A STUDY ON THE FUNCTIONAL INTERACTION DURING THE FORMULATION OF SHELF STABLE SEAFOOD CRACKERS

Thesis submitted in part fulfilment of the requirements for the degree of **Master of Fisheries Science in Fish Processing Technology** to the Tamil Nadu Fisheries University, Nagapattinam

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CERTIFICATE

This is to certify that the thesis entitled, "**A study on the functional interaction during the formulation of shelf stable seafood crackers**" submitted in part fulfillment of the requirements for the degree of Master of Fisheries Science in Fish Processing Technology to the Tamil Nadu Fisheries University, Nagapattinam is a record of bonafide research carried out by Miss. Reshma Ramesh, MFT 15059 (FPT) under my supervision and guidance and that no part of this thesis has been submitted for the award of any degree, diploma, fellowship or similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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TO

THE ALMIGHTY

AND TO MY LOVING

FAMILY, GUIDE &

TEACHERS

Abstract

ABSTRACT

Title	:	A study on the functional interaction during the formulation of shelf stable seafood crackers
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Snack foods have become a significant part of the diet of many individuals, especially children, and can influence their overall nutrition. Most of the snacks available in the market are mainly based on cereals, which are high in calorie and low in protein content. For this reason, snacks like fish crackers with high protein content should be developed as a supplementary diet. Fish crackers were prepared by blending lean fish (*Nemipterus japonicus*) along with tapioca, corn and sago in six different proportions. Fish and tapioca were taken in proportions of 0:70, 10:60, 20:50, 30:40, 40:30, 50:20 by keeping the proportion of corn and sago constant (15:15). The samples were examined for their physico- functional properties as well as the molecular interaction upon formulation and processing. The products were also subjected to proximate analysis as well as comparative sensory evaluation so as to determine the nutritive value and consumer acceptance. The physico – functional properties like linear expansion, volume expansion, oil absorption and crispiness decreased whereas bulk density

increased with the increase in the fish concentration. A sharp decrease in the Water Absorption Index (WAI) and a sharp increase in the Water Solubility Index (WSI) were noticed. FTIR spectral analysis was undertaken to examine the functional interaction that took place during the formulation and processing of fish crackers. The sensory evaluation conducted by the expert panels suggested that 40% fish incorporated crackers with a protein content of 12.65% is having a better overall quality and is considered to be the best formulation.

The gelatinization conditions of the fish crackers were optimized in order to improve the expansion, bulk density and crispiness using Response Surface Methodology (RSM). The process variables were steaming time (20, 40, 60 min); gel setting time (12, 18, 24 h) and drying temperature (40, 50, 60ºC) and the responses taken were linear expansion, bulk density and crispiness. The average values ranged from 51.29 to 71.14% for linear expansion, 0.24 to 0.40 g/cm^3 for bulk density and 2.71 to 9.01 N (hardness value) for crispiness of the products. The optimum condition predicted by RSM to have high linear expansion of products was at a steaming time of 40 min, a gel setting time of 24 h and a drying temperature of 40º C. To have a maximum crispiness, the steaming time has to be increased as 60 min. This study enabled to optimize few important process conditions that are not examined earlier to improve the expansion and crispiness of the fish crackers. The storage studies conducted on both dried and fried samples suggested that the samples are safe and shelf stable for a period of 3 months.

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Introduction

I. INTRODUCTION

Seafood are rich sources of high quality protein valued for their functional properties and nutritional value. Among these, fish is the most important and cheap source of protein, omega-3 fatty acids, low calories and minerals such as calcium and phosphorus. Polyunsaturated fatty acids in fish have several health beneficial effects in reducing risks of cardiovascular diseases and curing certain types of cancers (Guler *et al*., 2008). They are also essential for the normal growth and development and to prevent coronary artery diseases, arthritis, diabetics, hypertension, and other inflammatory and auto immune disorders (Simopoulos, 2000). These potential effects have drawn the attention of many food processors, medical community and consumers and have promoted the increased number of food products incorporated with fish.

Snacks are considered to be the fastest growing segments of the food industry and their consumption has significantly increased over the decades due to several reasons like rapid urbanization, changing life style, increase in number of nuclear families and working women, media penetration and higher disposable incomes (Thakur and Saxena, 2000). Convenience, manageable portions and satisfaction of short term hunger are the basic criteria for snack foods. Snacking is defined as a problem free consumption of miniature portioned, solid or liquid products in hot or cold form that needs little or no preparation intended to satisfy the occasional pangs of hunger (Kumar *et al*., 2012). Snacks have been classified into three, as first generation (conventional potato chips and baked crackers), second generation (directly expanded), and third generation (semi, half or intermediate) products (Harper, 1981).

Snack foods have become a significant part of the diet of many individuals, especially children, and can influence their overall nutrition (Shukla, 1994). Most of the snacks available in the market are mainly based on cereals, which are high in calorie and low in protein content (Rhee *et al*., 2004). They also lack essential amino acids like tryptophan, threonine and lysine. If they are taken in large quantities, they can suppress the appetite for the main meal. For this reason, snacks with high protein content should be developed as a supplementary diet. Many works have been conducted in this field so as to improve the nutritive value of the snack products by incorporating some protein sources extracted from plants or animals (Bhattacharya *et al*., 1990; Park *et al*., 1993; Rhee *et al*., 2004).

Chemical composition of fish plays a role in ensuring the nutritive value besides the finest quality flavour, odour and texture of the snack foods. Incorporation of fish into the snack foods also gives a novel opportunity for profitable utilization of fish and thereby improves the nutritional status of the human population in a country. Research are being undertaken by the nutrition scientists and food technologists incorporating fish to enhance the nutritional value of staple foods. Fish meat based snack foods are rarely available in Indian markets.

Different varieties of snack foods are available to consumers in the markets, which include cookies, biscuits, pies, sticks, breads, curls, crackers etc. Among these, crackers draw a major attention. Crackers are defined as thin crisp biscuits or wafers, made of unsweetened and unleavened dough. They are the food of choice for school going children, adolescent girls and high mobility groups and consumers of every age groups from 7 to 70. Hence, supplementing the cracker with fish should be considered in terms of a healthy and balanced diet. Fish crackers are very much popular in South- East Asian countries (Suknark *et al*., 1998) and are known by different names such as *keropok* in Malaysia, *kerupuk* in Indonesia, *kaewkrab pla* in Thailand and *banhphongtom* in Vietnam (Kyaw *et al*., 2001). Fish cracker is produced by gelatinisation of starch in dough made from fish and starch, which is shaped into cylindrical roll, steamed, cooled, sliced, dried and packed (Siaw *et al*., 1985). Upon frying in hot oil, the dried slice will get expanded to produce a low density porous product (Siaw *et al*., 1985; Tongdang *et al*., 2008). Deep fat frying is considered to be the oldest and the most common method of frying as it results in specific texture and flavour (Gazmuri and Bouchon, 2009).

Starch serves as a functional ingredient that contributes to the expansion of the product. The expansion is directly related to the crispiness, which determines the acceptability of fish cracker (Yu, 1991a). Normally, cassava starch is used for the preparation of fish crackers, as it gives high expansion. Tapioca is considered as the staple food for people in many countries and it is the main ingredient along with mince fish for the preparation of fish crackers. However, other starches like corn, wheat and rice or sago can also be used (Mohammed *et al*., 1989, Yu, 1993, Tongdang *et al*., 2008). Due to high proportion of starch, fish crackers do not have any resemblance with other sea food analogs.

Usually marine non-fatty fishes are used for the preparation of fish crackers but fresh water fishes can also be used. Thread fin breams (Nemipteridae) is a better choice for the preparation of crackers. They are found in tropical waters of the Indian and western Pacific Oceans. They are marketed mainly as fresh, frozen, steamed, dried-salted, dry-smoked, fermented or made into fish balls and fish meal. Some complications such as low grade protein gel, colour problem and lipid oxidation are often associated with the incorporation of fish species with high dark/ red muscle and fat content (Park and Lanier, 2000). Development of sensorily acceptable fish crackers of high nutritional quality can be a successful strategy for the consumption of fish as well as a very good alternative for the high calorie, low nutritional ready- to- eat food.

Many studies have been conducted on the formulation of fish crackers and examination of their physico- chemical properties by the researchers. But no studies have been conducted on the formulation of fish crackers with the mixture of three starches such as tapioca, corn and sago at different combinations. The optimization of the gelatinization parameters like steaming time, gel setting time and drying temperature to improve the physico-functional properties of the crackers were also not studied earlier by the scientific workers. Also, no data is available on the molecular interaction of the starch and fish protein molecules as analysed by FTIR. Hence, the present study was planned to develop 'fish crackers' from low fatty fish *viz*. thread fin bream (*Nemipterus japonicus*) with the following objectives.

1) To formulate the processing conditions for the formation of sea food crackers.
2) To evaluate the effect of different flour characteristics on the formation of crackers.
3) To evaluate the physico- functional properties of the crackers.
4) To examine the stability and safety of the crackers during storage.

Review of Literature

II. REVIEW OF LITERATURE

2.1. Formulation of fish crackers

2.1.1. Fish

Fish and shrimp are used as the main protein sources for cracker preparation. As compared to shrimp, fish cracker is found to be less expensive due to its low price. Generally, marine fishes are used for the preparation of crackers. Different species of fish have been experimented by different scientists for the preparation of crackers (Table 1). Besides this, the wash water protein from fish ball processing is also used for fish cracker formulation (Yu *et al*., 1994). Crackers are also produced from the shrimps and examined for the moisture and oil content and some other physical characteristics (Nguyen *et al*., 2013).

Table 1: Fishes used for the preparation of fish crackers

Source material	**References**
Fish protein hydrolysate	Yu and Tan (1990)
Sardine (*Clupea leiogaster*)	Yu (1991a)
Snapper (*Lutjanus* spp), Yellow pike conger (*Ophiocephalus micropeltis*), Feather back (*Notopterus chilata*)	Peranginangin *et al.* (1997)
Jewfish (*Johnius soldado*)	Kyaw *et al.* (1999; 2001a)
Powdered surimi	Huda *et al.* (2000, 2001)
Big-eye (*Brachydeuterus auritus*)	King (2002)
Dory fish (*Pangasius hypophthalmus*)	Huda *et al.* (2009)
Grass carp (Ctenopharyngodon idella)	Wang *et al*. (2012)

Kaewmanee *et al*. (2015) conducted studies on the effect of fish species on the characteristics of fish crackers and indicated that the colour and texture of the cracker are affected by the type of fish used as the raw material. The type of fish species also affected both the technical characteristic of intermediate products and the resulting cracker. Scanning electron microscopic studies on fish crackers revealed that the lean fish yield more porous product compared to the crackers formulated with fatty fish.

2.1.2. Starch

While focussing only on one protein source such as fish, the final quality of the product is primarily dependent on the quality of the starch as well its changes during processing and the interference of the other components. The molecular structure as well as the content of the starch greatly depends on the plant source and the conditions of growth or environment, storage and also on the fractionation and purification processes. The tropical crops like sweet potato and tapioca were used for the preparation of extruded fried fish crackers by Noorakmar *et al*. (2012). Different kinds of starches used for the preparation of the fish crackers are given in the Table 2.

Table 2: Different starches used for the preparation of fish crackers

Type of starches	**References**
Cassava	Mohamed *et al.* (1989); Cheow *et al.* (1999); Kyaw *et al.* (2001a, b); Huda *et al.* (2001); King (2002); Cheow *et al.* (2004); Tongdang *et al.* (2008); Huda *et al.* (2009); Saeleaw and Schleing (2010, 2011)
Corn flour	Mohamed *et al.* (1989)
Sago	Mohamed *et al.*(1989); Cheow and Yu (1997); Cheow *et al.* (2004); Tongdang *et al*. (2008)
Rice flour	Mohamed *et al.* (1989); Yu (1993)
Wheat	Cheow *et al.* (2004)

Pregelatinised starch	Yu and Low (1992)
Mixed starches	Mohamed *et al.* (1989); Tongdang *et al.* (2008); Saeleaw and Schleining (2010)

Cassava starch is obtained from the root of cassava plant (*Manihot esculenta, Crantz*) whereas sago starch is obtained from sago palm (*Cycas revolute*). Properties of these starches have been reported by many researchers (Sriroth *et al*., 1999; Hoover, 2001, Singh *et al*., 2003; Singhal *et al*., 2008). A higher expansion was noticed in cassava crackers than those made from the sago starch (Mohamed *et al*., 1989; Tongdang *et al*., 2008). The high amylose content of the sago promotes retrogradation resulting in a stronger gel and thus resists the expansion. When cassava is replaced with mung bean starch, a lower expansion was obtained (Mohamed *et al*., 1989). Mixing of other kind of starches with cassava had also decreased the expansion of the crackers (Mohamed *et al*., 1989; Tongdang *et al*., 2008; Saeleaw and Schleining, 2010). Mixtures of four types of flour (cassava, wheat flour, waxy and non-waxy rice) were used by Saeleaw and Schleining (2010) and they observed that a high fraction of cassava showed high expansion. In order to reduce the cooking time of cracker, pre-gelatinised tapioca starch was used for making crackers (Yu and Low, 1992), and these crackers will get more easily cooked than those made with native starch.

Amylopectin plays a key role on swelling of starch granules, while amylose acts an inhibitor and diluent (Leach *et al*., 1959). The ratio of amylose-amylopectin present in starch play an important role in the expansion of the snack (Wang, 1997). Mohamed *et al*. (1989) compared the expansion of the crackers made from different flours having different amylose-amylopectin ratio.

The starch granules will be enriched with amylopectin since all the amylose will be leached out during cooking process (Hermansson and Svegmark, 1996). Interaction between the amylose and lipid during gelatinization also affects the water uptake during heating and thereby reduces the swelling of the starch granules, which in turn will affect the expansion of the cracker. Few studies have revealed that protein and lipid inhibit the swelling power of starch (Leach *et al*., 1959; Pomeranze, 1991).

2.1.3. Seasonings

Salt, sugar, black pepper, garlic and monosodium glutamate are used as seasonings during cracker preparation. Salt helped to improve the puffed quality of rice–based snack (Jomduang and Mohamed, 1994). Salt plays an important role on protein solubility and starch gelation; and it is normally added at 2% of total ingredients. Salt helps the protein to disperse in starch (Cheow *et al*., 1999). Low salt content (0 to 20 g kg^{-1}) has significantly reduced the linear expansion of the fish crackers (Cheow *et al*., 1999) Sugar and MSG do not play much role in the visco-elastic properties (Cheow and Yu. 1997) and add less effect to the gelatinization temperature of fish–salt mixture than salt (Cheow *et al*., 1999).

2.1.4. Mixing

Mixing is the crucial step in the cracker preparation that helps the ingredients to be well mixed throughout to obtain a good quality product. Salt and other seasonings have to be mixed with fish and then, the starch is added (Cheow *et al*., 1999). This has been consistently practiced in commercial as well as household preparation of crackers. If salt is added after mixing of starch with fish, the aggregation of fish protein takes place and a less dispersion of fish protein in the starch system occurs, which results in the less expansion of the fish crackers (Cheow *et al*., 1999). Preliminary works have been conducted by Cheow *et al*. (2004) on the importance of mixing time on cracker expansion.

2.1.5. Cooking

Cooking process involves hydrothermal heating by steaming or boiling in water. Cooking results in the gelatinization of the starch and thereby leads to the formation of gel. Gelatinization depends on the steaming time and temperature (Kyaw *et al*., 1999; Tongdang *et al*., 2008), as well as the water and starch content of the dough (Kyaw *et al*., 2001b). The starch could be easily cooked in the presence of excess water.

Degree of starch gelatinization in the cracker dough has positively influenced the expansion of the crackers (Tongdang *et al*., 2008). The expansion of cassava crackers steamed for more than 60 min at 100^{o}C got decreased due to the fragmentation of starch as revealed by decreased water absorption index and increased water solubility index (Tongdang et al., 2008) and decreased gel strength (Kyaw et al., 1999). Irreversible changes like breaking of hydrogen bonds, water uptake, swelling of granules, melting of crystallites or double helices, bi-refringence loss and solubilisation in starch granules took place during the process of gelatinization.

2.1.6. Chilling

After cooking, the cracker gel is allowed to cool in an ice box or refrigerator, to obtain harder gel and to enable easy slicing. Retrogradation of the cooked starch will take place during this process. During retrogradation, extensive re-association of the polymers of gelatinized starch takes place (Atwell *et al*., 1988). It leads to precipitation, gelation and changes in consistency and opacity (Hermansson and Svwegmark, 1996). It also involves the re-association of amylose molecules to form double helical chain segments and its aggregation, followed by slow crystallization of the short chains of amylopectin (Biliaderis, 1992). The long term changes in the firmness of starch gels on storage are due to the crystallization of amylopectin (Ring *et al*., 1987). The starch type and its composition, storage temperature and time are the factors responsible for the retrogradation of starch gel.

2.1.7. Drying

After cooling, the drying process is practiced. The purpose of drying is to reduce the moisture content of the crackers, which determines the stability of the crackers during storage. An upper limit of 12% moisture content is imposed by the standards (Thai industrial standard, 1987) anticipating microbial spoilage at high moisture.

2.1.8. Frying

Deep fat frying in hot oil is applied in order to puff the intermediate product. This is a traditional as well as a very popular method in cracker production, both in house hold and commercial scale (Gazmuri and Bouchon, 2009). Half-finished chips with about 12% moisture content could form good quality crackers upon deep fried processing (Saeleaw and Schleining, 2011). During frying process, heat and mass transfer, water evaporation and formation of the cellular porous structure (Bhat and Bhattacharya, 2001), loss of moisture content (Krokida *et al*., 2000), protein denaturation, starch gelatinization and colour development (Maneerote *et al*., 2009) take place. Instead of frying, a puffing machine has been developed and used for the cracker production (Kok *et al*., 2004).

The published reports have documented the following time and temperature for frying as 200°C for 15 s (Cheow *et al*., 1999; Kyaw *et al*., 2001a);

180-200°C for 1-2 min (Yu and Low, 1992); 150°C for 3 min (Huda *et al*., 2001); 190°C for 30s (Tongdang *et al*., 2008); 180-200°C for 1 min (Huda *et al*., 2009); 160°C for 10s (Saeleaw and Schleining, 2010). Studies conducted by Saeleaw and Schleining (2011) showed that the besides the frying temperature, frying time had decreased the moisture content of fried products significantly. Increased frying temperature leads to rapid evaporation of the water molecules from the dried product. Krokida *et al*. (2000) reported that the water loss and the oil uptake increased with the increase in the frying temperature and frying time. The increasing frying temperature tends to decrease the oil uptake as the product spends less time in the fryer (Moyano and Predreschi, 2006; Rossell, 2001).

2.2. Proximate composition of fish crackers

Several studies have revealed that a moisture content of 9-13% is required for semi-finished chips to produce porous and crispy crackers (Nurul *et al*., 2009; Yu, 1991). Omobuwajo (2003) found that the moisture content was slightly higher (1.93%) in the experimentally produced crackers compared to the commercial crackers (1.35%). Neiva *et al*. (2011) produced fish crackers using minced fish of two different species by frying and microwave cooking and their moisture contents were 0.48 $\pm$ 0.06% and 3.12 $\pm$ 0.15%, respectively. In a study conducted by Chang *et al*. (2013), the moisture contents of cuttlefish crackers dehydrated at different temperatures of 70, 60 and 50ºC were 9.63, 9.67 and 11.71%, respectively after 3 hours of drying. The moisture content has a significant role in the expansion of the crackers as the expansibility of the cracker occurs due to the increase in the water evaporation volumes during the rapid heating process. The vaporized moisture creates a mass transfer on the surface and inside of the samples creating porous structures on crackers.

The protein content of the fish crackers increased with the increase in the concentration of the fish as observed by several authors (Yu, 1991b; *Huda et al.*, 2000; King, 2002). In a study conducted by Neiva *et al*. (2010), the mean protein content of the microwave processed fish crackers (MFC) was 14.70%, whereas the mean protein content of the fried fish crackers was (FFC) 10.86%. As a result of the increase in the protein content, the carbohydrate concentration in crackers with more fish to starch ratio reduced. It was reported that the commercial fish crackers contained carbohydrate in the range of 65–80 % (Huda *et al*., 2007).

On the other hand, the fat content of the crackers increased with increase in the fish concentration, as reported by Peranginangin *et al*. (1996) and King (2002), but at different rates. The lipid content of the FFC was found to be higher (26.11%) as compared to the MFC crackers (0.42%) (Neiva *et al*., 2010). In a comparative study conducted by Omobuwajo (2003), the proteins and minerals were higher in the crackers produced experimentally (6.16% and 1.38% respectively) than those produced commercially (2.57% and 0.68%, respectively). The total calorie of the fried crackers was 65% higher than that of the microwave processed crackers.

2.3. Physico- functional properties

2.3.1. Linear expansion

The expansion ability of the crackers is determined as linear expansion (Yu *et al*., 1981), which is the most critical factor related to sensory qualities. It results from the rapid expansion of water and consequent expansion of the starch granules and vacuoles, when the crackers are exposed to high temperatures (Cheow *et al*., 1999b; Cheow *et al*., 2004; Kyaw *et al*., 1999, 2001a). An acceptable quality of puffed cracker should have high expansion and crispiness, as well as low oil uptake.

The fish content and the starch ratio greatly influence the degree of linear expansion of fish crackers (Yu, 1991a, b; Huda *et al*., 2009). With the increase in the fish content, the linear expansion tend to decrease (Yu *et al*., 1994; Cheow *et al*., 1999; Huda *et al*., 2001; Kyaw *et al*., 2001b). In the presence of starch, the protein forms a continuous gel like structure appearing as filler, resulting in the reduction of expansion (Cheow *et al*., 1999a). Use of poor quality fish also results in lower expansion.

The other important factors that affect the expansion include the gel strength of the dough formed during the steaming stage and the protein content that affects the gel formation and strength by interfering with the vacuole formation or degradation during water evaporation (Badrie and Mellowes, 1992). Among the various types of flour used for making fish crackers, tapioca starch was found to be affecting the linear expansion greatly. Complete gelatinization was noticed in the fish crackers with 15% fish meat, when steamed for 100 min as compared to those containing less than 10% fish meat (Kyaw *et al*., 2001b). A study conducted by Chang *et al*. (2013) indicated that the samples dehydrated at higher drying temperatures and longer drying times showed higher expansibility. Noorakmar *et al*. (2012) reported that higher expansion of the fish crackers was obtained with low amount of orange sweet potato flour addition. Excessive steaming time had also resulted in the poor linear expansion of the crackers (Kyaw *et al*., 1999).

