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Functional and structural characteristics of chocolate flavoured biscuits incorporated with surimi powder from *Nemipterus* species

A. Brita Nicy*[a], P. Velayutham[a], P. Ganesan[a], S. Balasundari[b], N. Neethiselvan[a], R. Jeya Shakila[a] and V. Kanaga[a]

[a]Department of Fish Processing Technology, Fisheries College and Research Institute, Thoothukudi-628 008, Tamilnadu, India

E-mail: britanicy@gmail.com

[b]Department of Fish Processing Technology, Dr. M. G. R. Fisheries College and Research Institute, Ponneri, Tamilnadu, India

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Surimi, the concentrated myofibrillar protein prepared from *Nemipterus* species of fish was dried by two methods viz. freeze drying and oven drying and was powdered. The freeze dried surimi powder and oven dried surimi powder were used at 10%, replacing maida to bake chocolate flavoured biscuits, separately. Fourier Transform Infrared Spectroscopy of the ingredients and biscuits were performed and assignments of the peaks were done. The presence of shifts of peak was observed around 1638 and 1550 cm^{-1} in biscuits as compared to the ingredients, suggesting a change in protein structure. Microstructure analyses of biscuits were performed by Scanning Electron Microscopy. Uniformity and compaction of the product was observed in all the biscuits. A layer of lipid molecules was visible in all the biscuits and the difference between images of control and surimi powder added biscuits were not prominent, suggesting that the microstructure of biscuits were not affected by the incorporation of surimi powder.

Keywords: Fish biscuit, surimi biscuit, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, microstructure.

Introduction

Fish protein is known for its higher digestibility and often considered better than other animal proteins but the easily perishable nature of fish causes a significant portion of the fishery resource to remain unutilized as human food[1]. Even though fish protein can be incorporated in many traditional ready-to-eat products, the possibility of inclusion of fish into sweetened bakery products such as biscuits remained unexplored until recently. Nevertheless, some researchers attempted to incorporate fish fillet powder and fish protein concentrate into biscuits[2,3]. However, literature regarding FTIR spectral studies and imaging of microstructure of fish incorporated biscuits are few. Hence, the study aims to assign the FTIR peaks of different ingredients viz. cocoa powder, maida, sugar, shortening, oven dried surimi (ODS) powder and freeze dried surimi (FDS) powder, and also the final products viz. control biscuit, biscuits incorporated with FDS powder and that incorporated with ODS powder, and to examine the microstructure of the final products.

Results and discussion

Fourier transform infrared spectroscopy of biscuits:

FTIR spectrum was analysed to examine the functional interaction that took place during the formulation and processing of biscuits. The FTIR spectra were collected in the transmission mode using FTIR spectrometer for the different ingredients viz. cocoa powder, maida, sugar, shortening, FDS powder and ODS powder, and also the final products viz. control biscuit, biscuits incorporated with FDS powder and that incorporated with ODS powder.

The important FTIR peaks for cocoa powder were obtained at 3410, 2924, 2853, 2361, 2341, 1745, 1531, 1443, 1383, 1252, 1157, 1022, 780, 669 and 466 cm^{-1} for which the probable assignments are O-H, C-H/N-H, C-H (symmetric)/N-H, N=H, C-O vibration, C-H stretching/C=O/Asp-95, N-H, in-plane mode of N-H, C-H bending, C-F stretching, C-F stretching/C-O/C-N stretching, C-O, C-H out-of-plane vibration, C-Br/N-H wagging of amines and vibrations of PO_4 re-

spectively. Four more peaks were obtained at 3809, 3788, 3680 and 3657 cm^{-1} which are unassigned.

The important FTIR peaks for maida were obtained at 3397, 2928, 2361, 2341, 1657, 1548, 1425, 1382, 1243, 1155, 1081, 1020, 929, 860, 764, 707, 669, 576 and 529 cm^{-1} for which the probable assignments are O-H, C-H/N-H, N=H, C-O vibration, C=C/N-H in-plane bending, N-H out-of-plane, C-O stretching and OH deformation of the carboxyl group, C-H bending, C-O, C-F stretching/C-O/C-N stretching, P=O stretching/C-F stretching/C-O/C-N stretching, C-O, CH=CH_2 (CH_2 out-of-plane wag), C-H (bending)/N-H wagging of amines, C-C, C-H, C-Br/N-H wag amines, C-I and C-I, respectively. Three more peaks were obtained at 3891, 3808 and 3772 cm^{-1} which are unassigned.

