushchik, N. N. (2012). Comparison of polyunsaturated fatty acids content in fillets of anadromous and locked Sockeye salmon *Oncorhynchus nerka*. *Journal of Food Science*, 77 (12): 1303–1310.

Gülçin, İ., Elmastaş, M., Hassan, Y. A. (2012).Antioxidant activity of clove oil – A powerful antioxidant source. *Arabian Journal of Chemistry*, 5:489–499.

Gülçin, İ., Şat, İ. G., Beydemir, Ş., Elmastaş, M., Küfrevioğlu, Ö. İ. (2004). Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). *Food Chemistry*, 87:393‑400.

Ito, N., Hirose, M., Fukushima, G., Tauda, H., Shira, T., Tatematsu, M.(1986). Studies on anti-oxidant. Their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem. Toxical*., 24:1071–108.

Kirema-Mukasa, C. T. (2012). Regional fish trade in eastern and southern Africa-Products and Markets: A Fish Traders Guide (SmartFish Working Papers). Smart Fish, Commission De L'Ocean Indien.

Masa, J., Ogwok, P., Muyonga, J. H., Kwetegyeka, J., Makokha, V.& Ocen, D. (2011). Fatty acid composition of muscle, liver, and adipose tissue of freshwater fish from Lake Victoria, Uganda. *Journal of Aquatic Food Product Technology*, 20: 64–72.

Minihane, A. M., Givens, D. I., Gibbs, R. A. (2008). Health Benefits of Organic Food. In: Givens, I., Baxter, S., Minihane, A. M., Shaw, E. (Eds.). Effects of the Environment. CABI, Oxford, UK. pp. 19–49.

Mwanja, M. T., David, N. L., Samuel, K. M., Jonathan, M. and Wilson, M. W. (2010). Characterisation of fish oils of mukene (*Rastrineobola argentae*) of nile basin waters – Lake Victoria, Lake Kyoga and the Victoria Nile river. *Tropical Freshwater Biology*, 19(1):49 – 58

Naz, S., Sheikh, H., Siddiqi, R., Sayeed, S.A. (2004). Oxidative stability of olive, corn and soybean oil under different conditions. *Food Chem*., 88:253–259.

Nielsen, N. S., Debnath, D., & Jacobsen, C. (2007). Oxidative stability of fish oil enriched drinking yoghurt. *Int. Dairy J.*, *17*:1478–1485.

Owaga, E. E., Onyango, C.A. and Njoroge, C. (2010). Influence of washing treatments and drying temperatures on proximate composition of *dagaa* (*Rastrineobola argentea*). *African Journal of Food, Agriculture, Nutrition and Development*, 10:2834–2844.

Robert, A., Mfilinge, P., Limbu, S. M. and Mwita, C. J. (2014). Fatty acid composition and levels of selected polyunsaturated fatty acids in four commercial important freshwater fish species from Lake Victoria, Tanzania. *Journal of Lipids*, 2014: 1–7.

Secci, G., Borgogno, M., Lupi, P., Rossi, S., Paci, G., Mancini, S., Bonelli, A. and Parisi, G. (2016). Effect of mechanical separation process on lipid oxidation in European aquacultured sea bass, gilthead sea bream and rainbow trout products. *Food control,* 67:75-81.

Shah, M. A., Bosco, S. J. D. & Mir, S. A. (2014). Plant extracts as natural antioxidants in meat and meat products. *Meat Science*, 98, 21–33.

Shan, B., Cai, Y. Z., Sun, M. & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53:7749–7759.

Shan, B., Cai, Y. Z., Sun, M. & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53: 7749–7759.

Shantha, N. C., & Decker, E. A. (1994). Rapid, Sensitive, Iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.*, 77: 421–424.

Shobana, S. & Akhilender, N. K. (2000). Antioxidant activity of selected Indian spices. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 62:107–110.

Sidhu, K. S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Regul. Toxicol. Pharmacol*. 38: 336–344.

Slavin, M., Dong, M., & Gewa, C. (2016). Effect of clove extract pre-treatment and drying conditions on lipid oxidation and sensory discrimination of dried omena (*Rastrineobolaargentea*) fish. *International Journal of Food Science & Technology*, 51(11): 2376-2385.

URT. (2015). The United Republic of Tanzania. Ministry of Livestock and Fisheries development; Fisheries Development Division: Fisheries Annual report. Livestock and Fisheries development.

Venkateshwarlu, G., Let, M. B., Meyer, A. S., Jacobsen, C. (2004). Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *J. Agric. Food Chem.*, 52: 311−317.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, 52:4026–4037.

Yang, J., Guo, J. and Yuan, J., (2008). In vitro antioxidant properties of rutin. *LWT Food Science and Technology,* 41:1060-1066.

Zheng, W., and Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem*., 49: 5165–5170.