2.3.2. Bulk density

Bulk density is an indicator of puffing of cracker. Expansion of cracker, in terms of bulk density is determined using sesame seeds displacement method (Sahin and Sumu, 2006). Bulk density has a significant negative correlation to linear expansion. The effects of temperature (140,150 and 160ºC) and duration of frying (5, 10 and 15s) on cracker expansion, bulk density and moisture content of the dried product showed that with the increase in frying time and temperature, the linear expansion significantly increased with a corresponding decrease in the bulk density (Saeleaw and Schleining, 2011a). The air cell formation during frying is related to the expansion of the product. Higher the degree of expansion, the higher air cells will be formed (Saeleaw and Schleining, 2011). Noorakmar *et al*. (2012) reported that in a cracker having 40% fish contents, the addition of orange sweet potato flour with the tapioca starch had decreased the volume expansion. Also, an increase in the addition of orange sweet potato flour at low fish content increased the bulk density.

2.3.3. Oil absorption

The oil absorption was significantly higher for the fried extruded fish crackers with 30% fish content than those with 40% fish content (Noorakmar *et al*., 2012). Oil uptake is an important parameter controlling the quality, crispiness and colour of the crackers. A study conducted by Nurul *et al*. (2009) on the effect of different ratios of dory fish to tapioca flour showed a decrease in oil absorption with the increase in the ratio of fish to starch.

2.3.4. WAI and WSI

Water absorption index (WAI) and water solubility index (WSI) were determined mainly to examine the degree of gelatinization and to understand the extent of molecular breakdown during formulation of fish crackers (Zhu *et al*., 2010). The WAI measures the amount of water occupied by the starch granules after swelling in excess water, which gives information on the granular integrity. The WSI determines the solubility of free polysaccharides released from the starch granule during gelatinization. There existed a positive correlation between starch solubility and extent of molecular breakdown. In a study conducted by Noorakmar *et al.* (2012), as the amount of the sweet potato flour with the tapioca starch increased, WAI of the extruded fish cracker snacks decreased. Also WSI of extruded fish crackers increased with the addition of orange sweet potato flour to tapioca starch.

2.3.5. FTIR studies

Fourier- transform infrared spectroscopy (FTIR) is used to examine the interaction of functional groups during formulation and processing of the food products. Wilson *et al*. (1988) conducted FTIR analysis of wheat starch gels during the retrogradation process. Studies on gelation of amylose and amylopectin was conducted by Goodfellow *et al*. (1990). The retrogradation of potato starch was studied by van Soeast *et al*. (1994) using FTIR and the spectra showed that C-C and C-O stretching region (1300-800) is more sensitive to retrogradation. An in-situ observation of pressure- induced gelation of starches was studied by Rubens *et al*. (1999) with FTIR in the diamond anvil cell. No such study was so far conducted on the FTIR analysis of fish crackers prepared using tapioca, corn and sago starches.

2.3.6. Texture

Texture is perceived during the oral processing and is significantly influenced by the nature of the food, especially for puffed crackers. A texture measurement system based on an acoustic vibration technique was developed by Taniwaki *et al*. (2006), having a high sampling rate (80 kHz), enabling a high resolution measurement based on the probe speed of 22 mms^{-1} that was similar to the actual mastication speed. Sensory perceptions of food are greatly influenced by taste, flavour, odour, texture, appearance etc and crispiness is an important parameter and a unique characteristic to be controlled in deep–fat fried products (Thanatuksorn *et al*., 2007). Low break down force values shown by the expanded cereal foods is due to their porous nature (Bhat and Bhattacharya, 2001). The textural properties like crispiness and hardness of crackers are greatly influenced by the air cells or pores present in the product (Ngadi *et al*. 2009). Chang *et al.* (2013) noticed that the lowest hardness was observed in samples dehydrated at 50ºC for 6h, 60 ºC for 4.5h and 70 ºC for 3 h. Some studies demonstrated that for simultaneous measurement of force displacement and sound pressure generated by the fracture of a crispy food sample, an acoustic envelope detector (AED) was coupled with the texture analyser (Chen *et al*., 2005; Saeleaw and Schleining, 2011a).

2.4. Optimization of gelatinization conditions of fish crackers using RSM

Food scientists have undertaken research to improve the degree of expansion and crispness of crackers with the incorporation of food additives such as sodium pyrophosphate and sodium bicarbonate (Peranginangin *et al.,* 1996), optimization of steaming time (Kyaw *et al.,* 1999), thickness (Peranginangin *et al.*, 1996) and ratio of fish to starch (King, 2002). Effect of steaming time on the linear expansion of fish crackers was studied by Kyaw *et al*. (1999) stating that a steaming time of 20- 30 min is sufficient to cook the keropok gel. Optimization of processing conditions to reduce oil uptake and to enhance physico- chemical properties of deep fried rice crackers has been conducted by Maneerote *et al*. (2009). Association between textural profile and surface electromyographic behavior of microwavable cassava cuttlefish crackers with various expansion ratios have been studied by Chang *et al*. (2013). However, to the best of our knowledge, there are no reports on the influence of gel setting time and drying temperature on the linear expansion of the product.

2.5. Storage stability studies

2.5.1. Quality analyses

The thiobarbituric acid reactive species (TBARS) is widely used as an indicator of the degree of lipid oxidation in food. Several studies have been conducted on the formation of TBARS in different meat and fish products by various researchers (Singh *et al*., 2011, Chidananadaiah *et al*., 2009, Kumar and Tanwar, 2011, Bhat *et al*., 2010). They have found that the TBARS values increased significantly upon storage, which is mainly attributed to the oxygen permeability of packaging material that had led to lipid oxidation of foods (Brewer *et al*., 1992).

2.5.2. Microbiological analyses

In chicken meat patties, an increase in TPC was reported with the increase in storage period (Kumar *et al*., 2007). Similar findings were also reported by Chidanandaiah *et al*. (2009), Kumar and Tanwar (2011), Bhat *et al*. (2010), Bhat and Pathak (2011), Bhat *et al*. (2013a) and Bhat *et al*. (2013b) in meat patties, chicken nuggets, chevon Harrisa, mutton Harrisa, chicken seekh kababs and chicken meat balls, respectively. In the meat snacks an increase in

TPC was noticed at every storage level analysed at different intervals (Singh *et al*., 2011). Raja *et al*. (2014) had conducted microbiological analyses on the aerobically packaged fish curls containing different flours and found that the TPC significantly increased upon storage. Coliforms were not detected in aerobically packaged fish curls incorporated with different flours upto 21 days of storage while they were present in all the samples from 28 days of storage. The yeasts and moulds were not detected in the curls upto 14 days of storage, whereas it appeared from day 21 onwards (Raja et al., 2014).

2.6. Sensory evaluation of fish crackers

Sensory perceptions of the food are influenced by appearance, texture, taste, flavour, colour etc. The colour is an important attribute that attract the consumers before they taste a product (Francis, 1991). King (2002) conducted studies on the development and sensory acceptability of crackers made from the big- eye fish and found that no significant difference in flavour and crispiness of the different formulations of fish with starch was observed. However, it was found that the colour and overall acceptability of 40% and 50% fish incorporated snacks were significantly better than 60% fish incorporated crackers. In the study conducted by Neiva et al. (2010), the sensory scores for the fried crackers are more than the microwave processed crackers. In the microwave processed fish crackers, the colour was the attribute which received a lower score of 6.4 due to the fact that microwaved crackers are paler than fried ones. Kaewmanee *et al*. (2015) reported sensory scores of the finished puffed crackers and indicated that the oiliness and likability of flavour did not depend on the fish species used for making the crackers.

Materials & Methods

III. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Raw materials

Marine fish *(Nemipterus japonicus)* were purchased from the local fish market of Tuticorin, India and brought to the laboratory in iced condition for processing. Fish was semi-dressed by removing the head, viscera, fins and tail. Tapioca flour, corn flour, sago flour and other ingredients were obtained from the local supermarket. Refined sunflower oil was purchased from the local shop.

3.1.2. Media and Chemicals

Chemicals used for the analysis of proximate composition and biochemical characteristics were of analytical grade. Microbiological media from Hi Media Laboratories Pvt. Ltd., Mumbai, India was used for the microbiological analyses of fish crackers.

3.1.3. Instruments

Electronic balance, food processor (BRAUN, Germany), steam cooker (PRESTIGE, India), refrigerator (Whirlpool, India), hand slicer (ANJALI, India), hot air oven (TECHNICO, Chennai, India), horizontal sealing machine (SYS PAC, Delhi), SOCS PLUS-SCS4 and KEL PLUS-KEL FLOW (PELICAN, Chennai), muffle furnace (Servo, Salem, India), water bath (INLAB Equipments Pvt. Ltd, Chennai, India) , UV–VIS spectrophotometer (V-530, Jasco, Japan), refrigerated centrifuge (Eppendorf, Germany), iD3 ATR-FTIR Spectrophotometer (iS5 NICOLET, Thermo

Scientific, USA), colorimeter (Color Flex, Model No D/8-S, Hunter Associates Laboratory Inc., Reston, VA, USA), texture analyser (LLOYD Instruments, England), water activity meter (Aqua Lab LITE, Decagon Devices Inc, Pullman, USA), autoclave (Technico Laboratory Products Pvt Ltd., Chennai, India), laminar air flow chamber (Class II, KLEAN ZONE Systems, Chennai, India) and incubator (SECOR Laboratory Istruments, Scientific Eng Corp, Delhi, India) were used for conducting various physico- chemical and microbiological analyses.

3.1.4. Packaging material

Metallized polyester aluminium pouches of size 12 **x** 14 cm purchased from Manhari Bags Pvt Limited, Haridwar, Uttarakhand were used for packing the fish crackers. They are having water vapour transmission rate and oxygen transmission rate of < $0.01 g/m^2/24h/37^{o}C/90\%RH$ and $<0.006 cc/m^2/24h$ at ATP respectively.

3.2. METHODS

3.2.1. Preparation of fish crackers

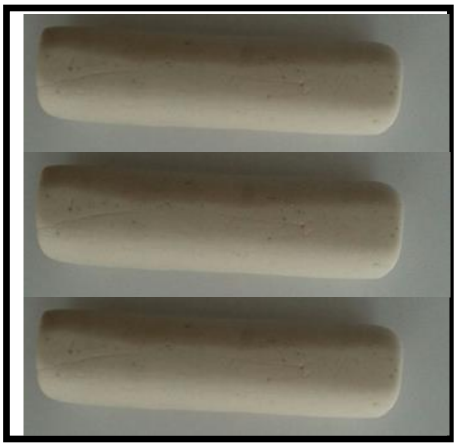
Semi dressed fish was steam cooked at a temperature of 100ºC for 10 min in a steam cooker and the fish meat was picked out manually in a hygienic manner. Cooking of fish was done to reduce the moisture and to improve the flavour. Different starch mixture was prepared using tapioca, corn and sago, keeping the proportion of corn and sago as constant (15:15). With the remaining 70% proportion, six different fish crackers were prepared at different fish to tapioca starch ratio viz. 0:70, 10:60, 20:50, 30:40, 40:30 and 50:20 and were

Plate 1:   Preparation of fish crackers

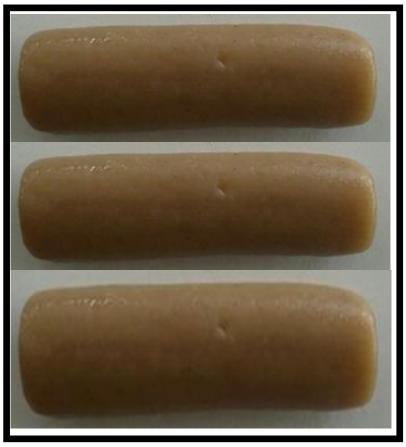
Raw material

Cooked meat

Mixing of fish meat with starch and ingredients



Preparation of cylindrical roll



Steam cooked roll

Sliced roll

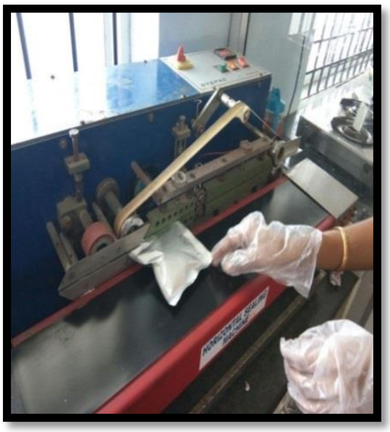
Plate 1: Preparation of fish crackers

Drying of slices

Dried slices

Frying of slices

Fried crackers



Packaging of crackers

Final product

designated as F0, F10, F20, F30, F40 and F50, respectively. Other ingredients used for cracker preparation were salt (2%), turmeric (1%), garam masala (3%) and pepper (1%), to improve the taste and flavour. The percentage of the ingredients was based on the weight of the fish and starch. Required water (50-100%) was used for the preparation of dough. All the ingredients were mixed and blended well in a food processor for 2-3 min until the dough was formed. The dough was then rolled manually into cylindrical shape and steamed at 100ºC in a steam cooker for 20 min. The steamed roll was then cooled at room temperature for 10 min and kept in the refrigerator (4ºC) overnight in order to harden the roll. On the next day, it was cut into round slice of 2 mm thickness using a hand slicer and dried in an oven at 50ºC. The dried slices were then fried using sunflower oil in a shallow pan for 4-5 s at 180-200ºC. After cooling at room temperature under fan, the finished products were packed in metallized aluminium pouches in a pack size of 10g and sealed using horizontal band sealer.

3.2.2. Proximate composition

Moisture content was determined by the hot air oven method (AOAC, 2005) by placing pre-weighed homogenized of fried and dried fish crackers in a hot air oven set at 100±5^{o}C for 12 h. The difference in their weights after drying was calculated and expressed as % of moisture.

Protein content was determined by Micro-Kjeldahl method (AOAC, 2005). For which, homogenized fish crackers was digested with conc. H_2SO_4 in the Kelplus digestion apparatus. Then, 5 ml of the digested sample was distilled in the presence of NaOH in the Kelplus distillation apparatus. The distillate was collected in 2% boric acid containing mixed indicator and then, titrated against standard 0.1N HCl to determine the total nitrogen. The crude protein content was calculated by multiplying the nitrogen content with the factor 6.25 and expressed as % of protein on wet weight basis.

Lipid content was determined by Automatic Solvent Extraction method (AOAC, 2005). Approximately 1-2 g of powdered fish crackers was placed in a thimble of socplus extraction apparatus and extracted with petroleum ether (60-70^{o}C) at 200^{o}C for 2 h. The extracted fat in the receiver beaker was then weighed after removal of the solvent to determine the crude fat as % on wet weight basis.

Ash content was determined as per the standard method (AOAC, 2005). For which, pre-weighed moisture free sample was taken in silica crucible and placed in a muffle furnace set at 550-600^{o}C for 12-16 h and the difference in the weights after ashing was calculated and expressed as % of ash on wet weight basis.

Carbohydrate content was determined by Phenol-Sulphuric acid method of DuBois *et al.* (1956). Homogenized sample was hydrolysed using 2.5 N HCl and then neutralised with solid sodium carbonate and centrifuged at 3000rpm for 5 min after making up the volume with distilled water. From that, 0.1 and 0.2 ml of sample solution was taken into two test tubes and made the volume to 1 ml with distilled water. To this add 1 ml of phenol and 5 ml of sulphuric acid. Test tubes were then placed in a water bath set at 80^{o}C for 20 min for colour development. The absorbance was then measured at 490 nm in a UV-Vis spectrophotometer. All determinations were carried out twice in triplicates. The total energy value expressed in Kcal was estimated using Atwater factors for protein, lipid and carbohydrates (Obiegbuna and Baba, 2001) as follows:

Energy value (Kcal) = (9 x fat content) + (4 x protein content) + (4 x carbohydrate content)

3.2.3. Physico - functional properties of fried fish crackers

3.2.3.1. Linear expansion

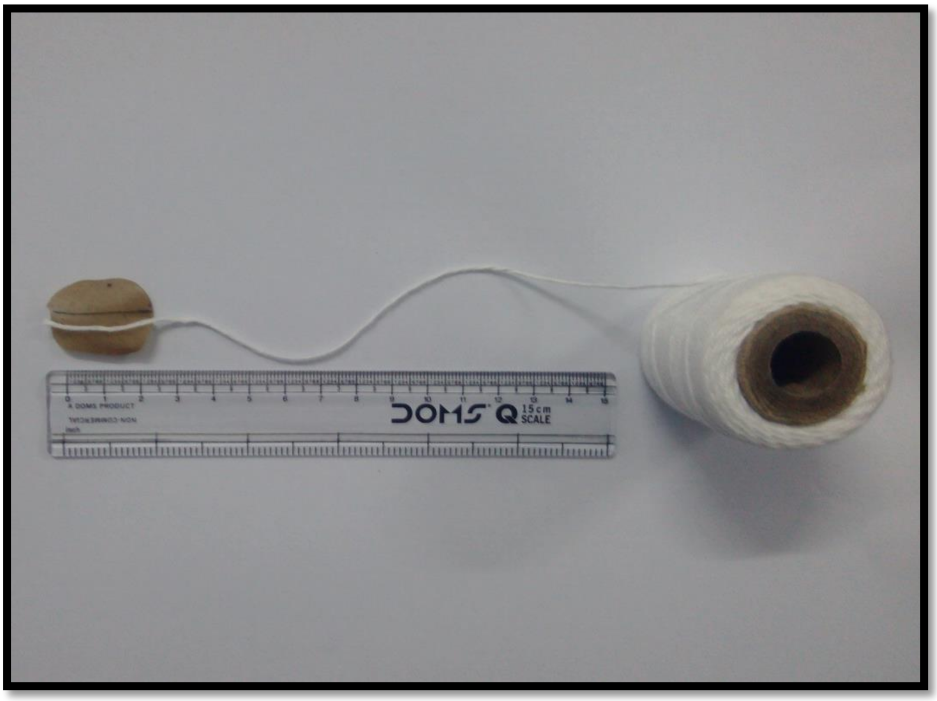
Linear expansion of the fried fish crackers was determined in triplicate as per the method of Yu (1991). The dried fish cracker was marked with three lines across using a permanent marker pen and the lines were measured before and after frying in oil at 180-200^{o}C. The percentage linear expansion was calculated as follows:

$$\text{Linear expansion (\%)} = \frac{\text{Length after frying} - \text{Length before frying}}{\text{Length before frying}} \times 100$$

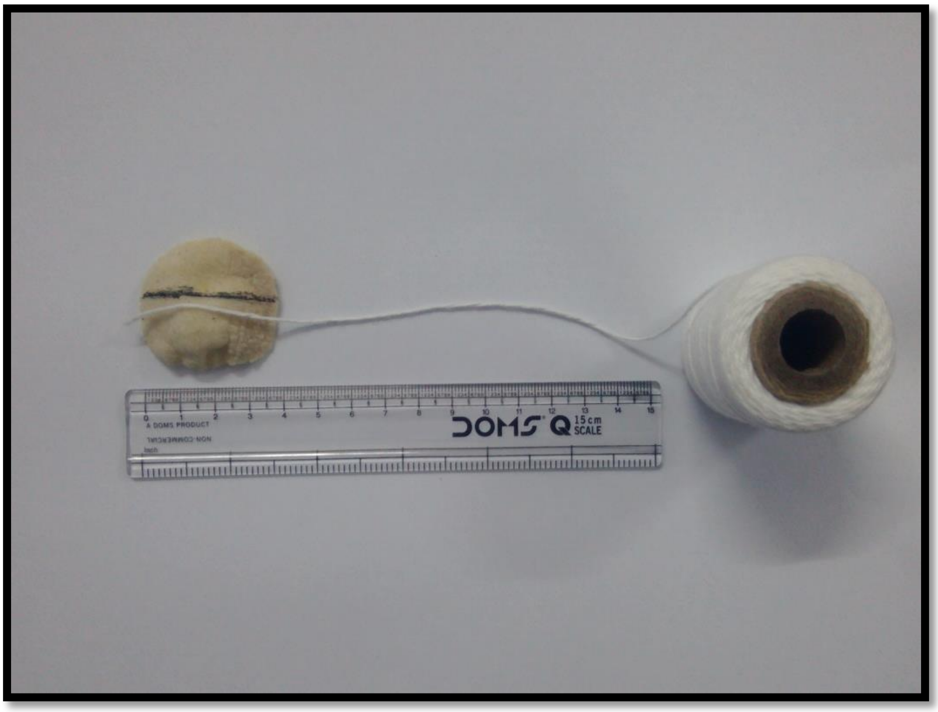
3.2.3.2. Volume expansion

Volume expansion of the fish crackers was determined in triplicate using sesame seed displacement method as described by Sahin and Sumnu (2006). Volume of an empty beaker was measured by determining how much quantity of sesame seed is required to fill the beaker. Then the dried fish crackers were added to the beaker and how much sesame seed got displaced on the addition gives the volume of the dried fish crackers. Similarly the volume of the fried fish crackers was

Plate 2: Image representation of linear expansion



Measuring length of crackers before frying



Measuring length of crackers after frying

determined. The difference in the volume of the fried and dried fish crackers gives the volume expansion. The volume expansion was calculated as follows:

$$\text{Volume expansion (\%)} = \frac{\text{Volume after frying} - \text{Volume before frying}}{\text{Volume before frying}} \times 100$$

3.2.3.3. Bulk density

Volume of the fish crackers was measured in triplicate as per the above method. Then, the mass of the cracker was measured using an electronic balance. The bulk density was calculated as follows:

$$\text{Bulk density } (\rho) = \frac{\text{Mass of the fish cracker (g)}}{\text{Volume of the cracker (cm}^3\text{)}}$$

3.2.3.4. Oil absorption

Oil absorption of the fried fish crackers was measured in triplicate as per the method of Nurul *et al.* (2009). The crackers were weighed before and after frying in hot oil. Then, they were ground and dried in hot air oven set at 105^{o}C for an overnight.

$$\text{Oil absorption (\%)} = \frac{\text{Dried sample weight after frying (g)} - \text{Dried sample weight before frying (g)}}{\text{Dried sample weight before frying (g)}} \times 100$$

3.2.3.5. Water absorption index (WAI) and Water Solubility Index (WSI)

WAI and WSI of the fried fish crackers were determined in triplicate as per the method of Anderson *et al*. (1969). For which, 2 g of fried crackers were suspended in 30 ml of distilled water and stirred well for 30 min occasionally at room temperature and centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge. The supernatant was decanted into an evaporating dish and allowed to dry in a hot air oven at 105^{o}C for overnight. The weight of the gel after removing the supernatant and the weight of the sediment after drying the supernatant were noted. The WAI and WSI were calculated as follows:

$$\text{WAI}\ (\%) = \frac{\text{Weight of the gel (g)}}{\text{Weight of the dry sample (g)}} \times 100$$

$$\text{WSI}(\%) = \frac{\text{Weight of the sediment (g)}}{\text{Weight of the dry sample (g)}} \times 100$$

3.2.3.6. Colour

Lightness (L*), redness/ greenness (a*) and yellowness/ blueness (b*) of the fried fish crackers were measured using Hunter Lab MiniScan® XE Plus Spectrocolorimeter with geometry of diffuse 8°(sphere-8 mm view) and an illuminant of D 65 optical sensor and 10° standard observer. The instrument was calibrated each time before its use. A white standard board was used for calibration.