The important FTIR peaks for sugar were obtained at 3562, 3389, 3014, 2971, 2940, 2913, 2724, 2363, 2913, 2126, 1461, 1431, 1369, 1346, 1323, 1238, 1209, 1128, 1069, 990, 942, 910, 867, 849, 731, 683, 642, 552 and 471 cm^{-1} for which the probable assignments are O-H, O-H, C-H (stretching), C-H, C-H, intramolecular O-H hydrogen bonds, intramolecular O-H hydrogen bonds, intramolecular O-H hydrogen bonds, intramolecular O-H hydrogen bonds, CH_2 scissoring modes, C-H, C-H, O-H, C-H, C-O stretch, O-H, C-O-C antisymmetric stretching, C-O stretching modes, C-C, CH=CH_2, O-C-C, CH=CH_2, C-O, O-C-C, ring glucofuran, O-C-O and O-C-O, respectively.

The important FTIR peaks for shortening were obtained at 2916, 2851, 1739, 1467, 1386, 1263, 1243, 1220, 1171, 1113, 964, 893 and 719 cm^{-1} for which the probable assignments are C-H/CH_2, C-H (symmetric), C=O, CH_2 bending modes, C-H bending, C-H, C-O, C-O-C antisymmetric stretching, C-O, C=O, C=O, carbohydrate radical from the triglyceride structure and $(CH_2)_n$ respectively.

The important FTIR peaks for FDS powder were obtained at 3414, 2926, 2364, 1655, 1546, 1447, 1403, 1132, 1051, 1020, 927, 833, 669 and 467 cm^{-1} for which the probable assignments are O-H, C-H/N-H, N=H, C=C/N-H in-plane bending, N-H out-of-plane, C-O stretching vibration, C-H bending, C-O-C antisymmetric stretching, C-O stretching modes, C-O, CH=CH_2 (CH_2 out-of-plane wagging), bending vibrations of C-H, C-Br/N-H wagging of amines and vibrations of PO_4, respectively. Two more peaks obtained at 3841 and 3753 cm^{-1} are unassigned.

The important FTIR peaks for ODS powder were obtained at 3386, 2926, 2362, 1647, 1553, 1537, 1453, 1136, 1051, 994, 926, 851 and 668 cm^{-1} for which the probable assignments are O-H, C-H/N-H, N=H, C=C/N=H , N-H , C=C in-plane vibration, C-O-C antisymmetric stretching, C-O stretching modes, CH=CH_2 (CH_2 out-of-plane wag), C-H (bending)/N-H wagging of amines and C-Br/N-H wagging of amines, respectively. Two more peaks obtained at 3832 and 3813 cm^{-1} are unassigned.

The important FTIR peaks for control biscuit were obtained at 3419, 2923, 2853, 2362, 2341, 1745, 1640, 1547, 1464, 1383, 1239, 1161, 1116, 1053, 921, 866, 722, 609 and 580 cm^{-1} for which the probable assignments are O-H, C-H/N-H, C-H (symmetric)/N-H, N=H, C-O vibration, C-H stretching/C=O/Asp-95, C=C/N=H, N-H out-of-plane, CH_2 bending modes, C-H bending, C-O/C-F stretch, C-F stretching/C-O/C-N stretching, C-F stretching/C-O/C-N stretching, C-O stretching modes, CH=CH_2 (CH_2 out-of-plane wagging), C-H (bending)/N-H wagging of amines and $(CH_2)_n$, υ_4 mode of the phosphate group and C-I, respectively. The peak obtained at 3774 cm^{-1} is unassigned.

