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Preservative effect of cactus pear fruit (*Opuntia* spp.) extract on the microbial quality of FPC-fortified functional oat cookies during storage

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Abstract

The present study focused on the development of functional oat-based cookies fortified with fish protein concentrate (FPC) derived from lesser tiger-toothed croaker (*Otolithes cuvieri*) and cactus pear fruit (*Opuntia* spp.) extract, and on evaluating their microbiological stability during storage. FPC was incorporated at 2% (w/w) to enhance protein content, while cactus pear extract, a rich source of antioxidants, dietary fiber, minerals, and natural flavor compounds, was added at varying levels (1-10%). Cookies were prepared, baked, and stored at room temperature, and microbial analysis—including total plate count (TPC), mold, and yeast—was conducted at 15-day intervals over a 90-day period. Results indicated that TPC, mold, and yeast counts gradually increased during storage but remained within permissible limits for all treatments, with fortified cookies exhibiting significantly lower microbial growth compared to the control. The highest level of cactus pear extract (10%) was most effective in inhibiting microbial proliferation. Fortification with FPC and cactus pear extract not only improved the nutritional profile of the cookies by enhancing protein, fiber, and micronutrient content but also contributed to improved storage stability. The findings suggest that these fortified cookies can serve as a health-promoting, shelf-stable snack suitable for health-conscious consumers.

Keywords: Oat-based cookies, fish protein concentrate, cactus pear extract, functional foods, microbiological stability, fortification

Introduction

Wheat-based products such as bread, cakes, and cookies are consumed worldwide in substantial quantities and constitute a major component of the human diet. Their widespread consumption is primarily attributed to their affordability, ease of processing, and simple formulations. In 2023, global wheat production reached approximately 777 million tonnes (Paterson, G., 2024) [8], underscoring the crop's critical role in ensuring food security and meeting the nutritional demands of the growing population. On the other hand, many bakery products are high in calories and fat but relatively low in protein, vitamins, and other essential nutrients (Ranhotra and Vetter, 1991) [9]. Conventional bakery products, which are primarily composed of carbohydrates and fats, can be nutritionally enhanced by increasing their protein content through the incorporation of fish protein concentrate. Biscuits and cookies are particularly suitable for protein fortification due to their wide acceptability, long shelf life, and favourable sensory characteristics. Consequently, the development of fortified biscuits, cookies, and other composite flour-based bakery products has emerged as a recent trend in the bakery industry aimed at improving nutritional quality.

The incorporation of oats and fish protein concentrate (FPC) in cookies offers significant nutritional and functional benefits. Oats enhance the nutritional profile of cookies by providing high levels of dietary fiber, essential amino acids, unsaturated fatty acids, vitamins, and minerals, while also serving as a gluten-free alternative to conventional starch-based ingredients. The inclusion of FPC substantially increases the protein content and improves the overall nutritional quality without adversely affecting product stability. Together, oats and FPC contribute to the development of protein-and fiber-enriched cookies with improved health attributes, making them suitable for health-conscious consumers and meeting the growing demand for nutritionally enhanced, shelf-stable snack foods.

The bioactive chemicals typically present in plants have been demonstrated to have anti-carcinogenic, antibacterial, and antioxidant properties [4-6]. The use of biologically active compounds derived from plants as functional ingredients in food, beverage, and cosmetic applications has gained considerable attention due to the growing preference for naturally sourced components. Plant-based bioactive compounds are widely recognized for their potential health benefits, including antioxidant, antimicrobial, and anticarcinogenic activities. Among these, cactus pear fruit (*Opuntia* spp.), belonging to the family *Cactaceae*, has attracted significant interest because of its high nutritional value and strong antioxidant potential. However, the fresh fruit has limited consumer acceptance, particularly among children, owing to its mucilaginous texture, acidity, astringency, and characteristic herbal flavour (Cruz *et al.*, 2021) [5]. As a result, processing cactus pear into value-added products such as cookies presents a viable approach to enhance its utilization and consumption. Incorporating cactus pear as a functional ingredient in cookie formulations not only helps preserve its nutritional, medicinal, and nutraceutical properties but also positions cookies as an effective carrier for bioactive compounds, thereby improving their nutritional quality and imparting additional functional benefits.

