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## Effects of orange peel extract on microbiological quality during storage of functional oat cookies fortified with fish protein concentrate from lesser tiger toothed croaker (*Otolithes cuvieri*)

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### Abstract

The goal of this work was to prepared functional oat cookies that were fortified with fish protein concentrate from lesser tiger toothed croaker (*Otolithes cuvieri*) and orange peel extract. Demand for various categories of healthy snacks is quickly rising across the world, and dietary fiber-enriched oat cookies enriched with proteins are undoubtedly popular among these items. Orange peel extract have exhibited bioactive effects such as antioxidant, antibacterial, antifungal, and anti-inflammatory activities, which provide desired properties to the product. Furthermore, the positive impacts on consumer health make them potentially ideal candidates for food supplements. Cookies microbiological quality was evaluated. According to the findings of this investigation, there was a substantial ( $P < 0.05$ ) rise in TPC, Mould, and Yeast in the cookies during storage. The TPC value of the control sample revealed a higher count of 2.29 log cfu/g after 90 days, but the O-10% samples revealed the lowest count. The mould count was not identified in any of the samples tested for up to 30 days. The control sample had the highest mould count, whereas the O-10% sample had the least count during the storage. The initial yeast count was not identified for up to 15 days, with the greatest count found in the control sample followed y O-1% sample and the lowest count found in the O-10% sample.

**Keywords:** Fish protein concentrate, cookies, fortified, microbiological quality, shelf life

### Introduction

Croakers forms a commercially important fishery in the state of Gujarat constituting an annual average catch of 0.43 lakh tonnes (5.74% of state marine catch) during 2019 [1]. The total annual landings of croakers in the year 2019 was 1.36 lakh tonnes (3.82% of the total marine fish catch of India). Croakers forms the most predominant group (21.42%) in the demersal landings of Gujarat state. More than 50% of the catch is harvested by multiday trawlers followed by mechanized dol netters and OBM gill netters [1].

Fish is high in nutrients and easy to digest. They provide high-quality proteins that are well-balanced in amino acids, polyunsaturated fatty acids, vitamins (A, B, and D), and minerals (P, Mg, Se, and I) [2]. Fish powder is a concentrated protein-rich fish product. It's a great nutritional supplement that may be used to fortify a wide variety of cereal goods, providing a healthy dose of easily digested proteins. Several studies have been conducted to incorporate fish protein concentrate (FPC) rich flour into wheat-based biscuits through fortification in order to improve their nutritional value by increasing protein and EAA contents [3].

Fish protein concentrate (FPC) is a dried and stable fish product that is suitable for human consumption and contains more protein than the original fish meat. FPC was created for human nutrition by turning a fish mince into a protein concentrate that has no similarity to the original raw material. In the food sector, FPC is used to create reformed and ready-to-eat food items. Fish cookies are flour-based items that have been completely combined with fish protein concentrate. It could have a lengthy shelf life with no negative effects. However, the use of fish protein concentrate as a dietary source is still in its early stages. Because baked foods such as crackers, cookies, and biscuits are commonly used and frequently stored for extended periods before eating, the keeping quality of these products is critical.

In the food sector, there is an increasing need for various sorts of health-oriented and shelf-stable food goods. As a result, fibre-enriched cookies with natural antimicrobial and antioxidant properties derived from plant extracts can help meet this need. Cookies are one of the most popular snacks around the world due to their ready-to-eat and easy-to-store nature,

availability in a variety of flavours, and low cost. Oats are a nutritious alternative to starch-based components because they are gluten-free and high in fibre, vital amino acids, unsaturated fatty acids, vitamins, and minerals. Many studies have found that diets high in soluble fibre from whole oats may minimise the risk of heart disease by reducing cholesterol. Oats, an essential source of dietary fibre-glucan, has been shown to decrease cholesterol and is made up of glucose molecules in long linear glucose polymers [4]. Aside from its high dietary fibre content, oat bran is also high in phenolic compounds, which have been linked to a lower risk of developing chronic diseases due to their high antioxidant capacity.

The bioactive chemicals typically present in plants have been demonstrated to have anti-carcinogenic, antibacterial, and antioxidant properties [5-6]. Although synthetic preservatives were widely employed in the processing sector to regulate the process of lipid oxidation as well as microbiological growth, their use has recently decreased due to growing concern about the safety of such chemical additives. The orange peel (*Citrus sinensis*) is a waste by-product of citrus industries that includes nutrients and other bioactive components. It contains antioxidants, taste ingredients, fibre, and minerals.

In this background, present study is aimed to prepare functional oats cookies fortified with fish protein concentrate along with plant extracts and investigate its storage stability and also study offers opportunities to develop conventional bakery product which is fortified with fish protein concentrate from low value fish and natural preservatives from plant extracts.

