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Microbiological analysis of dietary fibre enriched prebiotic biscuit

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Abstract
This research study's primary goal was to assess the microbiological analysis of freshly made biscuits. In this study, control biscuits were made without prebiotics, while experimental biscuits were made with varying prebiotic dosages. Oat powder was utilised as a prebiotic. The nutritious value of bakery products and their potential for use in feeding programmes and emergencies sparked customers' curiosity. After analysis of SPC (CFU/g) and Yeast and Mould (CFU/g), it was found that the mean value of SPC of control (T_0) biscuit was 1.40 CFU/g and mean value of SPC (CFU/g) of experimental (T_1, T_2 and T_3) biscuit were 1.63, 2.00 and 2.20 (CFU/g) respectively. After descriptive statistical analysis of yeast and mould (CFU/g), it was found that the mean value of control (T_0) biscuit was 1.50 CFU/g and mean value of yeast and mould of experimental (T_1, T_2 and T_3) biscuit were 2.00, 2.30 and 2.40 (CFU/g) respectively.

Keywords: Bakery products, SPC, Yeast and mould, coliform, microbiological analysis, prebiotics, protein, oats

Introduction
A convenience food that falls under the genre of baked food is a biscuit. A biscuit is a crisp food that has less than 6% moisture and is created from weak gluten wheat flour as the primary raw material along with various auxiliary ingredients like sugar (or without), oil, and other excipients by flour blending, moulding, baking, and other procedures (Hu *et al*., 2022) [2].

There are primary and auxiliary ingredients in biscuits. Salt, egg, emulsifier, starter (sodium bicarbonate, ammonium bicarbonate), milk powder, and flavouring spices are optional secondary components. Among these, flour, fat/oil, sugar, and water are the basic major ingredients (Mancebo *et al*., 2015) [3].

In this study, a biscuit high in dietary fibre was prepared. Total dietary fibre is the part of a plant that withstands intestinal digestion in the human large intestine. Consuming a lot of dietary fibre is associated with a lower incidence of prevalent illnesses and diseases in contemporary civilisations because total dietary fibre has been found to have good effects on human health and physical function (Parveen, 2017) [5].

This research study was carried out to develop dietary fibre enriched prebiotic biscuit. As a source of dietary fibre and prebiotics, oats are used in this experimental biscuit.

Aims and Objectives
Keeping in view the above-mentioned importance of prebiotics, with health benefits of oats, a research study on "Microbiological analysis of dietary fibre enriched prebiotic biscuit" was carried out to analyse the microbial content of newly prepared biscuit.

Materials and methods
The experiments related to "Microbiological analysis of dietary fibre enriched prebiotic biscuit" carried out in the research laboratory of Nutrition, Mahishadal Raj College, W.B., India.

Procurement of raw material
For preparation of biscuit, the raw ingredients like Oat powder, Wheat Flour, sugar, oil, Baking Powder were purchased from local market of Mahishadal.

Treatment combinations (Mondal *et al*., 2022) [4].
T_0= Oats powder (0%): Wheat Flour (80 g) + Sugar (5 g) + Salt (0.90 gm) + Butter (5 g) +Water (10) Baking at 175^0C for 15 Mins.
T_1= Oats powder (10 g): Wheat Flour (70 g) + Sugar (5 g) + Salt (0.90 gm) + Butter (5 g) +Water (10) Baking at 175^0C for 15 Mins.
T_2= Oats powder (15g): Wheat Flour (65 g) + Sugar (5 g) + Salt (0.90 gm) + Butter (5 g) +Water (10) Baking at 175^0C for 15 Mins.
T_3= Oats powder (20 g): Wheat Flour (60 g) + Sugar (5 g) + Salt (0.90 gm) + Butter (5 g) +Water (10) Baking at 175^0C for 15 Mins.
No. of Treatment: 4 +1 =5
No of replication: 03
Total no of trials: 15

Flow chart for the preparation of biscuit (control biscuit) (Uchenna & Omolayo, 2017) [6]

Ingredient weighing (Wheat Flour, sugar, salt, butter and water)

↓

Mixing

↓

Cutting

↓

Baking

↓

Cooling

↓

Packaging

↓

Biscuit

Flow chart for the preparation of biscuit (experimental biscuit) (Uchenna & Omolayo, 2017)

Ingredient weighing (Wheat Flour, oats powder, sugar, salt, butter and water)

↓

Mixing

↓

Cutting

↓

Baking

↓

Cooling

↓

Packaging

↓

Biscuit

Microbiological analysis of analysis of final products
Preparation of Dilutions

Preceding elimination of the Biscuit pattern from its box, thoroughly and vigorously blend substance to ensure the sampling of delegate component. Before starting a Biscuit sample, wipe the pinnacle of box with a sterile material or cotton saturated with 70% alcohol. Then straight away before shifting check part of Biscuit, shake container, making 25 whole up-and-down/ again-and-forth actions of approximately one foot in 7 seconds. Choose dilution(s) in a way that the whole quantity of colonies on a plate can be between 30 and 300. After that do away with 1 ml of the Biscuit pattern with a sterile pipette and move it to the primary tube of diluents (nine ml). Allow approximately 2 – 4 seconds for the substance of the pipette to drain and lightly blow out the final drop. Rotate the test tube among arms of the hand to complete the integration. This makes a dilution of 1:10 in addition, a sequence of dilutions can be organized by way of moving 1 ml of the primary dilution (1:10) into another 9 ml dilution blank to get 1:one hundred dilution and so on. wherein the solids content material or viscosity of Biscuit samples exceeds that of complete milk e.g., cultured dairy products, put together the preliminary 1:100 (or1:100) dilution by means of wt. 1 g (or 11 g) aseptically into containing 99 ml of dilution blank.