In this context, the present study was undertaken to develop functional oat-based cookies fortified with fish protein concentrate and cactus pear fruit (*Opuntia* spp.) extract, and to evaluate the influence of the extract on their microbial characteristics during storage period.

Materials and Methods

Fish Sample Preparation

Freshly landed croaker (*Otolithes cuvieri*) was procured from the Veraval coast, Gujarat, India, and transported to the laboratory within 20 min under iced conditions in an insulated container. Upon arrival, the fish were dressed and thoroughly washed with chilled potable water (1-2 °C) prior to further processing.

Preparation of Fish Protein Concentrate (FPC)

Following dressing and washing, the fish muscle was cooked in hot water at 120-130 °C for 30 min. The cooked meat was subsequently separated manually and washed 4-5 times to ensure cleanliness and microbial safety. Excess moisture was removed by wrapping the meat in a clean cloth and pressing gently. The prepared meat was then spread uniformly on aluminium trays and dried in a hot air oven at 60-70 °C until a constant weight was achieved. The dried material was finally pulverized to obtain fish protein concentrate.

Preparation of Cactus Pear Fruit (*Opuntia* spp.) Extract

Cactus pear fruits (*Opuntia* spp.) were dried and processed prior to extraction. A known quantity (300 g) of the dried fruit material was extracted using 600 mL of an ethanol-water mixture (80:20, v/v) and subjected to continuous mechanical agitation at room temperature for 72 h. The resulting extract was filtered through Whatman No. 40 filter paper, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 45 °C to remove ethanol. The remaining aqueous extract was collected and stored in dark glass bottles at 4 °C until further use (Ma *et al.*, 2008) [7].

Preparation of Oat-Based Cookies

Oat-based cookies were prepared using oatmeal powder along with other ingredients including sooji, sugar, vegetable oil, cardamom powder, and coconut powder. Fish protein concentrate (FPC) and cactus pear fruit (*Opuntia* spp.) extract were incorporated as fortifying agents. FPC was added at a fixed level of 2% (w/w), while cactus pear fruit (*Opuntia* spp.) extract was incorporated at varying concentrations. The required quantities of dry ingredients were blended uniformly, followed by the addition of oil and the respective extract to form a homogeneous dough by manual mixing.

Five formulations were prepared: C (control, without plant extract); FPC-fortified oat cookies without plant extract; C1 (cookies fortified with 1% cactus pear fruit extract); C2 (cookies fortified with 2% cactus pear fruit extract); C3 (cookies fortified with 3% cactus pear fruit extract); and C4 (cookies fortified with 10% cactus pear fruit extract). The dough was molded into uniform shapes, and coconut powder was sprinkled on the surface prior to baking. The cookies were baked in a microwave oven at 110 °C for 10 min and allowed to cool to room temperature. The baked cookies were then packaged in polypropylene-polyester laminated pouches (300-gauge thickness) and stored at room temperature. Samples were analyzed at 15-day intervals over a storage period of 90 days.

Microbiological Analysis

Sample preparation

A 10 g portion of the cookie sample was aseptically weighed into a sterile plastic bag and mixed with 90 mL of sterile phosphate buffer solution. The sample was homogenized using a stomacher blender at 250 rpm for 1 min to obtain a uniform suspension, corresponding to a 10^{-1} dilution. Serial dilutions were then prepared by transferring 1 mL of the 10^{-1} dilution into 9 mL of sterile diluent to obtain 10^{-2} and 10^{-3} dilutions for subsequent microbiological plating and enumeration.