3.2.3.7. Hardness

Hardness value was measured in triplicate in terms of Newton (N) using a texture analyser equipped with a spherical probe of diameter 7mm, for determining the crispiness of the fried fish crackers. The trigger and speed of the probe were set at 0.05N and 10mm/min, respectively. The dried slices were fried in oil and the fried crackers were put above a support rig and penetrated using the spherical probe.

3.2.3.8. FTIR Analysis

Attenuated Total Reflection- Fourier Transform infrared spectroscopy (ATR-FTIR) spectra of individual ingredients viz. tapioca, corn, sago, fish as well as dried and fried fish crackers were determined using an iD3 ATR-FTIR Spectrophotometer. Potassium bromide (KBr) disks were first prepared with each sample in order to place onto the crystal cell and the cell was clamped into the mount of the spectrometer. The signal was collected from a range of 500-4000 cm^{-1} in 32 scans at a resolution of 4 cm^{-1} and was rationed against a background spectrum recorded from the clean empty cell at 25°C.

3.2.4. Optimization of processing conditions by RSM

To optimize the steaming time, gel setting time and drying temperature during processing of fish crackers, Box-Behnken Design of Response Surface Methodology (RSM) was used. Three levels of steaming time (A), gel setting time (B) and drying temperature (C) were selected. Based on the previous experiments conducted and from articles referred, a steaming time of 40 min, a gel setting time of 18 h and a drying temperature of 50ºC were chosen as the centre points. Linear expansion, bulk density and crispiness were taken as output variables and were estimated as described earlier. Coded values and corresponding values of the independent variables are given in Table 3. The Design Expert 7.0 software was used to obtain Box- Behnken quadratic design model, which gave 17 runs to perform the analysis. Response surface and contour plots were generated using Design Expert 7 software.

Table 3: Experimental design with 3 parameters

Variables	Codes		
	-1	**0**	**1**
A (Steaming time, min)	20	40	60
B (Gel setting time, h)	12	18	24
C (Drying temperature, ºC)	40	50	60

3.2.5. Shelf stability studies on fish crackers

Fish crackers both dried and fried made with fish to starch ratio of 40:60 were chosen for studying the shelf stability. They were packed and stored in a clean dry place for a period of three months. Periodically samples were drawn for analysis to examine the various quality, microbiological and sensory analysis.

3.2.6. Quality analyses

3.2.6.1. Water activity

Powdered samples (2g) were taken up to half of the sample cup and tested by water activity meter previously calibrated with lithium chloride standards.

3.2.6.2. TBARS

TBARS of the fried fish crackers was determined as per the method of Raghavan and Hultin (2006). Crackers were homogenized with 7.5% TCA at the ratio of 1:3 and centrifuged at 9000 rpm for 10 min. The supernatant was filtered using Whatman No.1 filter paper. Two ml of the filtrate was mixed with equal volumes of 0.02M TBA reagent, kept in the boiling water bath for 40 min, cooled and the absorbance was measured at 535 nm in the UV-Vis spectrophotometer. TBARS value was calculated using the following formula and expressed as mg of malonaldehyde equivalent / kg of sample.

$$\text{TBARS (mg of malonaldehyde / kg of sample)} = \frac{A \times 72.03 \times V \times 1000 \times TV \times 1000}{156000 \times 1000 \times VE \times W}$$

where, A = absorbance at 535 nm; 72.03 = molecular weight of malonaldehyde; V = volume of reactants (TBA+TCA extract); TV = total volume of extract; 156000=Molar extinction co-efficient of malonaldehyde; VE = volume of extract added to TBA solution; W = weight of the sample (g).

3.2.7. Microbiological examination

For the enumeration of bacteria, 25 g of fish crackers was taken aseptically and homogenized with 225 ml of sterile saline (0.85% NaCl). The homogenized sample was then serially diluted using 9 ml diluent prior to pour plating. From each dilution, inoculum was taken for enumerating total plate count, total staphylococci and total fungal count. For the enumeration of total coliforms, 3 tube MPN technique was followed. The media, methodology, incubation period and time adopted for the enumeration and isolation of bacteria are given in Table 4.

Table 4: Media, methodology, incubation period and time adopted for the enumeration and isolation of bacteria

S.NO	Target bacteria	Media/Broth	Method	Incubation	Reference
1	Total plate count (TPC)	Plate count agar	Spread plate	48 h at 37ºC	APHA, 1976
2	Total fungal count (TFC)	Potato dextrose agar	Spread plate	48 h at 37ºC	APHA,1976
3	Total Staphylococci (TS)	Baird Parker agar	Spread plate	48 h at 37ºC	APHA, 1976
4	Total coliforms (TC)	1. Lauryl sulphate tryptose (LST) broth 2. Brilliant green lactose bile (BGLB) broth 3. Ecoli (EC) broth	MPN method	48 h at 35ºC (LST) 48 h at 35ºC (BGLB) 48 h at 44.5±2ºC (EC)	AOAC, 1998
		4.Levine's eosin-methylene blue (EMB) agar	Streaking	18-24 h at 35± 2ºC	

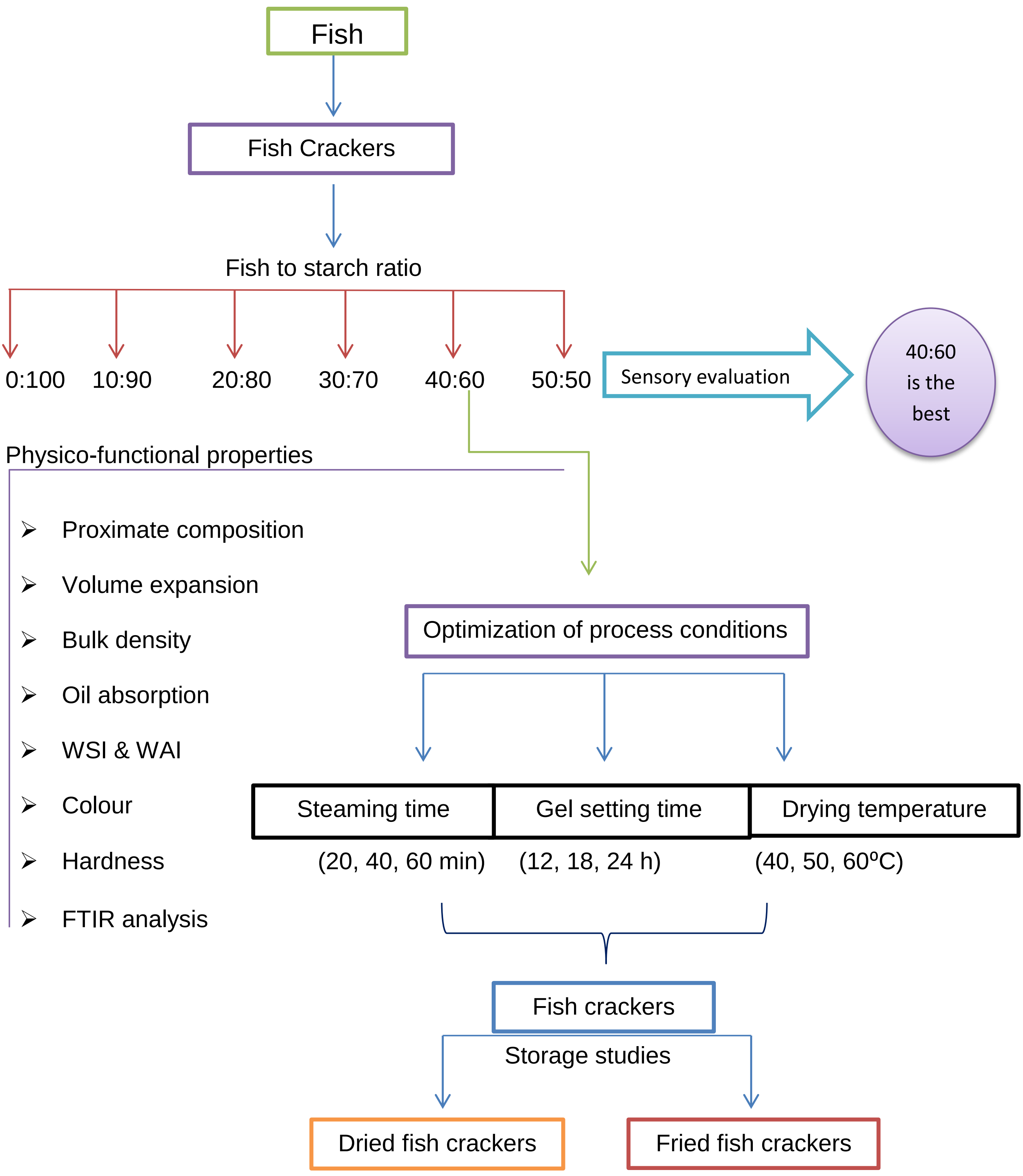
3.2.8. Sensory evaluation

Sensory evaluation was carried out for the fried fish crackers by 20 subject experts available in the department, who have put more than 5 years of experience in Fish Processing Division. They were asked to indicate their opinion on a 9 point hedonic scale (9 for excellent to 1 for extremely poor). The experts analysed each product for their colour, odour, taste, crispiness, appearance to determine the overall quality. Score sheet for sensory evaluation of fish crackers is given in annexure 1.

3.2.9. Statistical analysis

Data for optimization of gelatinization conditions of fish crackers was analysed using response surface methodology (Design Expert 7.0 software). Analysis of variance was performed for the response surface quadratic model and the model F value of 5.96 implied that the model was significant. The model terms were significant at $p < 0.05$. The results of sensory, physical, biochemical and microbiological characteristics of the fish crackers were interpreted statistically using one way analysis of variance. Significant difference between the means of triplicates were determined by Duncans multiple comparison test using the SPSS 16.0.

Experimental design



Results

IV. RESULTS

Fish crackers were prepared with different fish to starch ratio inorder to choose the best one based on physico-functional properties and the results are presented below:

4.1. Nutritional composition of fish crackers

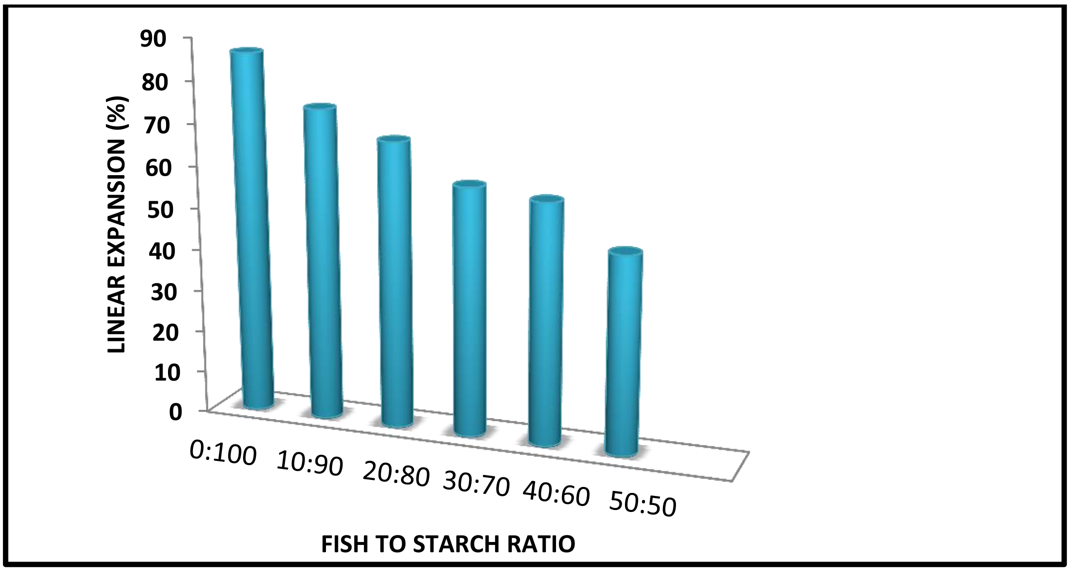
The proximate analysis of the fish was analysed. They had a moisture content of 75.01±0.75%, protein content of 18.05±0.01%, fat content of 2.30±0.02%, ash content of 3.41±0.08% and carbohydrate content of 0.21±0.01%. Also the quality of the fish used was very fresh. Proximate composition of the dried and fried fish crackers prepared at different fish to starch ratios were analysed to know their nutritious status (Tables 5 and 6). Dried crackers had their moisture contents in the range of 10.33 to 10.88%, while the fried ones from 2.37 to 2.73 %. The normal dried crackers without fish had 3.31% protein, while the fish crackers with 50% fish possessed 18.74% protein. Significant decrease in the carbohydrate content of dried crackers from 83.22% to 61.54% was recorded with the increase in fish to starch ratio. Similar decrease was also noticed in fried fish crackers from 70.73 to 56.29%. Fat content increased from 0.24 to 1.66% in dried crackers with the increase in fish to starch ratio. On the contrary, the fat content decreased in fried crackers from 24.61% to 19.52% with the increase in fish to starch ratio. Total minerals, determined as ash content also showed variation with the increase in fish to starch ratio. In dried fish crackers, it increased from 2.33% to 5.51% whereas in fried ones, it increased from 0.99 to 3.91 %. However a reduction in the ash content is noticed when the crackers got fried. There was no significant difference in the total energy values of dried fish crackers as it ranged between 336-348 Kcal, while in fried fish crackers, a significant decrease was noticed in the total energy values from 510 to 466 Kcal ($P<0.05$).

4.2. Physico- functional properties of fish crackers:

4.2.1. Linear expansion

There was a significant reduction in the linear expansion of fish crackers from 86.31± 9.05 to 46.69 ± 3.75% as the proportion of fish increased from 0 to 50% (Fig 1). The decrease was gradual at low fish concentration, and later remained almost same at 30 and 40% and finally, a sudden decrease in the expansion was noticed.

Fig 1: Linear expansion of fried fish crackers prepared with different ratios of fish and starch



4.2.2. Volume expansion

There was a significant decrease in the volume expansion of crackers from 800 ± 12.36 to 133 ± 10.73%, with the increase of fish to starch ratio (Fig 2). Although linear and volume expansion had a positive correlation, the decrease was not proportional. A drastic reduction had occurred with the addition of fish up to 20%, which later remained constant until 40% and again declined.

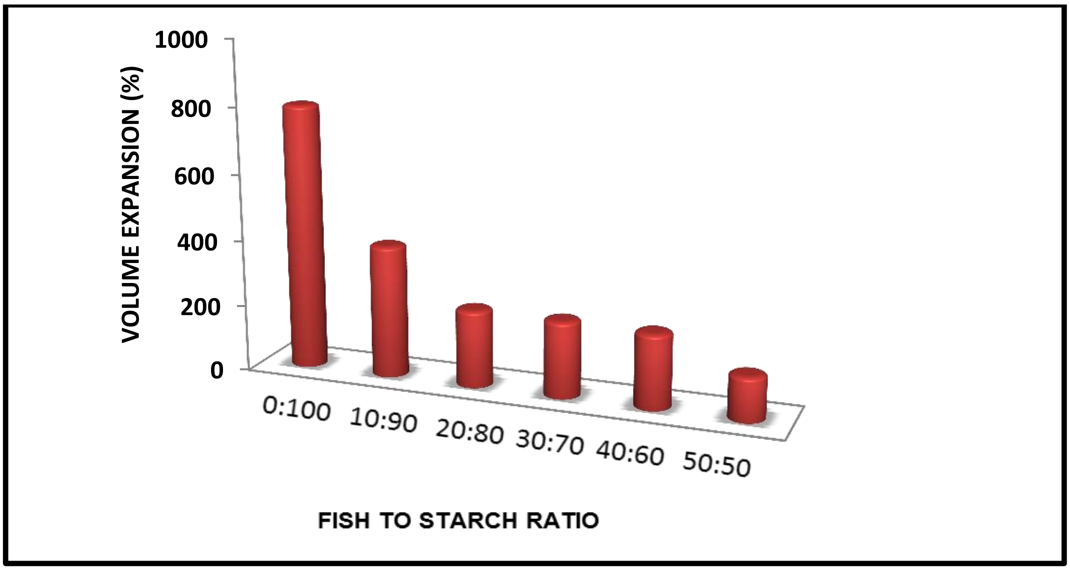
Table 5: Proximate composition of dried fish crackers prepared with different fish to starch ratio

DRIED CRACKERS	0:100	10:90	20:80	30:70	40:60	50:50
Moisture (%)	10.88 ± 0.02	10.77 ± 0.00	10.63 ± 0.04	10.58 ± 0.04	10.46 ± 0.07	10.33 ± 0.15
Protein (%)	3.31 ± 0.23	5.27 ± 0.27	6.42 ± 0.41	10.02 ± 0.42	14.04 ± 0.54	18.74 ± 0.38
Fat (%)	0.25 ±0.049	0.55 ± 0.04	0.66 ± 0.01	0.74 ± 0.04	0.98 ± 0.18	1.66 ± 0.02
Ash (%)	2.33 ± 0.01	2.37 ± 0.01	2.87 ± 0.04	3.37 ± 0.05	4.16 ± 0.18	5.51 ± 0.01
Carbohydrate (%)	83.22 ± 0.02	80.00 ± 0.10	77.94 ± 0.03	73.59 ± 0.02	69.01 ± 0.06	61.54 ± 0.04
Total energy (KCal)	348.325	346.028	343.356	341.077	341.015	336.12

Table 6: Proximate composition of fried fish crackers prepared with different fish to starch ratio

FRIED CRACKERS	0:100	10:90	20:80	30:70	40:60	50:50
Moisture (%)	2.73 ± 0.03	2.65 ± 0.714	2.52 ± 0.243	2.45 ± 0.22	2.38 ± 0.237	2.37 ± 0.06
Protein (%)	1.44 ± 0.08	4.84 ± 0.035	5.19 ± 0.064	8.65 ± 0.16	12.65 ± 0.041	16.41 ± 0.33
Fat (%)	24.61 ± 0.41	23.06 ± 0.06	22.62 ± 0.395	21.64 ± 0.32	20.99 ± 0.183	19.52 ± 0.41
Ash (%)	0.99 ± 0.01	2.22 ± 0.02	2.38 ± 0.02	2.58 ± 0.02	3.28 ± 0.023	3.91 ± 0.06
Carbohydrate (%)	70.73 ± 0.01	66.92 ± 0.05	65.09 ± 0.07	61.918 ± 0.02	58.65 ± 0.09	56.29 ± 0.02
Total energy (KCal)	510.125	494.536	484.619	476.967	474.187	466.475

Fig 2: Volume expansion of fried fish crackers prepared with different ratios of fish and starch



4.2.3. Bulk density

Bulk density of fish crackers increased with the increase in fish to starch ratio due to the decrease in the volume expansion (Fig 3). The values ranged from 0.07±0.03 to 0.51±0.06 g/cm^3. An inverse relationship between volume expansion and bulk density was noticed since both are negatively correlated.

4.2.4. Oil absorption

The crackers made without fish had high oil absorption and volume expansion (Fig 4). With the increase in fish to starch ratio, interestingly, the oil absorption decreased gradually from 39.70±3.94 to 24.38±1.27 %.

4.2.5. Water absorption index (WAI)

The WAI of fish crackers was more or less constant until the fish proportion was 40% and thereafter it showed a sharp decline (Fig 5). The WAI of crackers without fish was found to be 6.90%. The values for the fish crackers ranged from 6.89±1.17 to 4.70 ±1.13% with the least being observed with 50% fish concentration.