Preparation and Incubation of Plates

Use of fresh pipette and switch 1 ml of each required dilution into sterile petri dishes in duplicate. Permit 2-four seconds for the pipette to drain, touch the pinnacle of the pipette to dry vicinity within the petri dish to empty out the remaining drop. Then upload 10-15 ml of fashionable milk agar previously melted and cooled to 450 C. mix the substance of the plate very well while the medium remains liquid by lightly rotating the petri dishes and allow the agar to chill and set. Invert the plates and incubate at 370C for forty-eight h.

Counting of Colonies (SPC in CFU/g)

After 48 h remove the plate and pick the pair of plates having colonies between 30 and 300 on every plate and count number the variety of colonies by using a colony counter and judge the common of the counts in the 2 plates and multiply this by the dilution aspect and document as SPC (cfu/g).

Yeast and Mould (CFU/g)

It was done according to the process given by way of (APHA) fashionable technique for the examination of Dairy products (1992). Firstly, four check tubes were classified as 10-1, 10-2, 10-three and 10-4 respectively and nine ml of ringer's solution were taken in every test tube. After that 1 ml of the product was introduced to the take a look at tube categorized 10-1. Then 1ml of the diluted product changed into taken from 10-1 dilution and poured into the ten-2 dilution and the manner of serial dilution become continued until the dilution had reached up to ten-four. Then four petri-dishes each for 10-1, 10-2, 10-3and 10- four were labelled. 1ml of diluted product changed into poured from 10-1 into 4 petri-dishes every. The identical became repeated for the dilution 10-2, 10-three and 10-4. Then the sterilized Potato Dextrose agar turned into poured in each petri-dish and it was incubated at 37°C for 24-forty-eight hours. Sooner or later the colonies had been counted and the average was calculated.

Coliform count

It turned into achieved as consistent with the manner given through (APHA) popular technique for the exam of Dairy merchandise (1992). 5 ml of Mc Conkey's broth became taken in six test tubes and of each 10-1, 10-2, 10-3and 10-four and Durham's tube have been slipped in check tubes in inverted function. Then, the above check tubes were autoclaved at 15 lbs strain for 15-20 mins. After that 9ml of

ringer's solution turned into taken in 4 test tubes and turned into labelled 10-1, 10-2, 10-3 and 10-four. Then 1 ml of the product changed into introduced within the above 10-1. 1 ml of the diluted product became taken from 10-1 and poured into the ten-2 dilution and the manner of serial dilution turned into continued until the dilution had reached up to ten-four. From the above check tubes 1 ml of the diluted product was added to check tubes containing McConkey's broth. Sooner or later the take a look at tubes have been incubated at 37°C for 24-forty-eight hours and tested for the Coliform.

Statistical analysis

Factorial Analysis and Critical Difference (C.D.) were used for the physico-chemical and antioxidant parameters for developed cookies, and Two-Way Analysis of Variance (ANOVA) technique and Critical Difference (C.D.) were used for developed dough, in order to assess the statistical significance of the research findings. For all analyses, means and standard deviations were computed. The mean and standard deviation of three parallel measurements is used to express all data.

Results and discussions

This research study entitled "Microbiological analysis of dietary fibre enriched prebiotic biscuit" was carried out in the Laboratory of Nutrition, Mahishadal Raj College to develop dietary fibre enriched prebiotic biscuit by using oats in different percentage.

Table 1: Descriptive statistics of SPC (CFU/g) of control (T_0) and experimental (T_1, T_2, T_3) newly developed products

Treatments	T0	T1	T2	T3
observations N	3	3	3	3
mean	1.4000	1.6333	2.0000	2.2000
sample std. dev.	0.1000	0.3215	0.1000	0.1000
std. dev. of mean SE	0.0577	0.1856	0.0577	0.0577

After descriptive statistical analysis of SPC (CFU/g), it was found that the mean value of SPC of control (T_0) biscuit was 1.40 CFU/g and mean value of SPC of experimental (T1, T2 and T3) biscuit were 1.63, 2.00 and 2.20 (CFU/g) respectively.