Total plate count (TPC)

The total aerobic microbial count was determined using the standard method recommended by the AOAC (2006) [1] for microbiological analysis of food samples. Plate Count Agar (PCA) was used to enumerate the total aerobic bacteria. For this purpose, 1 mL of each appropriate dilution was aseptically transferred into sterile Petri dishes, and 18-20 mL of molten PCA was poured over the sample, mixed gently, and allowed to solidify. The plates were incubated at 37 ± 1 °C for 48 h. Following incubation, colonies were counted, and the total plate count was calculated using the formula.

$$\text{TPC (CFU/g)} = \text{Average No. of colonies} \times \text{Dilution factor}$$

Total fungal count (Yeast and Mold)

Yeast and mold enumeration was carried out using Rose Bengal Chloramphenicol (RBC) and Dichloran Rose Bengal Chloramphenicol (DRBC) media (AOAC, 2006) [1]. The media were poured into sterile Petri dishes and allowed to solidify at 56 °C. Subsequently, 0.5 mL of the appropriate sample dilution was spread evenly on the surface of the agar (AOAC, 1997) [2]. The plates were incubated at room temperature for 3-5 days. Mold colonies were identified by their rough, mycelium-covered surface, while yeast colonies

appeared as moist, mucoid growths. Fungal counts were calculated using the following formula:

$$\text{CFU/g} = \text{Average No. of colonies} \times \text{Dilution factor} \times 10$$

Statistical Analysis

All experiments were conducted in triplicate, and the results are presented as the mean of three independent replicates. Statistical analyses were performed using Microsoft Excel (version 2013), and relevant data were evaluated to determine variations among the treatments at different storage intervals.

Results and Discussion

Microbiological Characteristics

Total plate count (log cfu/g)

The initial total plate count (TPC) of the cookie samples exhibited a gradual increase over the storage period, remaining within acceptable limits (Table 1). The interaction between treatments and storage duration was found to be statistically significant ($p < 0.05$). At day 0, no detectable microbial growth was observed. After 15 days, the TPC values were 0.83 ± 0.13 , 0.77 ± 0.12 , 0.66 ± 0.22 , 0.67 ± 0.19 , and 0.63 ± 0.06 log CFU/g for C, C-1, C-2, C-3, and C-10, respectively. By the end of the 90-day storage period, TPC values increased to 2.29 ± 0.01 , 2.25 ± 0.01 , 2.19 ± 0.03 , 2.10 ± 0.02 , and 1.88 ± 0.03 log CFU/g for the same treatments. Despite this increase, all values remained within permissible limits, indicating the microbiological safety of the cookies throughout the storage period. No significant differences were observed among the experimental samples. These results are in agreement with the findings of (Abraha *et al.* 2018) [3], who reported that the TPC of wheat flour biscuits and fortified biscuits serves as a reliable indicator of microbial safety and hygienic quality.

Total Fungal Count

Mould count (log cfu/g)

Fungal growth was not detected in any of the treatments up to 45 days of storage and appeared only after 90 days. The interaction between treatments and storage duration was statistically significant ($p < 0.05$). At the end of the storage period, mold counts were 3.36 ± 0.41 , 2.36 ± 0.10 , 2.05 ± 0.23 , 1.99 ± 0.44 , and 1.86 ± 0.09 log CFU/g for treatments C, C-1, C-2, C-3, and C-10, respectively, with the highest fungal load observed in the control (C) sample (Table 2). These results indicate that fortification with cactus pear fruit (*Opuntia* spp.) extract effectively reduced fungal proliferation during storage.

Yeast count (log cfu/g)

No yeast was detected in any of the samples during the initial days of storage. Yeast growth was observed in all samples from the 30th day of storage, except in the C-3 and C-10 treatment. The interaction between treatments and storage period was statistically significant ($p < 0.05$). At the end of the storage period, yeast counts were 2.71 ± 0.76 , 2.24 ± 0.26 , 2.11 ± 0.24 , 2.05 ± 0.37 , and 1.85 ± 0.08 log CFU/g for treatments C, C-1, C-2, C-3, and C-10, respectively (Table 3). These results indicate that higher levels of cactus pear fruit (*Opuntia* spp.) extract contributed to reduced yeast proliferation during storage.