The important FTIR peaks for FDS biscuit were obtained at 3411, 2923, 2853, 2355, 1745, 1646, 1553, 1536, 1465, 1415, 1383, 1279, 1239, 1161, 1104, 1051, 992, 942, 921, 866, 722, 642, 579 and 470 cm^{-1} for which the probable assignments are O-H, C-H/N-H, C-H (symmetric)/N-H, C-O, C-H stretching/C=O/Asp-95, C=C/N=H , N-H , N-H stretching vibrations, CH_2 bending modes, C-O stretching and OH deformation of the carboxyl group, C-H bending, C-H, C-O/C-F stretching, C-F stretching/C-O/C-N stretching, C=O, C-O stretching modes, C-O stretching modes, C-C, CH=CH_2 (CH_2 out-of-plane wagging), C-H (bending)/N-H wagging of amines, $(CH_2)_n$, ring glucofuran, C-I and vibrations of PO_4, respectively. Three more peaks obtained at 3831, 3814 and 3684 cm^{-1} are unassigned.

The important FTIR peaks for ODS biscuit were obtained at 3412, 2923, 2853, 2359, 1745, 1642, 1551, 1464, 1416, 1383, 1239, 1161, 1116, 1052, 992, 942, 920, 866, 850, 722, 641, 607, 580, 552 and 470 cm^{-1} for which the probable assignments are O-H, C-H/N-H, C-H (symmetric)/N-H, C-H stretching/C=O/Asp-95, C=C/N=H, N-H, CH_2 bending modes, C-O stretching and OH deformation of the carboxyl group, C-H bending, C-O/C-F stretch, C-F stretching/C-O/C-N

stretch, C-F stretching/C-O/C-N stretching, C-O stretching modes, C-O stretching modes, C-C, CH=CH_2 (CH_2 out-of-plane wagging), C-H (bending)/N-H wagging of amines, C-H (bend)/N-H wagging of amines, $(CH_2)_n$, ring glucofuran, υ_4 mode of the phosphate group, C-I, O-C-O and vibrations of PO_4, respectively. Three more peaks obtained at 3895, 3847 and 3778 cm^{-1} are unassigned.

The O-H bond, probably from hydroxyl ion or water molecule between 3326 and 3560 cm^{-1} were prominent in biscuits. Common peaks around 2923 and 2853 cm^{-1} probably from C-H/N-H were present in all the biscuits. Shifts in peak around 1638 and 1550 cm^{-1} indicated changes in protein structure[4]. In the present study, the shifts of peak around 1638 and 1550 cm^{-1} were present in biscuits as compared to the ingredients which suggest a change in protein structure.

Microstructure of biscuit:

Scanning electron microscopic images of control biscuit, biscuits incorporated with 10% FDS powder and that incorporated with 10% ODS powder were recorded and those are given in Figs. 1 to 3, respectively.

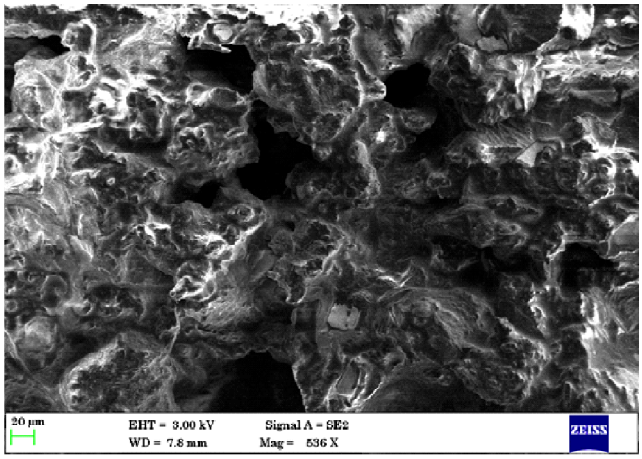

Fig. 1. SEM image of control biscuit.

In the present study, a layer of lipid molecules was visible in all the biscuits. Greater uniformity and compaction of the product was observed in control biscuit. Similar uniformity and compaction were observed in gluten free sweet biscuits elaborated with rice bran, broken rice and okara[5]. Scanning electron microscopy showed that biscuit produced with