## 2. Material methods

### 2.1 Fish sample preparation

Freshly landed Croaker (*Otolithes cuvieri*) at Veraval coast, Gujarat, India, transported to the laboratory without 20 minute in iced condition in insulated container. Up on arrival, fishes were dressed and washed with potable chilled water (1-2°C).

### 2.2 Preparation of FPC

After dressing and washing, the fish meat was cooked for 30 minutes in hot water (120-130°C). The meat was then collected by hand picking method and cleaned 4-5 times to make germ free FPC. The picked meat was first wrapped in a piece of cloth and then pressed to remove extra water. For the preparation of FPC, it poured onto an aluminium tray and dried in an oven at 60-70°C before being pulverised.

### 2.3 Preparation of orange peel and cactus fruit extract

The orange peel were dried and prepared before being used for extraction. 300g of orange peel were treated with 600 ml ethanol/water (80/20, v/v) mixture and vigorously shaken mechanically at room temperature for 72 hours. Following filtering (Whatman No.40), the filtrate was concentrated in a vacuum rotary evaporator at 45 °C until all of the ethanol was evaporated. The aqueous extract was stored at refrigerated (4 °C) in a dark glass bottle [7].

### 2.4 Preparation of oats cookies

Fortification of FPC and plant extracts, as well as other components such as oatmeal powder, cardamom powder, sooji, sugar, and vegetable oil, were used in the preparation of oat-based oats cookies. FPC and orange peel extract were used and then it combined a selected quantity, and dough was made by hand. FPC powder was added 2% in incorporated

with oats and orange peel extract for the cookies preparation. Five different formulations were made for preparation of cookies namely; C- Control, FPC fortified oats cookies without plant extract, O1 – Cookies fortified with 1% orange peel extract; O2 – Cookies fortified with 2% orange peel extract; O3 – Cookies fortified with 3% orange peel extract; O4 – Cookies fortified with 10% orange peel extract. Following that, oats cookies were given a shape by using a mould, and coconut powder was sprinkled all over the oats cookies. Later, the oats cookies were baked/cooked in a microwave oven at 110°C for 10 minutes while the temperature of the backing oats cookies was held at room temperature. Finally, the oat cookies were packaged in a bag (made of polypropylene and polyester with a thickness of 300 Gauge) and stored at room temperature before being examined at 15 day intervals till the 90 day storage study.

**TA:** Control, FPC fortified oats cookies without plant extract

**TB:** FPC fortified oats cookies with orange peel extract

## 2.5 Microbiological Analysis

### 2.5.1 Preparation of Sample

10g of sample weighed in a sterile plastic bag and add 90 ml of sterile phosphate buffer solution for homogenized the sample using a stomacher blender for 1 minute at 250rpm and diluted  $10^{-1}$ . 1 ml of the  $10^{-1}$  dilution was mixed with 9 ml of the appropriate for plating dilutions,  $10^{-2}$  and  $10^{-3}$ , for further dilution.

### 2.5.2 Total Plate Count (TPC)

The AOAC-recommended standard method for testing the microbiological characteristics of fresh fish was used (AOAC, 2006). The total aerobic count was determined by Plate Count Agar (PCA). In serial petri dishes, 1ml of each of the needed dilutions was transferred separately. On petri plates, 18-20 ml of sterile Plate Count Agar (PCA) was spread, stirred well, and allowed to solidify. After the petri plates were placed in the incubator. For 48 hours, the samples were incubated at 37 °C. The colonies were counted after 48 hours.

TPC (CFU/g) = Average No. of colonies x Dilution factor

### 2.5.3 Total fungal count (Yeast and Mould count)

For yeast and mould enumeration, Rose Bengal Chloramphenicol (RBC) and Dichloran Rose Bengal Chloramphenicol (DRBC) were used [8]. The media was put into the petri dishes and allowed to set at 56°C. The 0.5ml of appropriate sample dilution spreaded on the surface medium [9]. For 3-5 days, the plates were incubated at room temperature. Mould colonies have a rough surface that is covered in mycelial growth, whereas yeast colonies have a moist, mucoid surface.

CFU/g = Average No. of colonies x Dilution factor × 10

## 3. Statistical Analysis

The statistical analysis is based on three replications for each sample at each storage time. All the statistical analysis were performed using Microsoft Excel ver. 2013.

## 4. Results and Discussion

### 4.1 Microbiological Characteristics

#### 4.2 Total plate Count (log cfu/g)

The initial values of total plate count in samples exhibited a tendency of growing within the critical limit as the storage

duration increased (Table 1). The interaction effect of treatments and storage duration was found to be statistically significant ( $p < 0.05$ ). The total plate count value was not discovered for the 0 day period. After 15 days, the overall plate count value for C, O-1, O-2, O-3, and O-10 was 0.83 (log cfu/g), 0.7, 0.7, 0.62, and 0.59, respectively. The value raised at the end of the storage period (90 days) in treatments

C, O-1, O-2, O-3, and O-10 was 2.29 (log cfu/g), 2.26, 2.19, 2.12, and 1.92, respectively. The findings of the microbial analysis (TPC) were below the limit, and no significant difference was found between the experimental samples. Abraha *et al.* [10] showed in a similar study that the TPC count of wheat flour biscuits and fortified biscuits was a universal indication of safety and cleanliness.