Fig 3: Bulk density of fried fish crackers prepared with different ratios of fish and starch

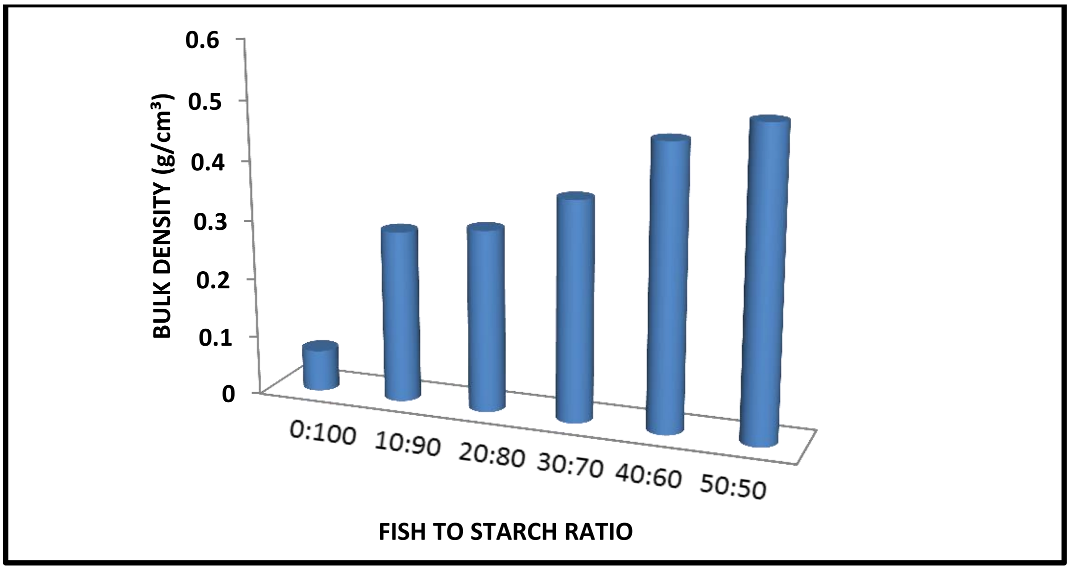


Fig 4: Oil absorption of fried fish crackers prepared with different ratios of fish and starch

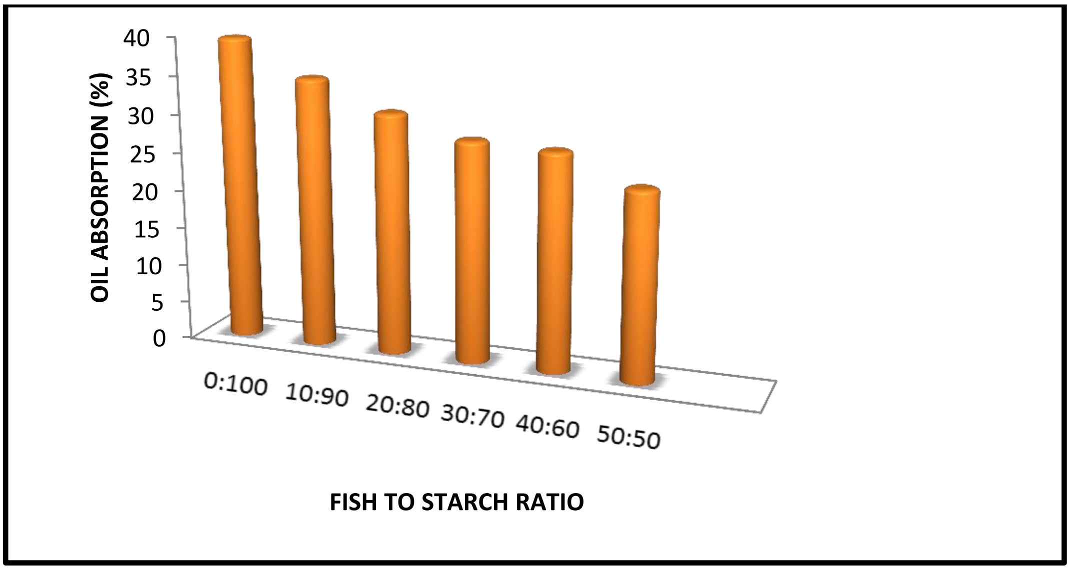
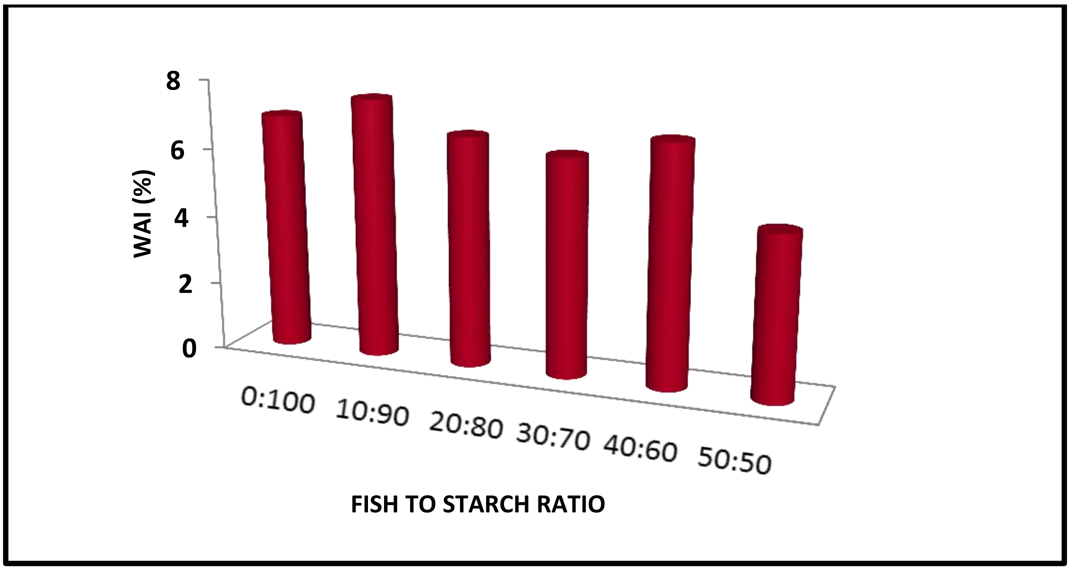


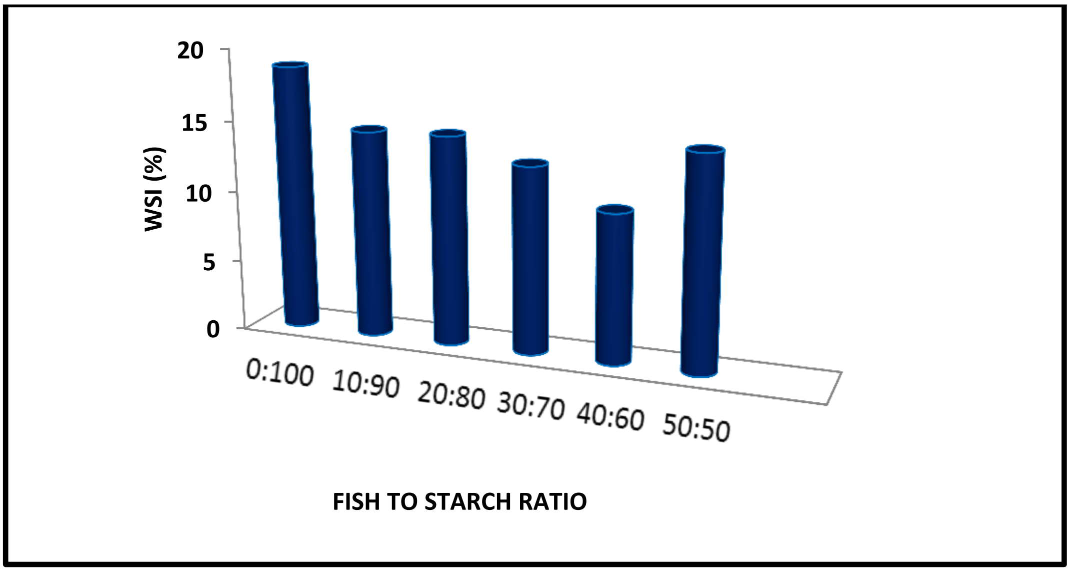
Fig 5: Water absorption index of fried fish crackers prepared with different ratios of fish and starch



4.2.6. Water solubility index (WSI)

The WSI of crackers without fish was more, which later decreased with the increase in fish proportion upto 40% from 18.66±2.13 to 10.41±0.95%, and again increased (Fig 6). The decline observed in WAI of the crackers with 50% fish coincided well with the increase in WSI.

Fig 6: Water solubility index of fried fish crackers prepared with different ratios of fish and starch



4.2.7. Colour

Colour characteristics of fried fish crackers are shown in Table 7. Colour values showed significant differences ($p < 0.05$) among the fried crackers. With the increase in the fish ratio or decrease in the starch ratio, L* value corresponding to lightness or whiteness of the product decreased and ranged from 66.11± 0.38 to 50.97± 0.06, whereas a* value corresponding to redness of the product and b* value corresponding to the yellowness of the product were found to be increased. The values of a* ranged from 2.51 ± 0.02 to 9.45 ± 0.07 and the values of b* ranged from 19.13 ± 0.02 to 28.55 ± 0.06 in the fried crackers.

Table 7: Colour of fried fish crackers prepared with different fish concentration

Fish to starch ratio	Lightness (L*)	Redness (a*)	Yellowness (b*)
0:100	66.11 ± 0.38 ^{f}	2.51 ± 0.02^{a}	20.38 ± 0.17 ^{b}
10:90	62.73 ± 0.07 ^{e}	2.69 ± 0.01 ^{b}	19.13 ± 0.02 ^{a}
20:80	57.64 ± 0.11 ^{d}	4.95 ± 0.03 ^{c}	22.20 ± 0.02 ^{c}
30:70	55.95 ± 0.09 ^{c}	5.77 ± 0.16 ^{d}	23.39 ± 0.23 ^{d}
40:60	54.23 ± 0.10 ^{b}	7.24 ± 0.08 ^{e}	26.69 ± 0.13 ^{e}
50:50	50.97 ± 0.06^{a}	9.45 ± 0.07 ^{f}	28.55 ± 0.06 ^{f}

*All the data were expressed as mean ± standard deviation. The different superscripts in the same column indicate that the values were significantly different at $p<0.05$.

4.2.8. Hardness

Hardness of the fried fish crackers slightly increased with the increase in fish to starch ratio, while a sharp increase was recorded when the fish proportion was 50% (Fig 7a). A representative graph on hardness measurement is also included (Fig 7b). In that, the number of break points in the force curve during hardness measurement was found to be 8. Hardness value is inversely proportional to the crispiness. Hardness value was found to be minimum (5.01N) for the normal crackers without fish, while for the fish crackers, the values ranged between 5.47±2.13 to 9.32±2.39N.

Fig 7a: Hardness of fried fish crackers prepared with different ratios of fish and starch

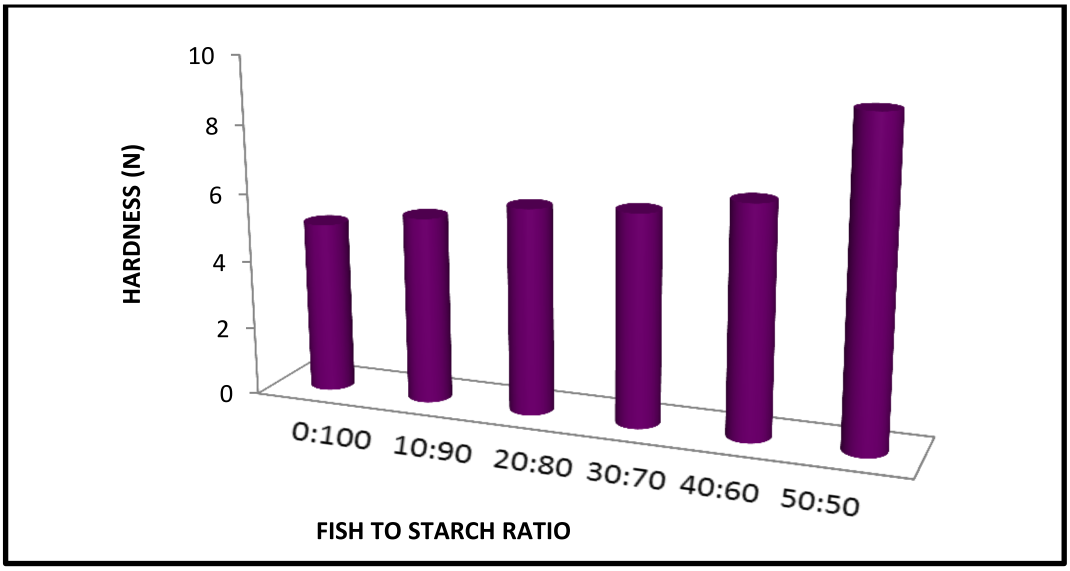
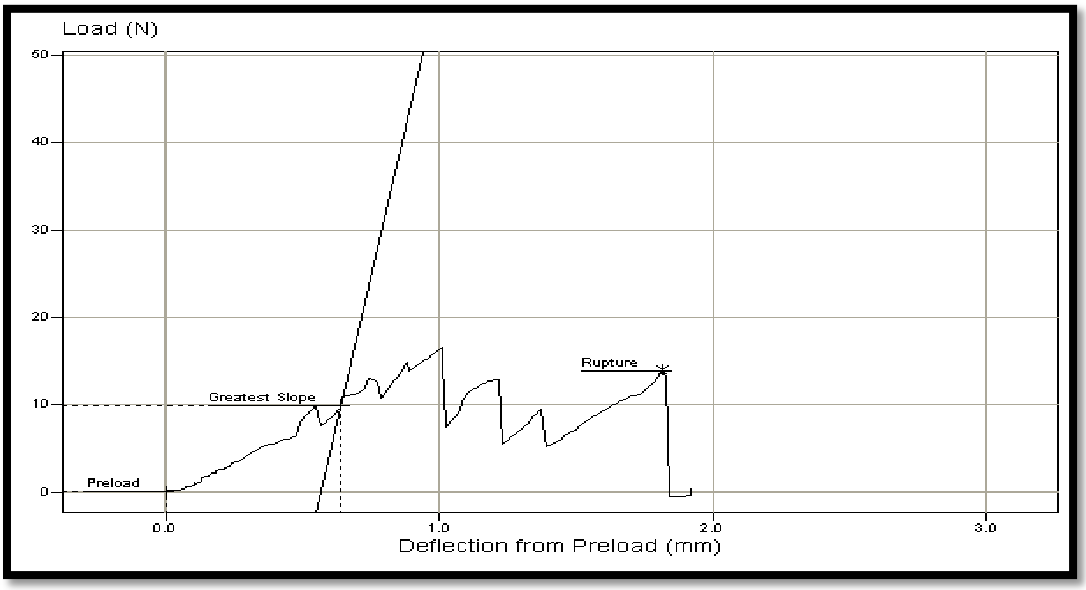


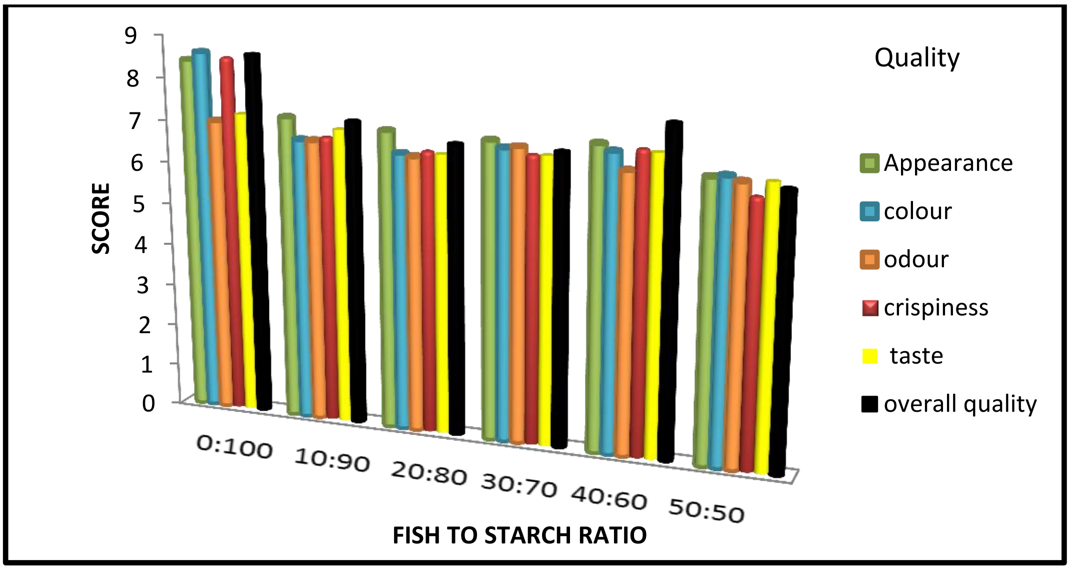
Fig 7b: A representative graph showing the hardness of fried fish crackers



4.2.9. Sensory changes

With regard to odour and taste, the average score values of fish crackers gradually increased with the addition of fish (Fig 8), however in crackers made with 50% fish, low scores were recorded due to more fishy odour and taste. The colour and crispiness scores decreased with the addition of fish, which in turn, also affected the appearance scores. However, the overall acceptance score of crackers made with 40% fish was found high.

Fig 8: Sensory score of fried fish crackers prepared with different ratios of fish and starch

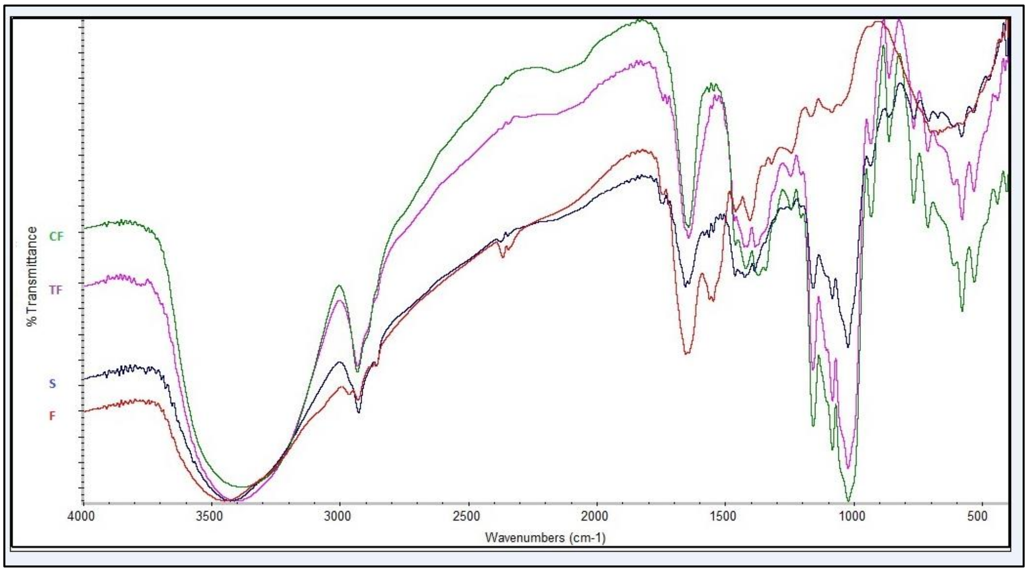


4.2.10. FTIR analysis

FTIR spectral analysis was undertaken to examine the functional interaction that took place during the formulation and processing of fish crackers. As a prelude, the FTIR spectra of the individual ingredients viz. tapioca, fish, sago and corn were analysed (Fig 9a). FTIR spectra of tapioca, corn and sago starch before gelatinization showed 8 typical major bands in the region of 800-1400 cm^{-1} but at slightly different frequencies. The major bands were found near 1420, 1380, 1242, 1155, 1080, 1019, 930 and 860 cm^{-1} wavenumber with slight shifts among the starches. Two bands at 1380 and 1241 cm^{-1} were missing in sago because of fewer frequencies. Common bands observed in starch and fish were found at around 3400, 2925, 1640-1651 and 1080 cm^{-1}. Besides this, spectra of fish contained bands at 2362, 1400, 1317, 1164 and 610 cm^{-1} wavelengths. FTIR spectral analysis of the formulated cracker was compared with the spectrum of the ingredients (Fig 9b). There existed a major change in the intensity of the typical 8 major bands of the starch in the formulated cracker. Also, the absorption bands at

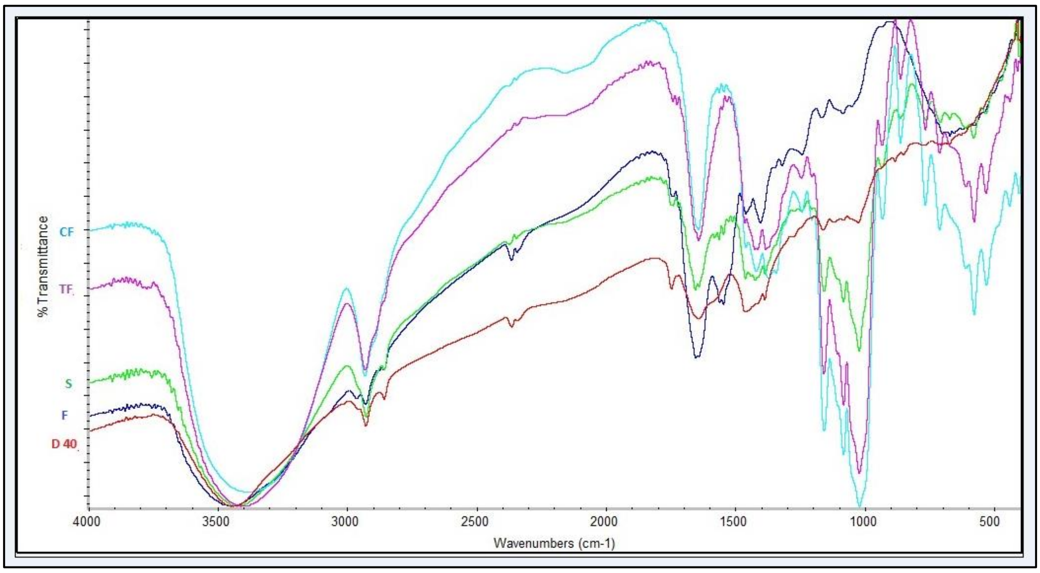
1155, 1080 and 1019 cm^{-1} showed a decrease in the bandwidth after gelatinization. The changes in the intensity of these bands were very prominent; and had diminished as small peaks. A great shift in the bands from wavenumbers 1380, 1155, 1080 and 1019 cm^{-1} to 1384,1158, 1085 and 1023 cm^{-1} was noticed (Table 8). Many of the peaks have disappeared after gelatinization. However, some major peaks at 3439, 2925, 2362 and 1642 cm^{-1}were present. Appearance of two peaks at 2854 and 1744 cm^{-1} were noticed upon gelatinization. Frying of dried crackers in vegetable oil had given a totally different spectrum for crackers (Fig 9c). Several new bands unique to vegetable oil used for frying had appeared prominently. They appeared at wavenumbers, 3426, 3009, 1746, 1654, 1378, 1238, 1099, 721 and 578 cm^{-1}. The FTIR analysis of fresh oil and the oil after frying was also analysed. Wave numbers for both fresh and fried oil occurred at peaks 3008, 2925, 2854, 1745, 1460, 1236, 1163, 1098 cm^{-1} (Fig 9d).