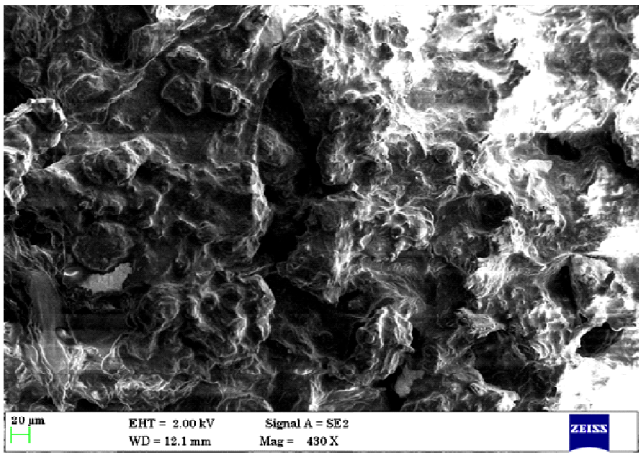

Fig. 2. SEM image of FDS biscuit.

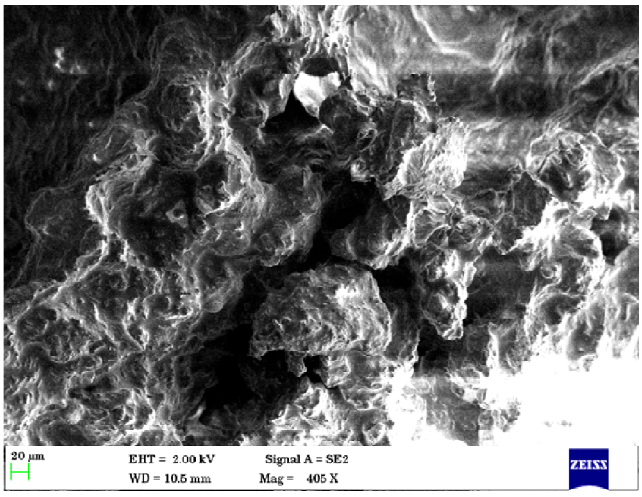

Fig. 3. SEM image of ODS biscuit.

palm mid fraction had a heterogeneous air cells and open internal microstructure[6]. Moreover, in the present study, the difference between images of control and surimi powder added biscuits were not prominent, suggesting that the microstructure of biscuits were not affected by the incorporation of surimi powder.

Changes in protein structure during baking were evident in the biscuits from the spectral studies. Layers of lipid molecules were visible in all the products. Greater uniformity and compaction of the product was observed in control biscuit. Similar studies can be extended to other bakery products such as bread and cake as well as other snack products.

Experimental

Materials:

Food grade ingredients viz. maida, cocoa powder, shortening (vanaspathi) and sugar were used for baking biscuits were of FSSAI standards and they were obtained from the local super market. Commercially available high quality surimi from *Nemipterus* species was procured from Gadre Marine Export Private Limited, Baikady village, Brahmavar, Udupi, Karnataka.

Preparation of biscuit:

Surimi from *Nemipterus* sp. of fish was purchased from commercial industry and was dried using two methods, freeze drying and oven drying and then were powdered. Biscuits were prepared with flour containing maida and cocoa powder as control. All the ingredients viz. maida (90 g), sugar (50 g), shortening (60 g) and cocoa powder (10 g) were weighed. Sugar and shortening were mixed thoroughly and all the other ingredients sifted together were added and mixed properly. Small balls were made and rolled into a sheet and was cut. It was kept in a baking pan greased with vegetable oil and baked at 160°C for 15 min. It was then cooled at room temperature. The biscuits were packed in aluminium lined polyethylene bags and stored at room temperature. Oven dried surimi (ODS) powder and freeze dried surimi (FDS) powder were added separately at 10% by replacing maida in the flour to prepare surimi chocolate biscuits.

Analysis of functional characteristics of ingredients and biscuits by FTIR:

Sample was placed on to the crystal cell and the cell was clamped in to the mount of FTIR spectrometer. Signals were collected from the range of 400–4000 cm^{-1} in 32 scans at a resolution of 4 cm^{-1} and were with reference to a background spectrum recorded from the clean empty cell at 25°C.

Analyses of structural changes of biscuits by SEM (Scanning Electron Microscopy):

Scanning electron microscope (ZEISS Sigma VP, Germany) was used to view biscuits in three dimensions and to determine the microstructure of the products. The bakery product sample was mounted on SEM stub using double sided adhesive tape and was coated with platinum. An accelerating potential of 5 KV was used during micrography.

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