**Table 1:** Change in TPC (log cfu/g) in cookies during storage

Storage days	Control	O-1%	O-2%	O-3%	O-10%
0	0	0	0	0	0
15	0.83 ± 0.13	0.7 ± 0.17	0.7 ± 0.17	0.62 ± 0.25	0.59 ± 0.11
30	1.73 ± 0.05	1.62 ± 0.15	1.56 ± 0.07	1.46 ± 0.15	1.36 ± 0.1
45	1.88 ± 0.03	1.75 ± 0.05	1.67 ± 0.06	1.63 ± 0.06	1.52 ± 0.07
60	2.02 ± 0.06	2.01 ± 0.05	1.92 ± 0.06	1.8 ± 0.04	1.63 ± 0.06
75	2.2 ± 0.05	2.14 ± 0.02	2.05 ± 0.02	1.98 ± 0.05	1.77 ± 0.07
90	2.29 ± 0.01	2.26 ± 0.03	2.19 ± 0.03	2.12 ± 0.02	1.92 ± 0.03

### 4.3 Total fungal count

#### 4.3.1 Mould count (log cfu/g)

The presence of fungus was not found until 45 days in all treatments, similar results were reported by Atitallah *et al.* [2], yeast in canned burgers during 2 months of storage at 4 °C, although it was recognised after 90 days. The interaction

effect of treatments and storage duration was found to be statistically significant ( $p < 0.05$ ). The mould count was determined to be 3.36 (log cfu/g), 2.23, 2.06, 1.88, and 1.79 at the conclusion of the storage period in treatments C, O-1, O-2, O-3, and O-10, respectively (Table 2).

**Table 2:** Change in mould (log cfu/g) in cookies during storage

Storage days	Control	O-1%	O-2%	O-3%	O-10%
0	0	0	0	0	0
15	0	0	0	0	0
30	0	0	0	0	0
45	2.36 ± 0.1	1.85 ± 0.22	1.73 ± 0.23	1.68 ± 0.14	1.42 ± 0.1
60	2.85 ± 0.54	2 ± 0.26	1.89 ± 0.19	1.75 ± 0.48	1.63 ± 0.58
75	3.19 ± 0.51	2.19 ± 0.2	2.58 ± 0.63	1.97 ± 0.28	1.73 ± 0.05
90	3.36 ± 0.41	2.23 ± 0.29	2.06 ± 0.23	1.88 ± 0.03	1.79 ± 0.1

#### 4.3.2 Yeast count (log cfu/g)

Initially, no yeast was found in any of the samples. Except for the O-10 samples, yeast was found in all samples after the 30th day. The interaction effect of treatments and storage term

was found to be statistically significant ( $p < 0.05$ ). The mould count was determined to be 2.71 (log cfu/g), 2.22, 2.13, 2.03 and 1.87 at the conclusion of the storage period for treatment C, O-1, O-2, O-3, and O-10, respectively (Table 3).

**Table 3:** Change in yeast (log cfu/g) in cookies during storage

Storage days	C	O-1%	O-2%	O-3%	O-10%
0	0	0	0	0	0
15	0	0	0	0	0
30	1.66 ± 0.05	1.46 ± 0.15	1.26 ± 0.24	0	0
45	2.12 ± 0.30	1.86 ± 0.22	1.56 ± 0.07	1.46 ± 0.15	1.35 ± 0.1
60	2.26 ± 0.3	1.99 ± 0.25	1.89 ± 0.19	1.75 ± 0.47	1.63 ± 0.57
75	2.66 ± 0.7	2.18 ± 0.31	2.10 ± 0.28	1.87 ± 0.11	1.25 ± 0.04
90	2.71 ± 0.76	2.22 ± 0.29	2.13 ± 0.25	2.03 ± 0.22	1.87 ± 0.09

### 5. Conclusion

The fish protein concentration (FPC) from lesser tiger toothed croaker (*Otolithes cuvieri*) and orange peel extracts were used to make the cookies. Orange peel extracts are an excellent natural source of antioxidants, flavouring compounds, fibre, and minerals. The Orange peel extracts had an antibacterial impact on cookies, its resultant the lower TPC, mould, and yeast count was observed during storage. Fortification of cookies by incorporating fish protein concentration (FPC) improved nutritional quality of cookies. It improves storage stability by controlling microorganism development.

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