Fig 9a: FTIR analysis of ingredients



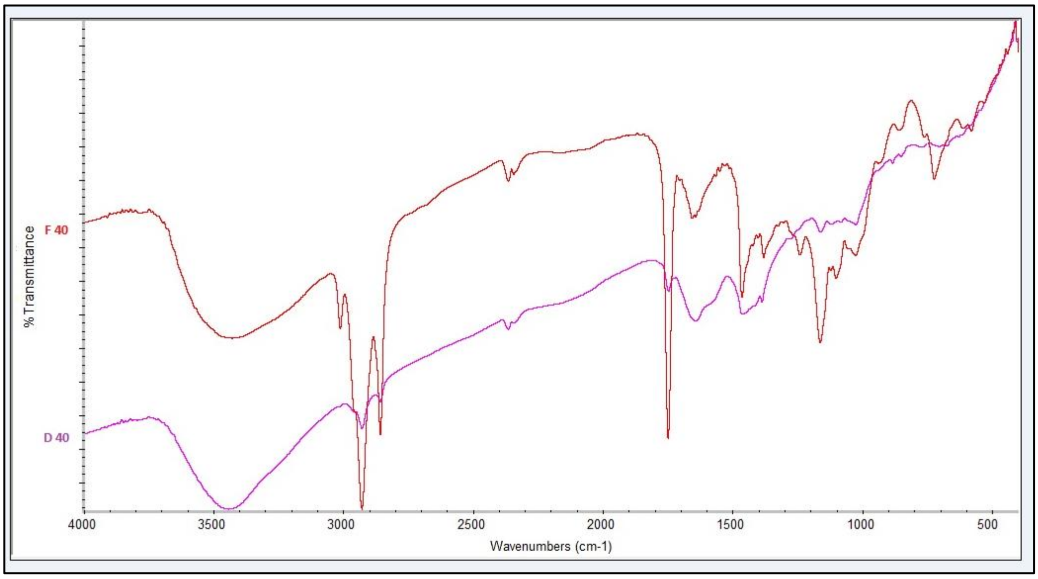
TF- Tapioca flour CF- Corn flour S-Sago F- Fish

Fig 9b: FTIR analysis of ingredients and formulated crackers



TF- Tapioca flour CF- Corn flour S-Sago F- Fish D40-Formulated crackers

Fig 9c: FTIR analysis of fried and dried crackers



D40- Dried fish crackers **F40- Fried fish crackers**

Fig 9d: FTIR analysis of fresh and fried oil

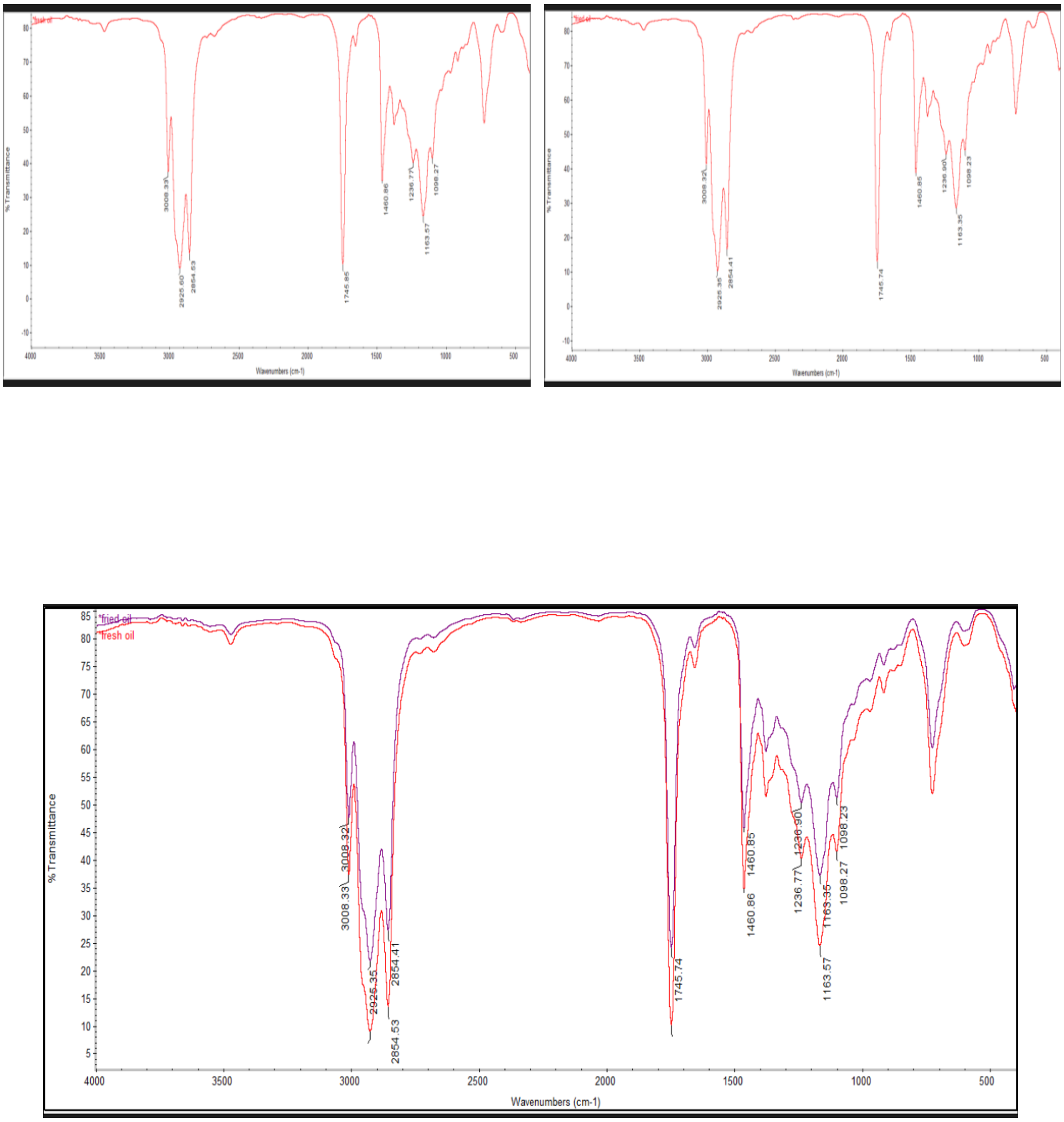


Table 8 : The comparison of shift in the functional groups present in the ingredients and in the formulated fish crackers

Bond type	Tapioca	Sago	Corn	Fish	Dried fish crackers	Fried fish crackers
	3772.73					
N-H	3410.05	3423.44	3383.41	3447.32	3439.61	3426.21
C-H (stretch)						3009.20
C-H	2927.85	2924.53	2929.07	2926.21	2925.79	2926.10
					2854.94	2854.75
				2362.37	2361.80	2361.72
C=O	1723.28	1738.75			1744.88	1746.09
C=C	1640.06	1653.51	1639.82	1651.61	1641.91	1654.46
		1544.10		1544.21		
				1457.82	1460.81	1462.24
	1422.66	1421.55	1418.45			
				1400.53		
N-O	1380.18				1384.67	1378.21
			1367.58			
				1317.42		
C-O	1242.98		1241.53	1240.46		1238.70
	1156.19	1155.75	1155.18		1158.93	1160.61
				1164.46		
	1080.05	1080.63	1080.18	1082.52		1099.15
	1019.25	1019.91	1018.63		1023.65	1023.82
	931.04	933.03	929.94			
C-H (bend)	860.73	861.32	860.74			
	764.29	763.76	764.35			
	709.20	706.83	708.91			
						721.47
C-Cl				609.55		
C-Br		577.26	575.73		578.57	

4.3. Optimization of gelatinization conditions of the fish crackers using RSM

The process parameters like steaming time, gel setting time and drying temperature were optimized to improve the physical properties like linear expansion, bulk density and crispiness of the fish crackers prepared with fish to starch ratio of 40:60. A steaming time of 40 min, gel setting time of 24 h and a drying temperature of 40ºC are found to improve the linear expansion, whereas a steaming time of 60 min was found to improve the crispiness of the product. In order to improve the bulk density, a gel setting time of 18 h was required.

4.3.1. Effect on the linear expansion

The results for linear expansion of fish crackers processed at selected process conditions are presented in Fig 10 (a-c). Linear expansion of fish crackers showed significant variation between steaming times of 20-40 min ($p < 0.05$) at the drying temperature of 40ºC (Fig 10a). The linear expansion was good at steaming times of 40 and 60 min. The linear expansion of the fish crackers showed significant difference at the drying temperatures of 40ºC and 50ºC ($p<0.05$), irrespective of steaming and gel setting times. It is noted that the linear expansion decreased at higher drying temperature (Fig 10a). There is significant difference in the linear expansion of fish cracker at higher gel setting times i.e. between 18 and 24 h ($p<0.05$), irrespective of drying temperature (Fig 10b). It was found that although the steaming time did not influence the gel setting time, the drying temperature of 40ºC gave maximum linear expansion at the gel setting time of 24 h (Fig 10c).

Fig 10a: Effect of steaming time and drying temperature on linear expansion of fish crackers

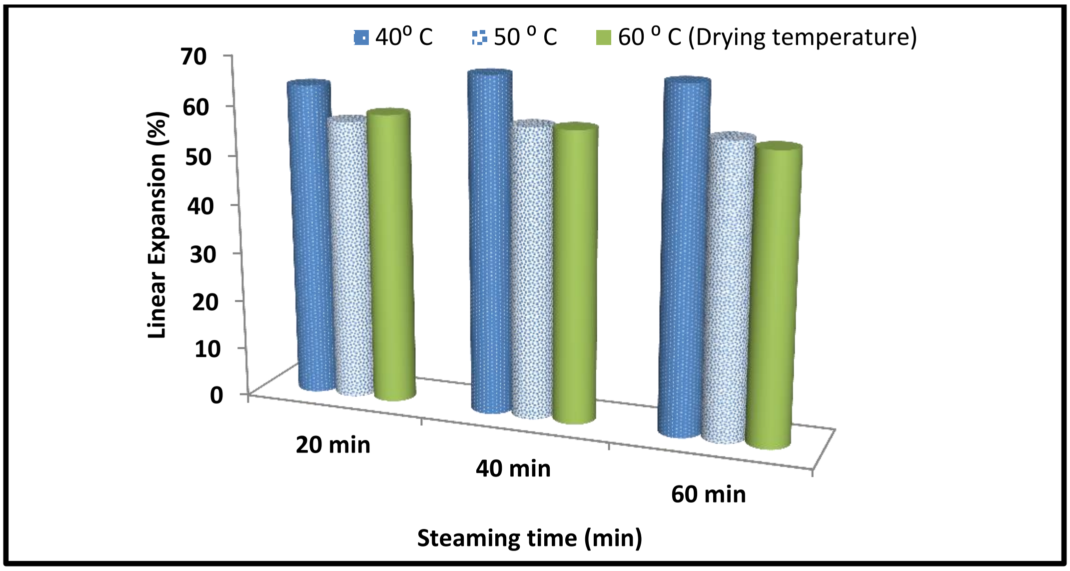


Fig 10b: Effect of gel setting time and steaming time on linear expansion of fried crackers

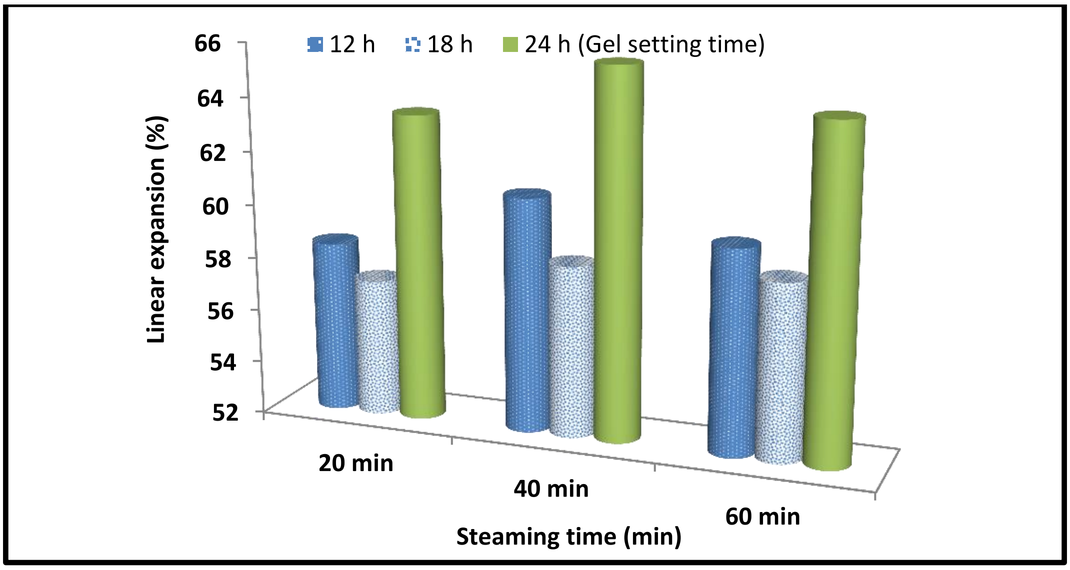
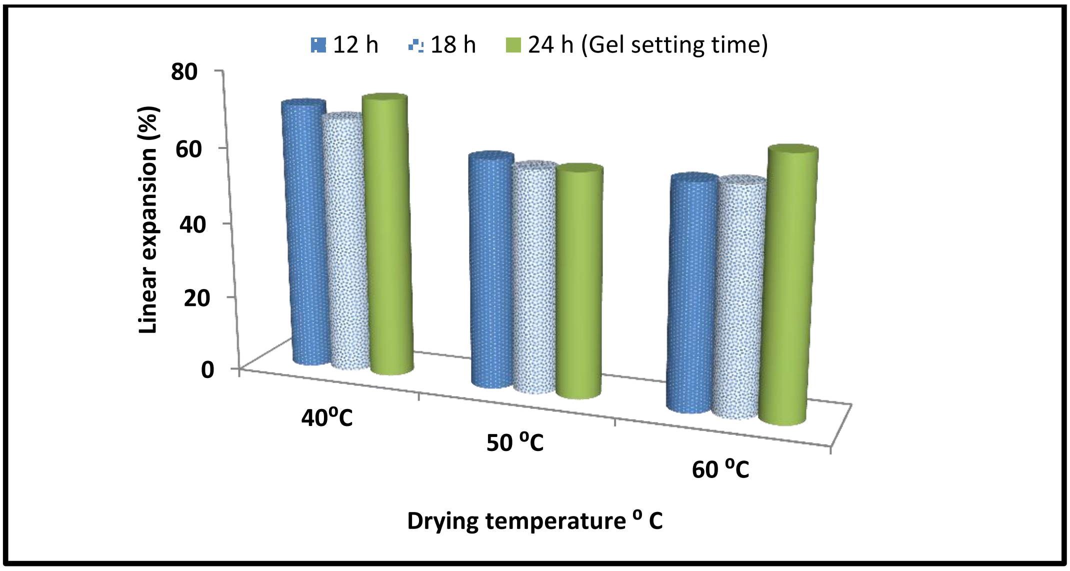


Fig 10c: Effect of drying temperature and gel setting time on linear expansion of fried fish crackers.



4.3.2. Effect on the bulk density

The results for bulk density of fish crackers made by the selected process conditions are presented in Fig 11a-c. The bulk density of fish crackers remained unchanged with an increase in drying temperature at a steaming time of 20 min; while at the steaming times of 40 and 60 min, it increased with the drying temperature (Fig 11a). Bulk density was found to be minimum at the steaming time of 40 min in fish crackers at a drying temperature of 40ºC, corresponding to the high linear expansion in the product. It was minimum at a gel setting time of 18 h at a steaming time of 40 min (Fig 11b). Bulk density did not increase at the drying temperatures between 40 and 50ºC, with the increase in gel setting time (Fig 11c). But, at a drying temperature of 60ºC, it slightly increased with the increase in drying temperature. Also, it decreased with the increase in gel setting time, irrespective of the increase in steaming time (Fig 11c). Bulk density was minimum at the gel setting time of 18 h and drying temperature of 40ºC with a steaming time of 40 min

Fig 11a: Effect of steaming time and drying temperature on bulk density of fried fish crackers

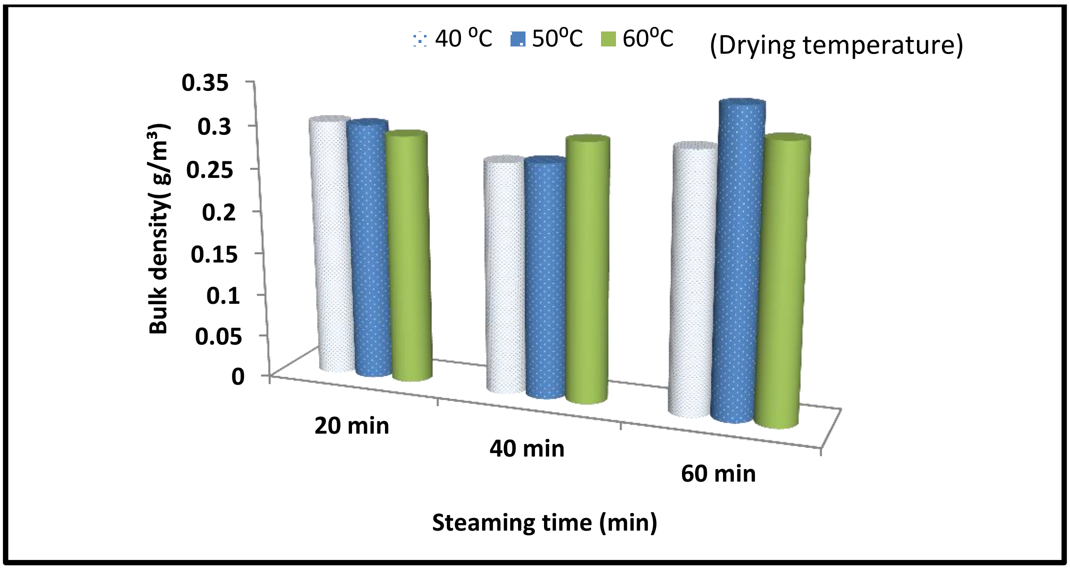


Fig 11b: Effect of gel setting time and steaming time on bulk density of fried fish crackers

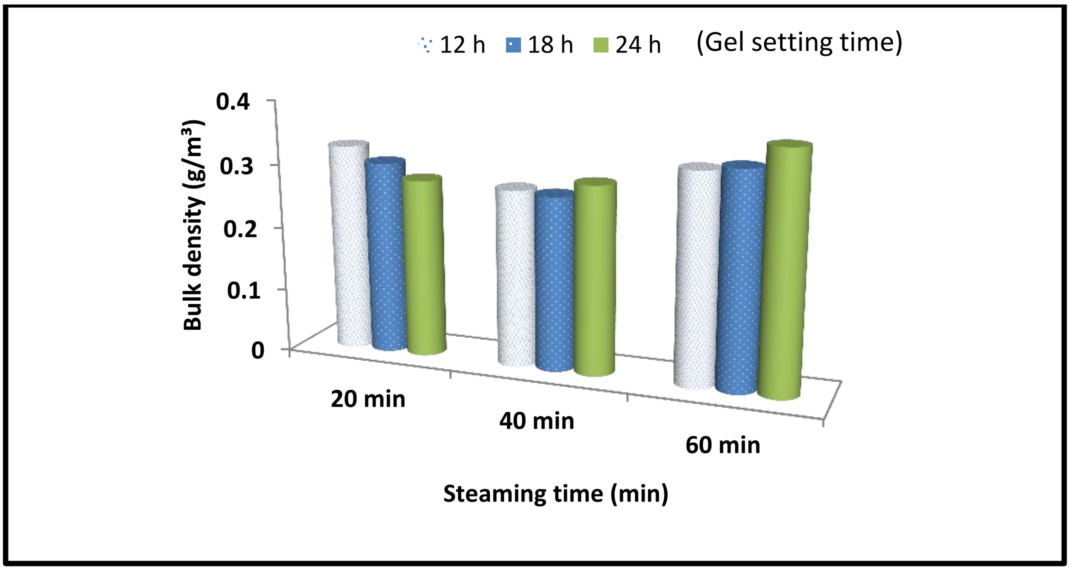
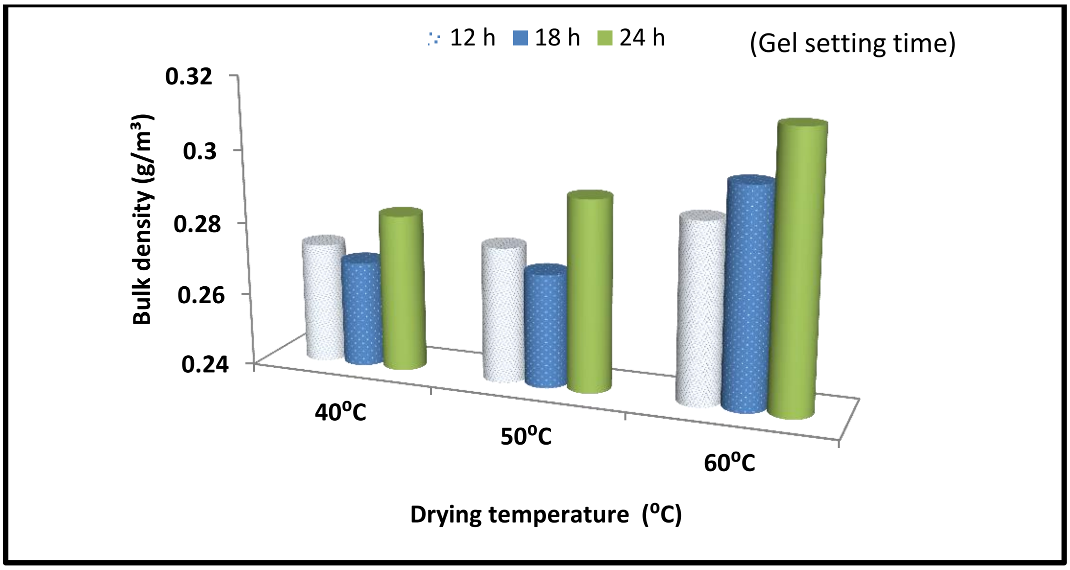


Fig 11c: Effect of drying temperature and gel setting time on bulk density of fried fish crackers



4.3.3. Effect on the crispiness

The results on the crispiness of fish crackers processed at different process conditions are presented in Fig 12a-c. Crispiness of the fried crackers at the drying temperatures of 40 and 50ºC increased with the increase in steaming time but not at the drying temperature of 60ºC. More crispy crackers were developed at a steaming time of 60 min with a drying temperature of 40ºC (Fig 12a). Crispiness decreased with the increase in drying temperature from 40 to 60ºC, irrespective of the gel setting time. Crispiness showed a different trend with the increase in gel setting time. At a gel setting time of 12 h, the crispiness was more, while at 18 h, it was less and further at 24 h, the crispiness improved, irrespective of the increase in steaming time (Fig 12b). The crackers were crispy at a gel setting time of 24h and a drying temperature of 40ºC (Fig 12c). Hence, it was found that a steaming time of 60 min produced more crispy crackers at a drying temperature of 40ºC but at the gel setting times between12 and 24 h.