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

Storage days	C	C-1 %	C-2%	C-3%	C-10%
0	0	0	0	0	0
15	0.83 ± 0.13	0.77 ± 0.12	0.66 ± 0.22	0.67 ± 0.19	0.63 ± 0.06
30	1.73 ± 0.05	1.59 ± 0.11	1.56 ± 0.07	1.42 ± 0.1	1.2 ± 0.17
45	1.88 ± 0.03	1.75 ± 0.05	1.67 ± 0.06	1.56 ± 0.07	1.42 ± 0.1
60	2.02 ± 0.06	1.98 ± 0.07	1.92 ± 0.06	1.75 ± 0.05	1.56 ± 0.07
75	2.2 ± 0.05	2.1 ± 0.02	2.05 ± 0.02	1.97 ± 0.03	1.75 ± 0.05
90	2.29 ± 0.01	2.25 ± 0.01	2.19 ± 0.03	2.1 ± 0.02	1.88 ± 0.03

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

Storage days	C	C-1 %	C-2%	C-3%	C-10%
0	0	0	0	0	0
15	0	0	0	0	0
30	0	0	0	0	0
45	2.36 ± 0.1	1.73 ± 0.26	1.69 ± 0.27	1.52 ± 0.07	1.43 ± 0.23
60	2.85 ± 0.54	1.91 ± 0.27	1.82 ± 0.11	1.75 ± 0.08	1.69 ± 0.09
75	3.19 ± 0.51	2.17 ± 0.23	1.95 ± 0.3	1.8 ± 0.04	1.82 ± 0.07
90	3.36 ± 0.41	2.36 ± 0.1	2.05 ± 0.23	1.99 ± 0.44	1.86 ± 0.09

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

Storage days	C	C-1 %	C-2%	C-3%	C-10%
0	0	0	0	0	0
15	0	0	0	0	0
30	1.66 ± 0.05	1.35 ± 0.1	1.20 ± 0.1	0	0
45	2.12 ± 0.30	1.82 ± 0.30	1.51 ± 0.07	1.35 ± 0.1	1.30 ± 0
60	2.26 ± 0.3	1.90 ± 0.26	1.75 ± 0.08	1.66 ± 0.1	1.59 ± 0.11
75	2.66 ± 0.7	2.06 ± 0.2	1.80 ± 0.03	1.75 ± 0.04	1.67 ± 0.01
90	2.71 ± 0.76	2.24 ± 0.26	2.11 ± 0.24	2.05 ± 0.37	1.85 ± 0.08

Common abbreviations

1. FPC (Fish protein concentrate)
2. TPC (Total plate count)
3. CFU (Colony Forming Unit)

Conclusion

The incorporation of fish protein concentrate (FPC) and cactus pear fruit (*Opuntia* spp.) extract into oat-based cookies effectively enhanced their nutritional and functional properties. Cactus pear extract, a rich source of antioxidants, dietary fiber, minerals, and natural flavor compounds, contributed to the antimicrobial activity of the cookies, resulting in reduced total plate counts, mold, and yeast during storage. Fortification with FPC and cactus pear extract not only improved the protein and micronutrient content but also enhanced storage stability by controlling microbial growth, demonstrating the potential of these ingredients to develop health-promoting, shelf-stable functional cookies.

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References

1. AOAC. Assn. of Official Analytical Chemists. Official methods of analysis of Association of Official Analytical Chemists International. 2006.

2. AOAC. Official Methods of Analysis (15th ed.), Association of Official Analytical Chemists, Washington, DC; 1997.
3. Abraha B, Mahmud A, Admassu H, Habte-Tsion HM, Xia W, Yang F. Production of biscuit from Chinese sturgeon fish fillet powder (*Acipenser sinensis*): A snack food for children. Journal of Aquatic Food Product Technology. 2018 Nov 26;27(10):1048-1062.
4. Ahn J, Grün IU, Mustapha A. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. Food Microbiology. 2007 Feb 1;24(1):7-14.
5. Cruz LL, García-Mateos R, Moncada CY, Corrales-García J. Sweetened nopal flakes: a functional snack. Journal of Applied Botany & Food Quality. 2021 Jan 1;94.
6. Kanatt SR, Chander R, Sharma A. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chemistry. 2007 Jan 1;100(2):451-458.
7. Ma YQ, Chen JC, Liu DH, Ye XQ. Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. Journal of Food Science. 2008 Oct;73(8):T115-120.
8. Paterson G. Food Outlook: Biannual Report on Global Food Markets. Interaction (Melbourne). 2024 Sep 1;52(3).
9. Ranhotra GS, Vetter JL. Foods considered for nutrient addition: snacks and confectioneries. Nutrient Addition to Food. 1991:319-345.