Fig 12a: Effect of steaming time and drying temperature on crispiness of fried fish crackers

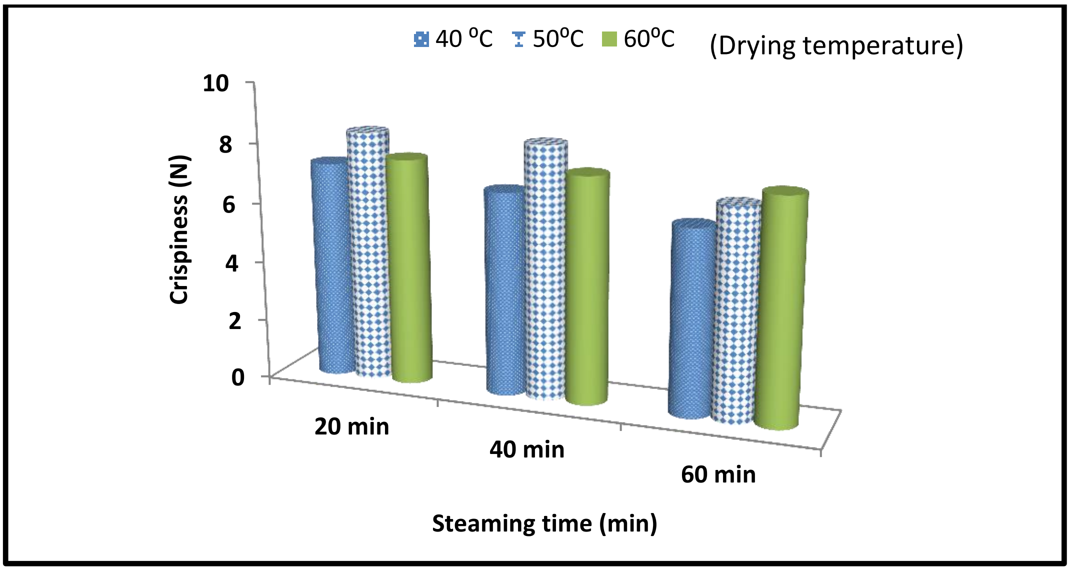


Fig 12b: Effect of gel setting time and steaming time on crispiness of fried fish crackers

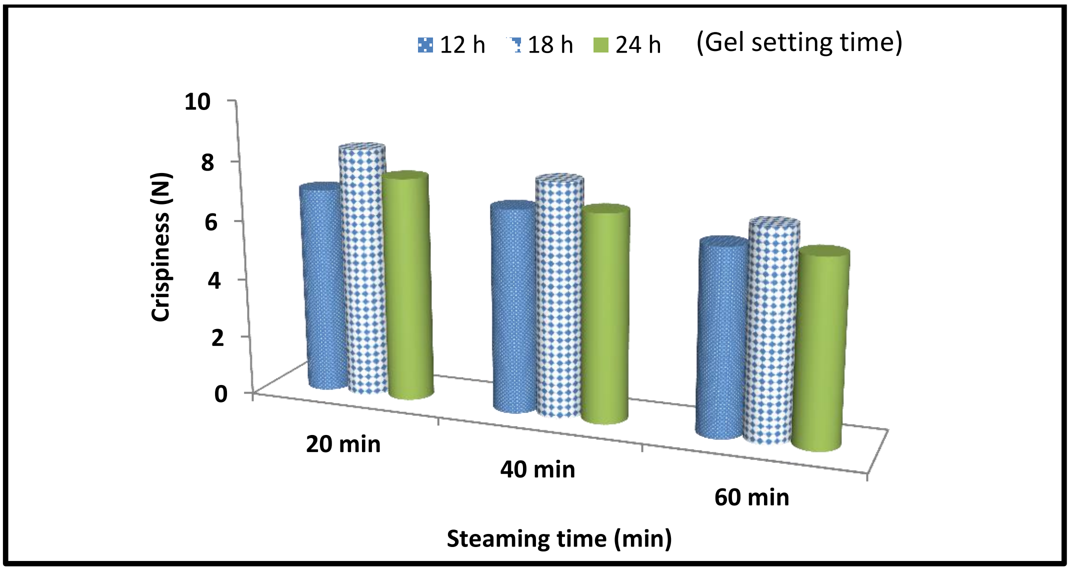
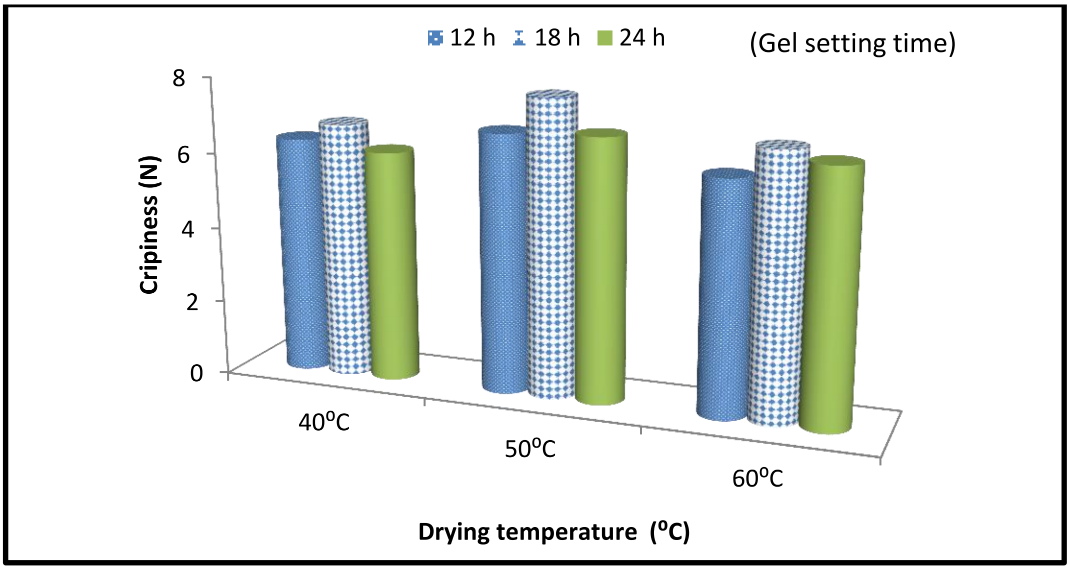


Fig 12c: Effect of drying temperature and gel setting time on crispiness of fried fish crackers



4.3.4. Model Parameters and Evaluation

The regression coefficients of quadratic equations for linear expansion, bulk density and crispiness were analysed by using ANOVA from Design Expert 7.0 software. The regression coefficient for the quadratic model is shown in Table 9. Statistical analysis of quadratic model indicates that p value of the model was <0.05, which explained that the model was significant and can be used for optimization of gelatinization conditions. The linear expansion of fish crackers was significantly affected by drying temperature and by gel setting time ($p<0.05$). The bulk density of fish crackers was however significantly affected only by the steaming time ($p<0.05$). The crispiness of fish crackers was significantly dependent on steaming time ($p<0.05$). The R^2 coefficient values of bulk density was high (>0.9), while those of linear expansion (0.86) and crispiness (0.84) were slightly low, however fitted quadratic equation. The p values of lack of fit were also large for all the three cases, which implied that it is not significant relative to the poor error.

Final equation in terms of coded factors is given as a) Linear Expansion = 58.40 + 0.18*A – 5.35*B + 2.22*C – 2.77*A*B + 0.29*A*C + 0.21* B*C –1.66*A² + 4 B² + 3.85*C² b) Bulk density = 0.27 + 0.020*A + 0.021*B – 1.875*C + 0.036*A*B +0.024*A*C – 2.750*B*C + 0.049*A² - 0.016*B² + 2.975*C² c) Crispiness = 7.81 – 0.74*A + 0.90*B + 0.22*C + 0.81*A*B – 0.27*A*C – 0.69*B*C – 0.14*A² - 1.27*B²- 1.40*C².

The validity of the models was confirmed by conducting the experiments under the optimum conditions. The predicted values calculated from these models are presented with the experimental values in Table 10.

Table 9: **The regression coefficients model**

Factor	Coefficient		
	Linear expansion (%)	Bulk density (g/m^3)	Crispiness (N)
Intercept	58.40	0.27	7.81
A –Steaming time	0.18	0.02*	-0.74
B –Drying temperature	-5.35*	0.02*	0.90*
C –Gel setting time	2.22	-1.88	0.22
AB	-2.77	0.04*	0.81
AC	0.29	0.02*	-0.27
BC	0.21	-2.75	-0.69
A^2	-1.66	0.05*	-0.14
B^2	4*	- 0.16	-1.27*
C^2	3.85*	2.975	-1.40*
R^2	0.86	0.92	0.84

***Parameter is significant to the regression model.

The contour plots of linear expansion (Y_1) , bulk density (Y_2), crispiness (Y_3) of fried fish crackers as affected by steaming time (A), drying temperature (B), and gel setting time (C) are shown in Fig 13 a-c. The linear expansion of fish crackers increased with the increase in the steaming time and the decrease in drying temperature as shown in Fig 13a_1. The expansion increased with the increase in gel setting time with a maximum at a steaming time of 40 min (Fig 13a_2) and a drying temperature of 40ºC (Fig 13a_3). The contour plots of linear expansion clearly indicated the optimum area providing desirable quality attributes of fish crackers. The bulk density of fish crackers decreased with an increase in steaming time but not with the drying temperature (Fig 13b_1). It has not much relationship with the gel setting time (Fig 13b_2). A slight increase in the bulk density was noticed as the drying temperature increased from 40-50ºC (Fig 13b_3). The contour plot of bulk density indicated the optimized parameters for obtaining the desirable bulkiness of the product, which mainly included the steaming time. The crispiness of fish crackers increased with the increase in steaming time but not influenced significantly by the drying temperature (Fig 13c_1). It was however not influenced by the gel setting time (Fig 13c_2) and drying temperature (Fig 13c_3). So, to have an expanded crispy fish cracker, the steaming time should be between 40 to 60 min. The optimum processing conditions of deep fried fish crackers were obtained using optimization tool in Design Expert 7.0 software by using experimental data and coded values as shown in Table 10. The target values of steaming time, gel setting time and drying temperature were set in the program. The results showed that the optimum conditions in both coded data and experimental data, leading to better linear expansion, bulk density and crispiness at a steaming time of 40 min, a gel setting time of 24 h and a drying temperature of 40ºC.

Fig 13 a-c. The contour plots of linear expansion, Bulk density and crispiness of fried fish crackers as affected by steaming time (A), drying temperature (B) and gel setting time (C)

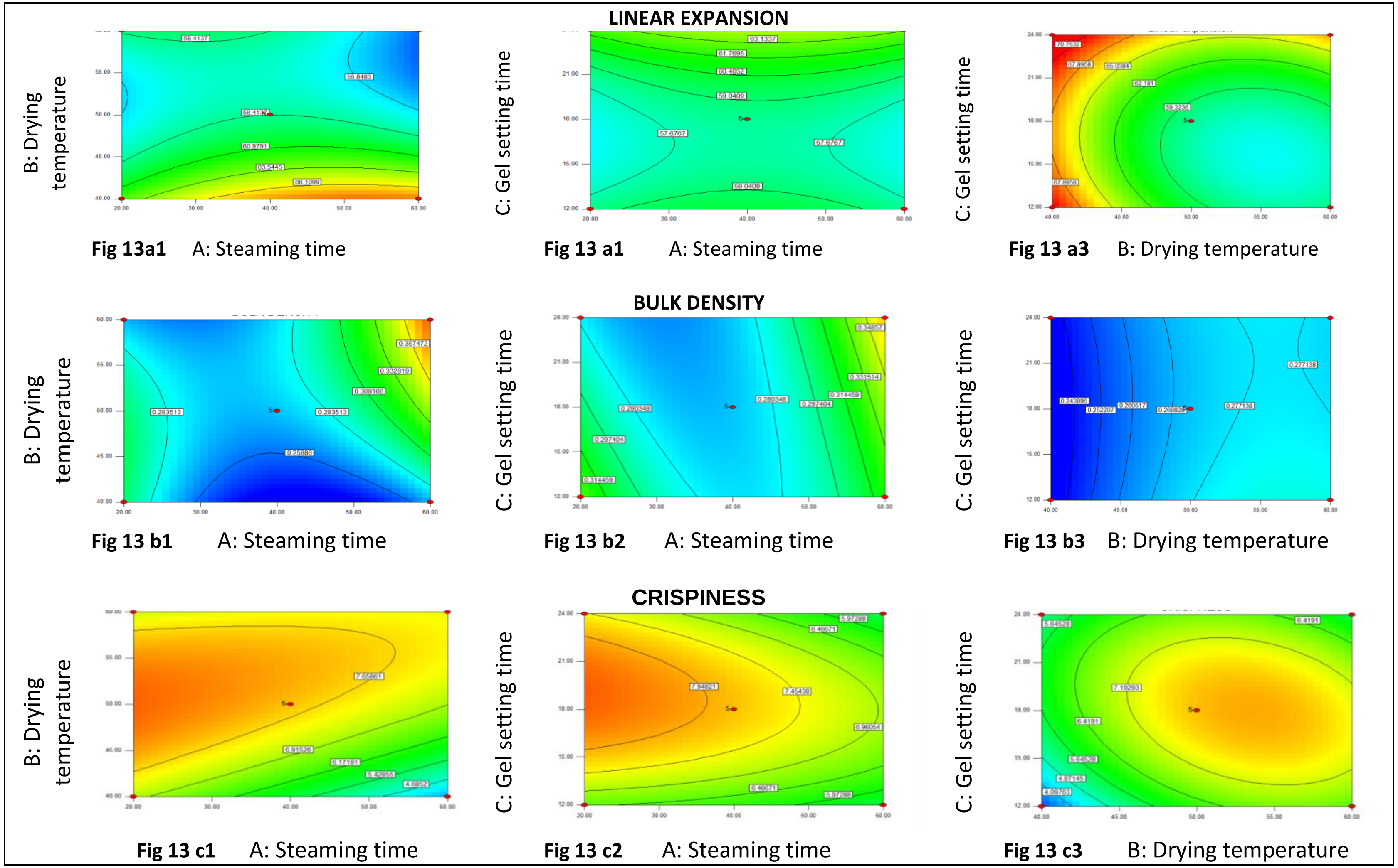


Table 10: **Box-Behkehn design for optimizing gelatinization conditions of fried crackers in coded units together with experimental data (Exp) and predicted values (Pred).**

Std order	A	B	C	Linear Expansion		Bulk density		Crispiness	
				Exp	Pred	Exp	Pred	Exp	Pred
1	1	0	1	66.32	64.14	0.351	0.366	5.211	6.189
2	0	0	0	56.54	58.40	0.286	0.271	7.615	7.808
3	0	0	0	58.74	58.40	0.265	0.271	6.969	7.808
4	-1	1	0	57.71	58.75	0.275	0.289	6.463	7.385
5	0	0	0	61.73	58.40	0.26	0.272	8.527	7.808
6	1	1	0	51.30	57.27	0.4	0.379	7.016	7.372
7	-1	-1	0	64.64	63.94	0.284	0.303	7.384	7.314
8	0	0	0	57.77	58.40	0.272	0.272	6.912	7.808
9	0	-1	1	70.30	73.23	0.259	0.285	4.654	6.101
10	-1	0	-1	55.42	58.53	0.345	0.328	6.779	7.113
11	1	0	-1	56.45	59.83	0.323	0.325	5.429	6.166
12	-1	0	1	64.14	63.24	0.277	0.283	7.623	7.555
13	0	-1	-1	71.14	70.62	0.241	0.273	2.710	6.301
14	0	1	1	67.27	67.08	0.272	0.312	6.179	6.625
15	1	-1	0	69.30	68.30	0.263	0.325	4.694	6.164
16	0	1	-1	61.78	58.36	0.265	0.287	6.982	6.661
17	0	0	0	57.22	58.40	0.276	0.271	8.277	7.808

4.4. Studies on the storage stability of fish crackers

4.4.1. Physico- chemical characteristics

Physico- chemical characteristics such as moisture content, water activity, crispiness and TBARS values of fish crackers processed under the optimized process conditions were analysed at an interval of 15 days during the storage period to determine their shelf stability.

4.4.1.1. Moisture content

Results showing the changes in the moisture content of the dried and fried fish crackers during storage are presented in Fig 14. A significant increase in the moisture contents of the dried fish crackers was noticed on storage whereas in case of fried ones, significant difference was noticed up to day 75 ($p<0.05$). The moisture content of dried crackers was 9.40±0.2 % on day 1 of storage, which increased to 11.53±0.09% on day 90. In case of fried fish crackers, the moisture content increased from 1.57±0.06 to 2.61±0.02% during the storage period.

4.4.1.2. Water activity (a_w)

Water activity of the dried and fried fish crackers gradually increased on storage and the results are presented in Fig 15. A significant increase in the water activity was noticed between day 30 and 45; also between day 60 and 75 in dried crackers ($p<0.05$). In fried fish crackers also, the water activity was initially 0.187±0.02, which increased intermittently between day 30 and 45; and also between day 60 and 75 ($p<0.05$) to a maximum of 0.310±0.02 on the final day of storage. The final a_w of dried and fried fish crackers were 0.555±0.01 and 0.310±0.02, respectively after 90 days of storage.

Fig 14: Moisture content of dried and fried fish crackers upon storage

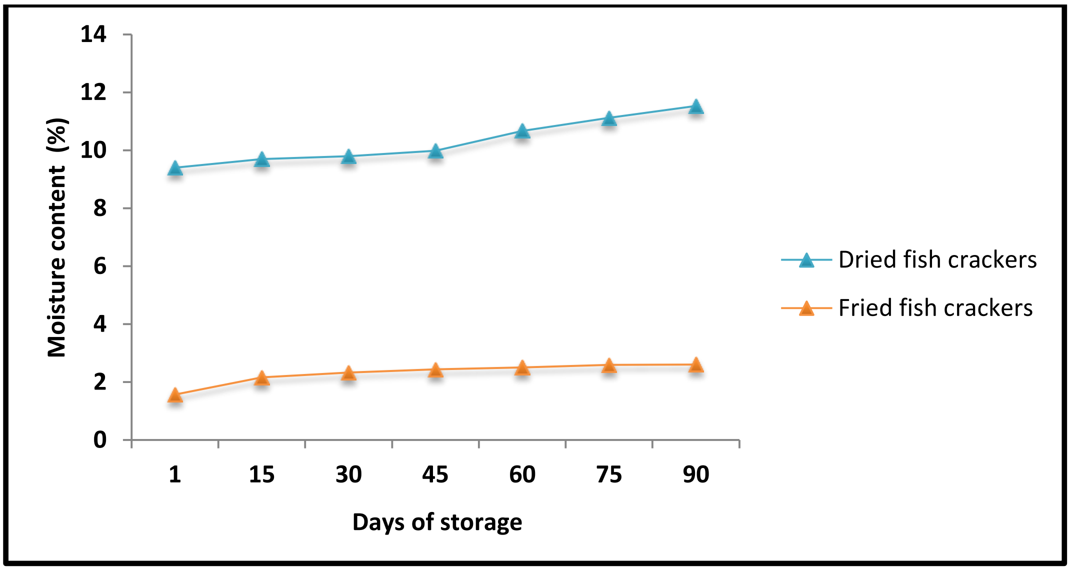
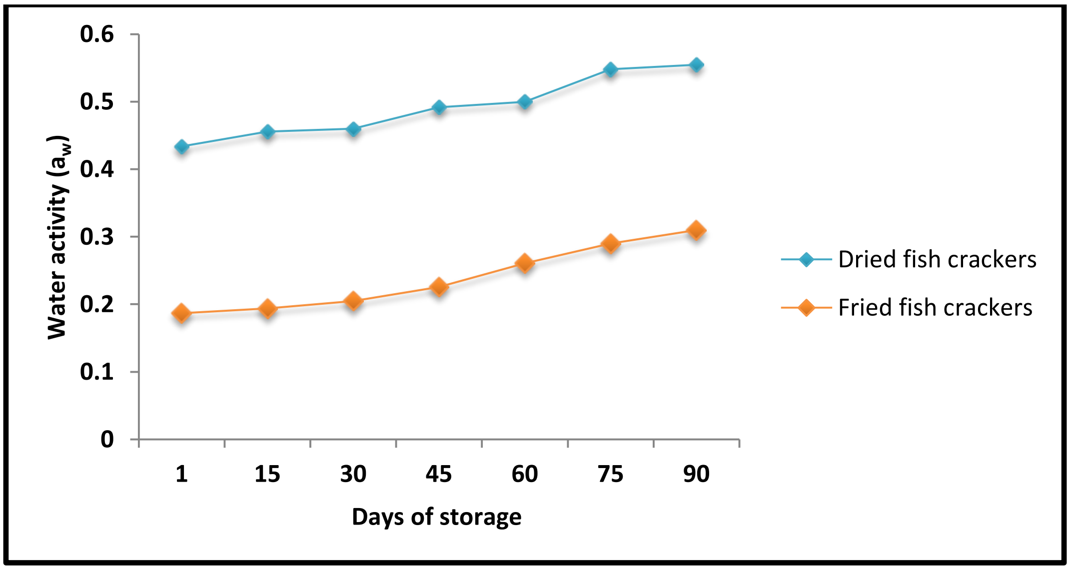


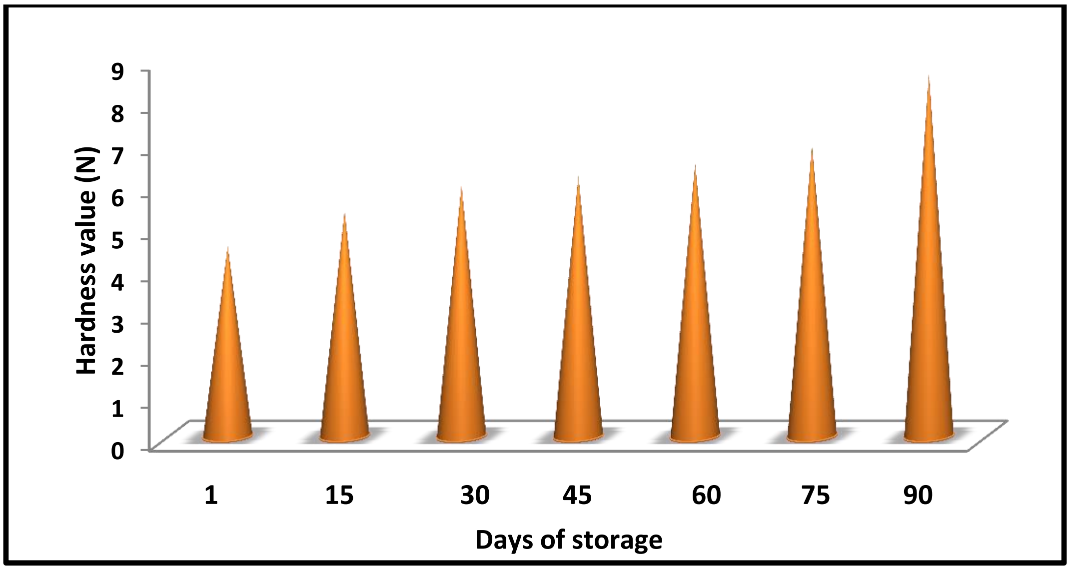
Fig 15: Water activity of dried and fried fish crackers upon storage



4.4.1.3. Crispiness

The results showing the trend of crispiness in fried fish crackers on storage is presented in Fig 16. Interestingly, it is noticed that there were no significant differences ($p>0.05$) in the values of the crispiness upto day 75, as the change was very gradual. The hardness value was 4.51±1.76 N on the initial day of storage, which increased to 8.61±2.57 N on the final day of storage. A prominent difference in the crispiness value was noticed only between days 75 and 90 of storage.

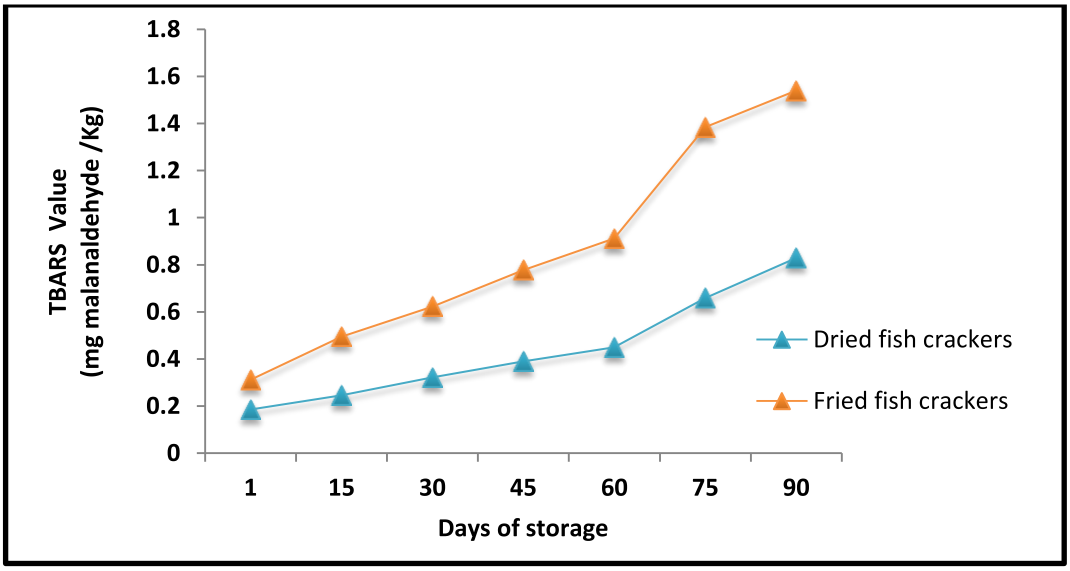
Fig 16: Crispiness of fried fish crackers upon storage



4.4.1.4. TBARS

A significant increase in the TBARS value was noticed during the storage period of both dried as well as fried fish crackers ($p<0.05$) (Fig 17). On the first day, the TBARS value was found to be 0.19 ±0.00mg malanaldehyde/kg in dried and 0.31±0.02mg malanaldehyde/kg in fried fish crackers; which increased to 0.83±.00 and 1.54±0.04 mg malanaldehyde/kg, respectively. A clear cut difference in the TBARS value of fried crackers was noticed as compared with dried crackers, with a sharp increase in the values beyond day 60.

Fig 17: TBARS of dried and fried fish crackers upon storage expressed in mg malanaldehyde /kg



4.4.1.5. Sensory score

Results of the sensory scores of the fried fish crackers upon storage are given in Table 11. A significant difference in the appearance of the crackers was noticed in day 15 ($p<0.05$). After that, such difference was noticed between day 45 and day 60 of storage. In case of colour, a significant difference was noticed on day 15 and later between day 45, 60 and 75. On the other hand, no significant difference in the odour score was noticed on day 15 but occurred between day 60 and 75. But a significant difference was noticed between days 60 and 75 of storage. In case of taste, the score showed a significant difference on day 60 with a score value of 5.27. The overall quality of the product decreased with the increase in the storage period from 8.0 to 4.2 on day 90. Significant difference in the overall quality score occurred on day 30, 60, 75 and 90 ($p<0.05$).

Table 10: Sensory score of the fried fish crackers upon storage

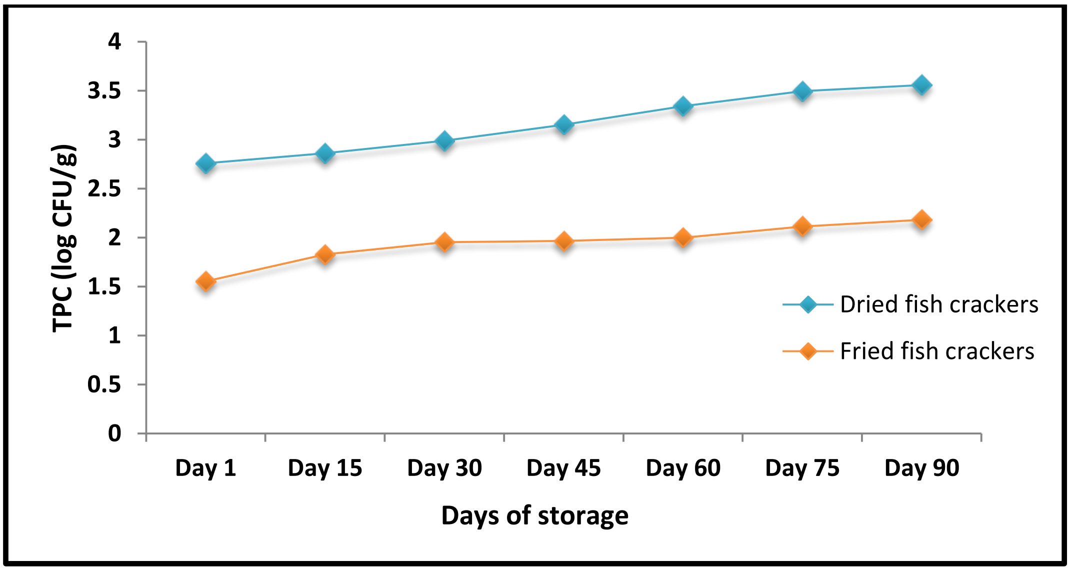
Days of storage	Appearance	Colour	Odour	Crispiness	Taste	Overall quality
1	8.47 ± 0.61^{d}	8.27 ± 0.77^{d}	8.00 ± 1.26^{d}	8.47 ± 0.81^{d}	8.27 ± 0.99^{d}	8.00 ± 0.89^{e}
15	7.53 ± 0.88^{c}	7.73 ± 0.77^{cd}	7.73 ± 0.93^{d}	8.20 ± 0.75^{cd}	7.33 ± 1.13^{cd}	7.93 ± 0.57^{e}
30	7.46 ± 0.72^{c}	7.07 ± 1.29^{c}	6.27 ± 1.69^{c}	8.13 ± 0.71^{cd}	7.00 ± 1.59^{c}	7.13 ± 0.96^{d}
45	7.46 ± 1.02^{c}	7.00 ± 0.63^{c}	6.20 ± 1.68^{c}	7.73 ± 1.18^{c}	6.63 ± 1.99^{c}	7.00 ± 1.03^{d}
60	6.40 ± 0.88^{b}	5.80 ± 1.20^{b}	5.60 ± 1.20^{bc}	6.67 ± 0.69^{b}	5.27 ± 0.99^{b}	5.73 ± 0.57^{c}
75	5.27± 0.99^{a}	4.60 ± 0.88^{a}	5.00 ± 1.21^{ab}	4.87 ± 0.88^{a}	4.93 ± 0.77^{ab}	5.00 ± 0.52^{b}
90	4.87 ± 0.62^{a}	4.27 ± 0.57^{a}	4.40 ± 0.49^{a}	4.73 ± 0.99^{a}	4.33 ± 0.87^{a}	4.20 ± 0.52^{a}

*All the data were expressed as mean ± standard deviation. The different superscripts in the same column indicate that the results are significantly different at $p<0.05$.

4.4.2. Microbiological analysis

Total plate count (TPC) of the dried and fried fish crackers was expressed as log CFU/ g and the results are given in Fig 18. The TPC increased significantly on storage in the dried fish crackers ($p<0.05$) from 2.76 to 3.56 log CFU/g. But, in case of fried fish crackers, a significant increase ($p<0.05$) in TPC was noticed only up to day 30 and thereafter the increase was marginal; and on day 75 and 90, again a significant increase ($p<0.05$) was observed as the value was 2.18 log CFU/g.

Fig 18: Total plate count of dried and fried fish crackers upon storage expressed in log CFU/g



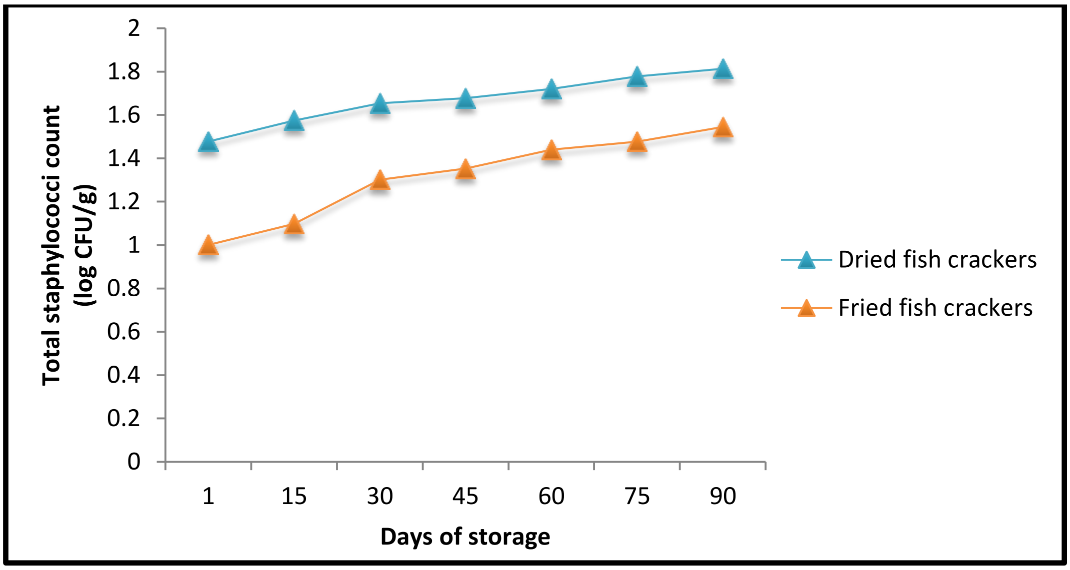
The results of total fungal count (TFC) of both dried as well as fried fish crackers are given in Table 12. Fungi were absent in the dried fish crackers until day 15 beyond which, very few colonies were detected, with values ranging from log 1 CFU to log 1.60 CFU/g on day 90. In case of fried fish crackers, no fungi were detected until day 75 of storage and only on the final day, 1.09 log CFU/g count was recorded.

Table 12: Total fungal count of dried and fried fish crackers upon storage expressed in log CFU/g

Days of storage	Dried fish crackers	Fried fish crackers
1	Absent	Absent
15	Absent	Absent
30	1.000 ± 0.001[a]	Absent
45	1.352 ± 0.039[b]	Absent
60	1.440 ± 0.031[c]	Absent
75	1.574 ± 0.024[d]	Absent
90	1.602 ± 0.045 [d]	1.097 ± 0.072[a]

Total Staphylococci count was initially 1.48 and 1 log CFU in dried and fried fish crackers, respectively (Fig 19). The counts increased significantly on storage and reached to 1.81 log CFU in dried crackers ($p<0.05$). In fried fish crackers, significant increase in the count was noticed between day 15 and 30 ($p<0.05$), and the values increased from 1.09 to 1.30 log CFU/g.

Fig 19: Total *Staphylococcus* count of dried and fried fish crackers upon storage expressed in log CFU/g



Total coliforms were not detected in the dried as well as fried fish crackers until day 75 of storage (Table 13). In case of dried fish crackers, very low coliform count of 0.036MPN/g was noticed only on day 90.

Table 13: Total coliform count of dried and fried fish crackers upon storage expressed in MPN/g

Days of storage	Dried fish crackers	Fried fish crackers
1	NP	NP
15	NP	NP
30	NP	NP
45	NP	NP
60	NP	NP
75	NP	NP
90	0.036	NP

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Discussion

V. DISCUSSION

5.1. Formulation of fish crackers at different fish to starch ratio

Formulation of fish crackers is the first and foremost step required for the preparation of fish crackers. It is very important to determine the physical as well as the nutritional value of the crackers because the difference in the fish: starch ratio, either increase or decrease the physico-functional qualities. Hence, a correct formulation of the product with right proportion of fish and starch is essential to have good physico- functional qualities in the fish crackers while retaining their nutritional properties.

Any snack product need to be analysed first for the biochemical compositional data to understand their nutritional quality. Hence, proximate analysis of fish crackers is very important besides analysing their nutritive content, to know the energy value and to determine the shelf life of the snack product. Moisture content is one of the important parameters that determine the shelf life of the crackers. Moisture content of dried fish crackers given in Table 5 was found to be well within the range recorded earlier by few authors (Yu, 1991a; King, 2002; Huda *et al*., 2007). A slight decrease in the moisture had occurred due to the increase in the fish to starch ratio, as the water molecules bound in the intermolecular spaces of the starch molecules have been replaced by the protein molecules due to its compactness which modulates the surface area (Diakova *et al*., 2007) Nutritive value of the fish crackers mainly depends on the protein content. The protein content increased with the increase in the proportion of the fish as observed earlier by several authors (Yu, 1991b; Peranginangin *et al.*, 1996; Huda *et al*., 2000; King, 2002). They have recorded a protein content in the range of 8 to 10%, in crackers with an addition of 40% fish. In this study, the fish crackers had a slightly higher protein (14%) at the same fish to starch ratio. Such differences exist due to the variation in the inherent biochemical composition of fish species. Thread fin bream (*Nemipterus japonicus*) used in this study, is a lean fish rich in protein content of 18.24±0.08%, moisture content of 74.72 ± 0.75%, fat of 2.78 ±0.14%, ash of 3.92±0.19% and carbohydrate content of 0.25± 0.001%. The Codex Alimentarius Commission classifies crackers as Grade I, if the protein is above 12% (CAC, 2001). According to that, the fish crackers made with 40% and 50% fish were Grade I snack products.

Carbohydrate is the major component in dried as well as fried fish crackers. Commercial fish crackers generally contain carbohydrates in the range of 65 – 80% (Huda *et al*., 2007; Nurul *et al.,* 2010; Neiva *et al*., 2011). Addition of fish to starch flour had caused proportionate reduction in total carbohydrates in both dried as well as fried forms, which in turn helped to improve the nutritive quality of the snack.

Fat is an another important parameter that showed drastic changes. The increase in the fat content of dried crackers with the increase in the fish:starch ratio was in agreement with the earlier reports (Gamble *et al.,* 1987; Yu, 1991; Peranginangin *et al*., 1996; King, 2002). Such increase had occurred due to the proportionate addition of fish meat in the dried crackers, which in fact was advantageous from the nutritional point of view, as fish contains more PUFA (Kris-Etherton *et al*., 2000; Kris-Etherton *et al*., 2002). The PUFA content of the muscle of *Nemipterus japonicus* and the sunflower oil were 36.2% (Nazeer et al., 2009) and 59% (British Pharmacopoeia Commission, 2005) respectively In the fried crackers, fat absorption takes place as the oil gets pulled into the pores to replace the lost water due to evaporation (Mellema, 2003). However, the decrease in the fat content has occurred in fish crackers with more fish content due to lower expansion of the products; which in turn might have limited the oil absorption in fried fish crackers.

The ash content of the fish crackers was also within the same observed range recorded by Huda *et al*. (2010). Upon frying, a reduction in the ash content was noticed mainly due to the migration of cooking oil to the matrix of fried crackers.

Total energy values were calculated based on Atwater system to know about the biologically available nutrients in the snack. The significant decrease noticed in the total energy values from 510 to 466 Kcal was mainly due to the reduction in the fat content. Although frying process aided in the uptake of fat from frying oil summing up the total calories, increasing the fish to starch ratio had helped to reduce the total calories significantly. However, the drastic increase in total protein did not reflect in the total calories of the snack product, as both protein and carbohydrates possess similar energy values.

5.2. Changes in the physico- functional properties of fried fish crackers

Linear expansion, volume expansion, bulk density, oil and water absorption, water solubility and crispiness are some of the important physico-functional properties that determine the quality of fried fish crackers.

A significant reduction in the linear expansion with the increase in fish to starch ratio from 86.31 to 46.69% (Fig1) was recorded, which was in accordance with the earlier findings (Nurul *et al*., 2009; Yu and Low, 1992). Saeleaw *et al*. (2010) stated that there existed a negative correlation between linear expansion and protein content; and a positive correlation between linear expansion and carbohydrate content. The decrease has occurred because of the interaction of the fish protein with the starch granules (Nurul *et al*., 2009). The values obtained for crackers with 40% fish were slightly higher (56.96%) than the values reported by Kaewmanee *et al*. (2015).

The important factors that might reduce the expansion are fish to starch ratio and the protein content (Tondang *et al*., 2008), as they affect the formation of gel, its strength; and also determine the vacuole formation and aggregation. Microstructure studies conducted by Cheow *et al*. (1999a) have ascertained the presence of large aggregates of fish proteins in poorly expanded fish crackers.

The degree of expansion also depends on the degree of gelatinization of starch and amylose to amylopectin ratio of the flour (Lachmann, 1969). A good quality cracker is required to have >50% amylopectin and 5.2% amylose (Yu, 1991a). Several workers have indicated that the presence of fish proteins can reduce the expansion of amylopectin in starch based foods during frying (Nurul et al., 2009; Singthong and Thongkaew, 2009). Other than protein and carbohydrates, high fat present in the fish may also reduce the expansion due to the formation of strong amylose – lipid complex at 105-139ºC (Stauffer, 1998).

The increase in the volume of crackers due to the removal of water results in porous nature of the products, which is termed as volume expansion. A significant reduction in the volume expansion recorded was in accordance with the decrease in the linear expansion. The expansibility of the crackers that occurred during heating process was due to the increased evaporation of water molecules (Douzals *et al*., 1996, Rossell, 2001). Simultaneous heat and mass transfer during expansion has resulted in the formation of porous structure (Bhat and Bhattacharya, 2001).

The increase in the bulk density with the increase in fish to starch ratio is due to the decrease in the volume expansion. There existed a negative correlation between bulk density and linear expansion proportionally (Taewee, 2011). According to Barrett *et al.* (1994), the strength and fracture properties of the crackers are influenced by the cellularity and bulk density. In fact, the fracturability is considered as the primary determinant of the sensory attributes such as crispiness and crunchiness of the snack.

Oil absorption characteristic of fish crackers is a concern from the points of nutritive value and shelf stability of the product. There exists a positive correlation between oil absorption and volume expansion (Suhaila and Hamid, 1994). The oil gets entrapped on the surface layer of the bigger “air cells” and causes expansion (Mohamed *et al*., 1988). The crackers made without fish had higher oil absorption due to their higher volume expansion. With the increase in the fish to starch ratio, the expansion of the product get reduced and thereby the oil absorption decrease as observed earlier by few authors (Nurul *et al*., 2009; Kaewmanee *et al*., 2015). The interaction that had occurred between amylose of starch and protein of fish during formulation of fish crackers could have blocked some of the functional sites which entrap more water molecules to cause expansion during the frying process. Hence high fish to starch ratio might had reduced the uptake of oil in crackers, due to less expansion which was in fact advantageous for making healthy snacks. Oil absorption into the product occurred not only during the frying process but also during the cooling period (Ufheil and Escher, 1996).

A more or less constant WAI noticed in fish crackers made with 10 to 40% fish proportion indicated that the amount of water occupied by the starch granules after swelling in excess water remains almost constant and thereafter the granular integrity is lost resulting in a sharp decline. There existed a positive correlation between starch solubility and extent of molecular breakdown (Singh *et al*., 2003). The addition of fish had eventually reduced the degree of starch gelatinization. The decline observed in WAI of the crackers made with 50% fish coincided well with the increase in WSI. This clearly stated that the addition of fish beyond 50% had led to molecular breakdown in starch – protein complex upon heating in oil at 180ºC (Huang *et al*., 2011).

The colour of fish crackers is a very important parameter that determines the consumer acceptance. The results indicated a decrease in L* value with the increase in the fish ratio (Table 7). Nurul *et al*. (2009) have also reported that an increase in fish ratio will contribute to darker products. Starch used for the preparation of fish crackers are of white colour while the fish flesh contains some pigments. Water soluble nitrogenous compounds like myoglobin, haemoglobin and hemocyanin contribute to the pink colour of the fish and shellfish (Haard *et al*., 1994). In addition, colour compounds are also formed due to the involvement of water released from the amino acid and in Maillard –type reactions (Sikorski and Pan, 1994, Wang *et al*., 2013). The decrease in the L* value of the fried food may be related to Maillard browning and caramelizaton that occur at a high frying temperature (Ngdi *et al*., 2009). In this study, the difference in L* value was mainly due to increase in the protein content and not related to the changes in starch. Hence, the presence of water soluble pigments and maillard reaction products perhaps could have contributed for low L* values in fish crackers.

High b* values indicate yellowish colouration of product. There are many factors that affect the colour of the fish crackers, such as the amount and type of the starch (Huda *et al*., 2001). On the contrary, redness (a*) value had decreased with an increase in fish to tapioca flour ratio according to Nurul *et al*., (2009). The type of additives and the thickness of the crackers can also influence the colour of the crackers (Rosmawaty *et al*., 1996). The type of flour used in fish cracker also affects the clarity of the fish crackers, which in turn affect the colour of the product (Mohamed *et al*., 1988). The a* and b* values also depend on lipids, myoglobin as well as haemoglobin present in the dark muscle. The changes in the a* values were mainly related to the oxidation of myoglobin, whereas the changes in the b* values is more related to the gelatinization and swelling ability of starch (Yang and Park, 1998; Pilar and Reyes, 2007). In this study, lightness (L*) value decreased with the increase in the ratio of fish meat, whereas redness (a*) and yellowness (b*) values increased with the fish to starch ratio (Table 7).

Hardness of the fish crackers was measured by texture analyser based on fracture behaviour and sound emission to determine the crispiness, which has an inverse relationship with hardness (Huda *et al*., 2009). Crispiness is the most important attribute for the snack product. Crispy products fracture at low force with multiple brittle fracture (Vincent, 1998; Luyten *et al*., 2004). Loss of crispiness in fish crackers with the increase in fish to starch ratio was also reported by Nurul *et al*. (2009). In fact, the air cell or pore distribution developed due to the contact of crackers with hot oil determines the crispiness (Ngadi *et al*., 2009; McDonough *et al*., 1993; Saelaw and Schleing, 2011). The sharp decrease in the crispiness of crackers made with high fish proportion (50%) has occurred due to the molecular breakdown upon heating in oil, as reflected by the increase in WSI and decrease in WAI. The leaching out of soluble polysaccharides and proteins from the strong complex matrix might have broken the large air cells or pores contributing to the loss of crispiness of the product.

Eventhough the changes examined in the physico- functional properties determine the acceptability and quality of the product, sensory evaluation of the snack based on their appearance, colour, odour, crispiness and taste gives the actual perception of the product from consumer point of view. Low scores for the crackers made with 50% fish were due to more fishy odour and taste. Loss of expansion and white coloration are some other attributes that have to be compromised to make fish crackers with additional nutritive value and taste. In this study, the crackers made with 40% fish had good taste and odour but slight loss in crispiness and hence, it was considered as the best snack product, taking their nutritive value into consideration.

The FTIR spectra of the individual ingredients analysed showed that all the starches contained 8 major peaks in the region of 800-1400 cm^{-1}, which is considered as the sensitive region for the conformation of polysaccharides (Wilson and Belton, 1988). The bands in this region were due to C-O and C-C vibrations, that are highly coupled, making assignment of individual bands difficult (Belton *et al*., 1986). However, based on model calculations and deuterium exchange methods for glucose, the assignments of the bands in this region have been done (Hineno, 1977). As the absorptions in this region arise largely from C-O stretching of the ring, linkaging C-O-C and C-OH groups, the position of these bands are similar in all carbohydrates (Belton *et al*., 1986). As most vibrations are within the isolated D-glucose residue and the contribution of inter-residue vibrations are very small, polymerization does not largely affect the vibrations (Cael *et al*., 1974). However, previous studies have shown that some bands are highly sensitive to polymer conformation (Wilson and Belton, 1988). The bands at 1047 and 1022 cm^{-1} are sensitive to the amount of ordered or crystalline and amorphous starch, respectively (van Soeast, 1995). These two bands were detected at 1080 and 1019 cm^{-1} wavelength in the three starches viz. tapioca, corn and sago, used in this study.

Fish contained typically 12 bands, the major ones are amide A (3447 cm^{-1}), amide B (2926 cm^{-1}), amide I (1651 cm^{-1}), amide II (1544 cm^{-1}), amide III (1240 cm^{1}), amide VI (609 cm^{-1}) besides other bands. The amide A band corresponds to N-H groups in hydrogen bonding and also O-H stretching of free water. Amide B is related to asymmetric stretching of CH_2 groups. Amide I peak results from C=O stretching related with secondary structure. Amide II band formed due to the bending vibrations of N-H groups and stretching vibrations of C-N groups. Amide III band corresponds to the vibrations in plane of C-N and N-H groups of amines. A band at 2362 cm^{1} is more related to the CO_2 stretching. Other typical bands appeared in fish were 1457, 1400, 1317, 1164 and 1082 cm^{1} (Staroszczyk *et al*., 2014).

FTIR spectra of the formulated fish cracker (Fig 9b) showed combination spectra with majority of the peaks associated with fish protein rather than starch. This may be due to the blocking of some of the functional groups present in the starch by the proteins present in the fishx. Gelation and retrogradation are the two processes that had occurred during formulation of fish crackers. The changes in the intensity of the IR spectral peak are found to be the major change during gelation (Rubens *et al*., 1999). The narrowing of the bands at 1155, 1080 and 1019 cm^{-1} can be interpreted as an evolution to a more uniform state of the amorphous phase. The greatest shift in the bands at 1156, 1080 and 1019 cm^{-1} can be assigned to C-O and C-C stretching and the C-C-O bending (Rubens *et al*., 1999). So during the gelation process, there will be changes in IR spectral intensity, band width and frequency, which is evident in this study. Disappearance of several bands of starches in the region from 700-1000 cm^{-1} was noticed due to an increased conversion of starch into amorphous material. This interaction gives to narrowing of some bands as noticed in IR spectra. Some additional peaks had formed after the gelation and retrogradation processes at 2854 and 2361cm^{-1}, which are more related to symmetric and asymmetric stretching of CH_2 and CH_3 groups, respectively. The peak at 1744 cm^{-1} is related to the oil peak formed as a result of emulsion formation due to the presence of ester carbonyl functional group of the triglyceride present in tapioca and sago (Van de Voort *et al*., 1994).

FTIR spectra of the fried fish crackers revealed the presence of oil in the final product (Fig 9c). The presence of oil is clearly evident through the sharp bands noticed between 3000-2800 cm^{-1}; as well at 1750 cm^{-1}. Typical bands of oil were found at wave numbers 3426, 3009, 1746, 1654, 1378, 1099 and 721 cm^{-1}. The frequency of cis double bond stretching vibration occurs at the band near 3009 cm^{-1}. Oils with high proportion of linolenic or linoleic acyl groups show high frequency data for this band than oils with high proportion of oleic acyl groups. The bands corresponding to 2925 and 2854 cm^{-1} are due to the symmetric and asymmetric stretching vibration bands of aliphatic CH_2 functional groups (Bellamy, 1975) and their frequencies were very high. The CH_3 functional group had given arise to symmetric and asymmetric stretching vibrations at slightly different wave number of 2962 and 2872 cm^{-1}, respectively. In this study, the bands are related to stretching vibrations of CH_2 groups.

The typical amide B band at 2361cm^{-1} of fish protein was found unaltered even after frying process gave an indication on their stability to high temperature. A high frequency band at 1746 cm^{-1} was due to ester carbonyl functional group of the triglycerides, which had slightly shifted to higher wave number upon frying indicating that an appreciable proportion of aldehyde functional groups are present in the oil. There were two bands at 1462 and 1378 cm^{-1} in fried crackers formed due to bending vibrations of CH_3 and CH_2 aliphatic groups. The band 1378 cm^{-1} had shown a shift to lower wavenumber, while the band at 1462 cm^{-1} had shifted to higher wavenumber after frying process. The band at 1160 cm^{-1} corresponds to saturated acyl groups and will experience sharp changes during oxidation process. Frying process had brought in several molecular conformation through the formation of new C-H aromatic ring stretching peak at 3009 cm^{-1}; as well as small peaks at 1239 and 1098 cm^{-1} and shifts in certain peaks indicating the loss of certain functional groups.

5.3. Optimization of gelatinization conditions

The process parameters like steaming time, gel setting time and drying temperature were optimized to improve the physical properties like linear expansion, bulk density and crispiness of the crackers.

5.3.1. Linear expansion

Linear expansion is the major parameter that needs to be taken as an important output parameter while performing RSM, to optimize the conditions such as steaming time, gel setting time and drying temperature. The rapid increase in molecular volume of water during evaporation is the concept during expansion of fried foods (Rossell, 2001). The steaming time of 40 and 60 min were found to be good for fish cracker formulation. Earlier, Kyaw *et al*. (1999) found that *Keropok* gel required a steaming time of 20 - 30 min for sufficient cooking to get better linear expansion and hard texture of gel. Similarly, 'Khao Kriap Waue', a Thai based snack required a steaming time of 20 min for complete gelatinisation of the dough (Jomduang *et al*., 1994). During steaming, the starch granules expand to their maximum size before fragmentation. Swelling or expansion of the starch molecules is due to the hydration of water molecules (Leach *et al*., 1959). When the swelling power increases greater amount of water will get entrapped in starch granule contributing to higher linear expansion upon frying (Cheow *et al*., 2004). The gelatinisation temperature of tapioca starch ranges between 52- 64 ºC (Pomeranz, 1991). So, degree of gelatinisation of the starch is one of the factors influencing the degree of linear expansion of snack products when immersed in hot oil (Lachmann, 1969) and the reduced expansion is mainly due to incomplete gelatinisation (Mohamed *et al*., 1989). According to Kyaw *et al.* (1999), with a steaming time of 20 min, the centre of keropok gel had acquired around 92ºC. This study indicated that a minimum steaming time of 40 min was sufficient to cause complete gelatinization of starch and to bring maximum expansion of fish crackers since higher steaming time will only result in the higher cost. A low drying temperature of 40-50ºC is more preferred to prevent case hardening of the dried product (Siaw *et al*, 1985), which otherwise leads to the poor expansion of the final product.

A significant difference in the linear expansion of fish cracker at higher gel setting times i.e. between 18 and 24 h, irrespective of drying temperature indicated that a high gel setting time provided more linear expansion in the fried fish crackers. Even though gelatinisation happens during the steaming process; the gel has to be perfectly set to form a hardened structure by storing in chilled condition for an appropriate duration. 'Retrogradation' of cooked starch will occur at the time of chilling, which is a reaction that takes place when the amylose and amylopectin chains are steamed and the gelatinized starch realign themselves on chilling resulting in setting of gel (Wang *et al*., 2015). When starch is heated and dissolved in water, crystalline structure of amylopectin and amylose is lost and then, they get hydrated to form a viscous solution. If viscous solution is further chilled at lower temperature for a long period, the linear molecules, amylose and linear parts of amylopectin retrograde and rearrange themselves again to a more crystalline structure. The crystallization of amylopectin plays a major role in the firmness of starch gels upon storage (Ring *et al*., 1987; Wang *et al*., 2015). So, starch retrogradation depends upon the duration of chilled storage. It has been earlier reported that firmness is more in cracker gel set for 24 h, as it improved the slicing of the gel, which in turn influenced the linear expansion of fried crackers.

5.3.2. Bulk density

The volume expansion of the fried product is greatly influenced by the escape of water during frying (Rossell, 2001). As the volume expands, the density decreases. Bulk density was found to be minimum at the steaming time of 40 min in fish crackers at a drying temperature of 40ºC corresponding to high linear expansion. The bulk density has a significant negative correlation to linear expansion (Saeleaw and Schleining, 2011). The optimised gel setting time to have the maximum bulk density is not in accordance with the optimised gel setting time of 24h required to have the maximum linear expansion. Badrie and Mellowes (1992) reported that crude protein of soybean flour/cassava extrudates correlated positively with bulk density and negatively with expansion. The presence of amylopectin in starch has positively correlated with bulk density in a Thai rice- based snack, Khao-Kriap-Waue (Jomdung and Mohamed, 1994).

5.3.3. Crispiness

The crackers were found to be more crispy at a gel setting time of 24h and a drying temperature of 40ºC. This is well matching with the result of linear expansion as the expansion is directly correlated with the crispiness of the product. Hardness has a negative correlation with crispiness. Increasing the linear expansion will increase the crispiness value of fried crackers, which is indicated by less hardness value (Peranginangin *et al*., 1997; Yu, 1991). It was observed that the optimised steaming time of 60 min in terms of crispiness was not in accordance with the optimised steaming time of 40 min in respect of the linear expansion of the product.

5.4. Storage stability of fish crackers

Examination of the changes in the physico- chemical characteristics of the fish crackers enables us to have an idea on their shelf stability during storage at ambient temperature.

5.4.1. Physical characteristics

The moisture content of both the dried as well as fried crackers increased during the storage period (Fig 14), mainly because of the absorption of water vapour from the atmosphere through the packages. As the packaging material is slowly permeable to the water vapour, migration of water vapour from atmosphere to the product had occurred resulting in an increase in moisture content. However, the final moisture contents of both the dried and fried crackers were well within the acceptable limit of (9-13%) as prescribed by Huda *et al*. (2007) for snack foods.

The water activities of the dried and fried fish crackers were 0.56 and 0.31, respectively after 90 days of storage (Fig 15). A number of factors such as presence of starch, processing and frying process (in case of fried ones) have been found to be responsible for the low water activity of snacks. If the water activity value (a_w) of the product is below 0.6, it is known to inhibit the microbial and fungal growth (Franco and Landgraf, 2004). As both the dried and fried crackers have a_w values <0.6; they are expected to be free from microbial and fungal attack. But a few count obtained during the storage study may be due to the handling process.

Crispiness is perceived when food is chewed between molars and is usually expressed in terms of hardness and fracturability. A distinct change in the crispiness value was noticed beyond day 75 of storage i.e. from 6.91±2.11 to 8.61±2.57N (Fig 16), which could have occurred due to the absorption of moisture by the fried crackers, as there was a progressive increase in their moisture contents. Hence, the permeability of the packaging material has great influence on the crispiness of the final product.

5.4.2. TBARS values

An increase in the TBARS values of dried and fried crackers indicated that oxidation of lipid had occurred during storage (Fig 17). The oxygen permeability of the packaging material had probably led to lipid oxidation (Brewer *et al*., 1992). However, the final TBARS values of both the dried as well as fried fish crackers were within the maximum permissible limit of 2 mg malanaldehyde/kg (Zanardi *et al*., 2004). Similar to this study, a gradual increase in the TBARS value of fish, beef and children snacks during storage was also reported by Ratanatriwong *et al*. (2011), Singh *et al*. (2011) and Park *et al*. (1993), respectively. Similar increase in the TBARS value was also reported in different meat snack products upon storage by Chidanandaiah *et al*. (2009) Kumar and Tanwar (2011), Bhat and Pathak (2011) and Bhat *et al*. (2010).

5.4.3. Sensory score

A linear decreasing trend in the colour and appearance of the fish crackers has been noticed with the progress of storage period (Table 11). Similar to this finding, a decrease in the colour and appearance scores of chicken and fish snacks was reported by Singh *et al*. (2011) and Ratanatriwong *et al*. (2011), respectively. The same trend was also recorded by Kumar and Sharma (2004) in chicken patties, Kilinc (2009) in anchovy patties, Chidanandaiah *et al*. (2009) in buffalo patties, Bhat *et al*. (2010) in chevon Harrisa and Bhat *et al*. (2013b) in chicken meat balls.

But, the taste and odour scores of the fish crackers decreased gradually with the progress of the storage period. This could be attributed to the increase in lipid oxidation, liberation of fatty acids and increase in microbial load (Sahoo and Anjaneyulu, 1997). A gradual loss of taste and odour might be due to the expected loss of volatile flavour compounds from spices and condiments upon storage of the product. The loss could also be related to the increase in TBARS value of meat products (Tarladgis *et al*., 1960). The decrease in the odour with the advancement of the storage might also be related to the dilution in fishy flavour. Similar decrease in the taste and odour of various other meat products upon storage was reported by Singh *et al*. (2011), Padda *et al*. (1989), Kumar and Sharma (2004), Bhat and Pathak (2009), Bhat *et al*. (2013a) and Bhat *et al*. (2013b).

Loss in crispiness scores had occurred due to absorption of moisture by the crackers with the increasing storage period. The decline in the overall acceptability scores may be related to the changes in scores of colour, appearance, odour, crispiness and taste. Similar findings in various fish and meat products have also been reported by Kumar and Sharma (2004), Bhat and Pathak (2009), Bhat *et al*. (2013a) and Bhat *et al*. (2013b).

5.4.4. Microbiological analysis

TPC includes all pathogens and non-pathogens and is used to determine the hygienic status of food products. A gradual increase in the total plate count was noticed during the storage of both the dried and fried fish crackers (Fig 18). Raja et al. (2014) have also noticed an increase in the TPC as the storage days progressed in treated fish curls. Such increase in the levels of TPC upon storage of various snacks was also reported by several other authors (Kumar et al. 2007, Chidanandaiah et al. 2009, Kumar and Tanwar, 2011, Bhat et al. 2010, Bhat and Pathak, 2011, Bhat et al. 2013a and Bhat et al. 2013b). The increase in TPC during the storage might be due to easy availability of carbohydrate – rich starch in the product that favours microbial growth (Raja et al. 2014). It was found that the TPC of the fried fish crackers is comparatively lower than those of dried ones. This was because of severe heat treatment process (frying) employed while processing. In none of the samples, TPC exceeded the permissible level of microbial standards (log 10^6 CFU/g) prescribed for cooked meat products (Jay, 1996).

Moulds can grow on stored food, making them unpalatable or toxic and thus found to be a major source of food contamination causing illness (Wareing, 2010). Fungi were absent initially in the dried as well as fried fish crackers upto day 15 of storage (Table 12). Due to the absorption of the moisture by the dried cracker, fungal growth began from day 30 and experienced until day 90 of storage. Fungi growth is always associated with the moisture content of the product. As compared to the dried fish crackers, fried crackers had very less moisture and hence, there was a delayed growth of fungi (i.e. on the day 90 only). Singh *et al*. (2011) reported that yeast and mould appeared in the chicken snacks on day 30 of storage and is more related to the availability of nutrients in meat. In this study, reason for the growth of fungi was mainly because of permeability of water vapour (moisture) through the packages, which in turn increased the water activity permitting the fungal growth. This can very well be curtailed by use of packages having good gas and water vapour barrier properties.

Presence of *Staphylococci* in food products indicates poor handling and processing practices, cross contamination, and inadequate cleaning and disinfection of equipments. An increase in the Staphylococci count upon storage was noticed in both the fried as well as dried crackers (Fig 19). Presence of *Staphylococci* on the initial day itself indicated that there is a mild post-process handling contamination; however the counts never exceeded the permissible limit of 2 log CFU /g in both the dried and fried fish crackers. The gradual increase in the Staphylococci count was due to the slow multiplication of the bacteria under minimal moisture condition. The acceptability of the product does not face any problem, as the counts were below the permissible limit even after 90 days of storage.

Coliforms are commonly estimated as an indicator of sanitary quality of food and water. Their presence indicates unsanitary conditions or practices during production, processing or storage. Coliforms were not detected in the dried as well as fried fish crackers until day 75 of storage (Table 13), which implied that low a_w (<0.6) did not allow the coliforms to grow. Absence of coliforms is mainly due to frying of crackers at 180 ± 5ºC, which is far above their thermal death point of 57ºC, and the hygienic practices adopted during the preparation and packaging of fish crackers (Raja *et al*., 2014). Similar findings have been reported in chicken snacks (Singh *et al*., 2011) and in fish curls (Raja *et al*., 2014) held at ambient temperature storage. Zero count of coliform for the products heated at high temperature were also reported by Kumar and Sharma (2004) in pork patties, Kandeepan *et al*. (2010) in buffalo meat keema, Bhat *et al*. (2010) in Chevon Harrisa, Bhat and Pathak (2011) in mutton Harrisa, Bhat *et al*. (2013a) in chicken seekh kababs and Bhat *et al*. (2013b) in chicken meat balls.

Very low levels of coliforms were detected in the dried fish crackers on day 90, which could be due to the slight increase in the moisture content of the final product.

The nutritional composition of both fried and dried fish crackers implies that it is a better alternative for the usual snacks foods available in the market. Also, the microbial analysis of the crackers clearly indicated that the products had minimal bacterial growth which was well within the prescribed standards and hence, confirms total safety of these products for consumers upto 90 days of storage.

Summary

VI. SUMMARY

Fish crackers were prepared by blending lean fish (*Nemipterus japonicus*) along with tapioca, corn and sago in six different proportions. Proximate composition, physico-functional properties of the crackers were examined. For the selected proportion of the fish and starch gelatinization conditions were optimized by performing RSM. Fish crackers prepared under optimized condition were examined for their shelf stability during their storage by conducting biochemical, microbiological and sensory analysis. The salient features observed in these studies are given below:

- Dried fish crackers had their moisture contents in the range of 10.33 to 10.88%, while the fried ones from 2.38 to 2.73%. Normal dried crackers without fish had 3.31% protein, while fish crackers made with 50:50 fish to starch ratio had 18.74% protein. Carbohydrate content of dried fish crackers reduced from 83.22% to 61.54% and that of fried crackers from 70.73 to 56.29% with the increase in fish to starch ratio. Fat content on the other hand, increased from 0.24 to 1.66% in dried crackers but in fried crackers decreased from 24.61% to 19.52% with the increase in fish to starch ratio. Total mineral content increased from 2.33% to 5.51% in dried fish crackers, whereas in fried ones, a reduction was noticed. The total energy values of dried fish crackers ranged between 336-348 Kcal, whereas in fried fish crackers, a decrease was noticed from 510 to 466 Kcal with the increase of fish ratio.
- A high linear expansion, volume expansion, crispiness and oil absorption was noticed in the fish crackers with less fish to starch ratio. The WAI of fish crackers was more or less constant whereas WSI decreased with the increase in fish

proportion upto 40%. The colour analysis indicated that with the increase in the fish ratio, L* value decreased from 66.11 to 50.97, whereas a* value and b* value increased from 2.51 to 9.45 and 19.13 to 28.55, respectively in the fried crackers.

- The overall quality of the product decreased with the increase in the storage period from 8.0 to 4.2 on day 90. A significant difference in the overall quality score occurred on day 30, 60, 75 and 90.
- The gelatinization conditions of the fish crackers prepared with 40:60 fish to starch ratio was optimized using RSM indicated that a steaming time of 40 min, gel setting time of 24 h and a drying temperature of 40ºC are found to improve the linear expansion, whereas a steaming time of 60 min was found to improve the crispiness and to improve the bulk density, a gel setting time of 18 h was required.
- Storage stability of dried and fried fish crackers prepared under optimized condition was analysed. The moisture content of dried crackers increased from 9.4 % to 11.53% on storage, while in fried fish crackers it increased from 1.57 to 2.61%. Water activity of the dried and fried fish crackers gradually increased to the final value of 0.555 and 0.310 a_w were respectively after 90 days of storage. The hardness value also increased from 4.507 N to 8.613 N on the final day of storage, and TBARS value increased from 0.18 to 0.83 in dried and from 0.312 to 1.540 mg malanaldehyde/kg, in fried ones. A sharp increase in the TBARS values beyond day 60 was noticed in fried crackers and the sensory quality of the product decreased with the increase in the storage period from 8.0 to 4.2 on day 90 and were found acceptable for consumption.

- With regard to microbiological quality, the TPC increased from 2.76 to 3.56 log CFU/g in dried crackers whereas in fried fish crackers, it had reached a value of 2.18 log CFU/g only. A very few fungal colonies were detected in dried crackers ranging from log 1 CFU to log 1.60 CFU/g whereas in fried fish crackers fungi was detected only on the final day. Total Staphylococci increased from 1.48 to 1.81 log CFU in dried crackers whereas in fried crackers from 1 log CFU to 1.544 log CFU/g. Total coliforms were not detected in the dried as well as fried fish crackers until day 75 of storage. Microbial analysis values of both the dried and fried fish crackers clearly indicated that the products had very low microbial growth within the permissible microbial standards and thereby confirm the safety of these products for consumers until 90 days of storage.
- FTIR spectral analysis was undertaken to examine the functional interaction that occurred during the formulation and processing of fish crackers. FTIR spectra of tapioca, corn and sago starch before gelatinization showed 8 typical major bands in the region of 800-1400 cm^{-1} but at slightly different frequencies. The major bands were found near 1420, 1380, 1242, 1155, 1080, 1019, 930 and 860 cm^{-1} wavenumber with slight shifts among the starches. There existed a major change in the intensity of the typical 8 major bands of the starch in the formulated cracker. Also, the absorption bands at 1155, 1080 and 1019 cm^{-1} showed a decrease in the bandwidth after gelation. Frying of dried crackers in vegetable oil had given a totally different spectrum for crackers. Several new bands unique to vegetable oil used for frying had appeared prominently. They appeared at wavenumbers, 3426, 3009, 1746, 1654, 1378, 1238, 1099, 721 and 578 cm^{-1}.

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