Table 2: One-way ANOVA of SPC (CFU/g) of control (T_0) and experimental (T_1, T_2, T_3) newly developed products

Source	sum of squares SS	degrees of freedom v	mean square MS	F statistic	p-value
treatment	1.1625	3	0.3875	11.6250	0.0028
error	0.2667	8	0.0333		
total	1.4292	11			

Table 3: significance and insignificance results of treatments

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
a vs b	2.2136	0.4482348	insignificant
a vs c	5.6921	0.0161120	* p<0.05
a vs d	7.5895	0.0029854	** p<0.01
b vs c	3.4785	0.1421749	insignificant
b vs d	5.3759	0.0217891	* p<0.05
c vs d	1.8974	0.5613897	insignificant

The results from the previous analysis showed that there was a nonsignificant difference between a (T0) and b (T1), b(T1) and c (T3); c (T2) and d (T3) and there was significantly difference between a (T0) and c (T2); b (T1) and d (T3) at p<0.05. and there are significantly difference between a (T0) and d (T3) at p<0.01.

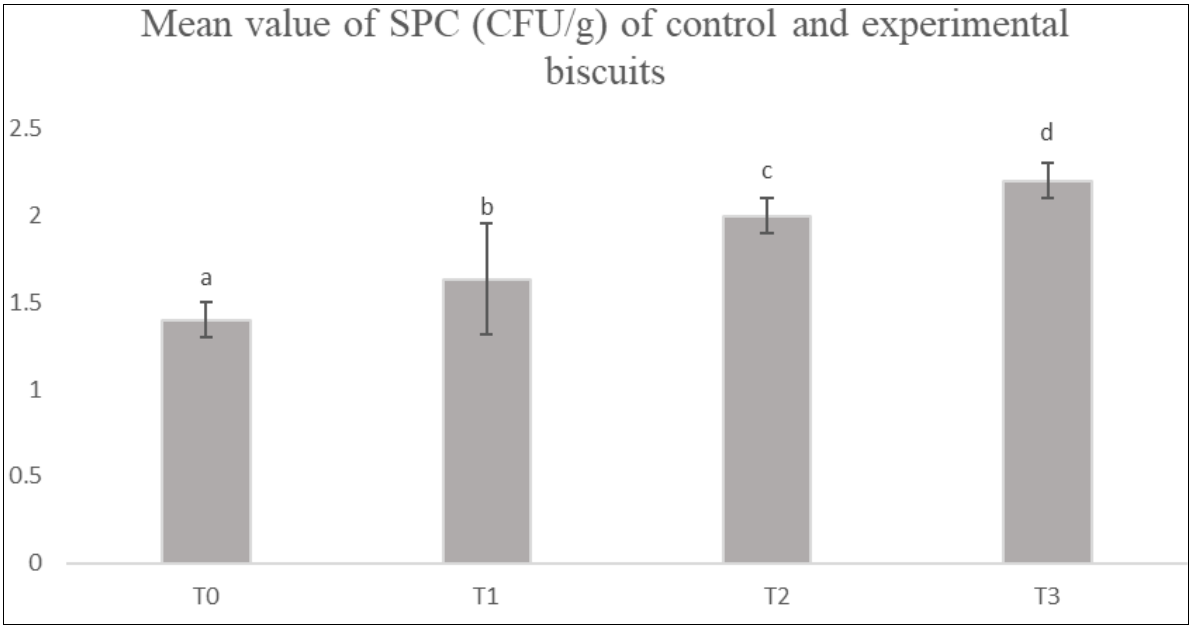

Fig 1: Graphical representation of SPC of newly prepared biscuit

Table 4: Descriptive statistics of yeast and mould (CFU/g) of control (T_0) and experimental (T_1, T_2, T_3) newly developed products

Treatments	T0	T1	T2	T3
observations N	3	3	3	3
mean	1.5000	2.0000	2.3000	2.4000
sample std. dev.	0.1000	0.1000	0.1000	0.1000
std. dev. of mean SE	0.0577	0.0577	0.0577	0.0577

After descriptive statistical analysis of yeast and mould (CFU/g), it was found that the mean value of control (T_0) biscuit was 1.50 CFU/g and mean value of yeast and mould of experimental (T1, T2 and T3) biscuit were 2.00, 2.30 and 2.40 (CFU/g) respectively.

Table 5: One-way ANOVA of yeast and mould (CFU/g) of control (T_0) and experimental (T_1, T_2, T_3) newly developed products

Source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	1.4700	3	0.4900	49.0000	1.7099e-05
error	0.0800	8	0.0100		
total	1.5500	11			

Table 6: significance and insignificance results of treatments

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
a vs b	8.6603	0.0012698	** p<0.01
a vs c	13.8564	0.0010053	** p<0.01
a vs d	15.5885	0.0010053	** p<0.01
b vs c	5.1962	0.0259323	* p<0.05
b vs d	6.9282	0.0052352	** p<0.01
c vs d	1.7321	0.6211878	insignificant

The results from the previous analysis showed that there was a nonsignificant difference between a (T0) and b (T1), a (T0) and c (T2); a (T0) and d (T3); b (T2) and d (T3) at p<0.01 and b(T1) and c (T2) at p<0.05. And there was insignificantly difference between c (T2) and d (T3).

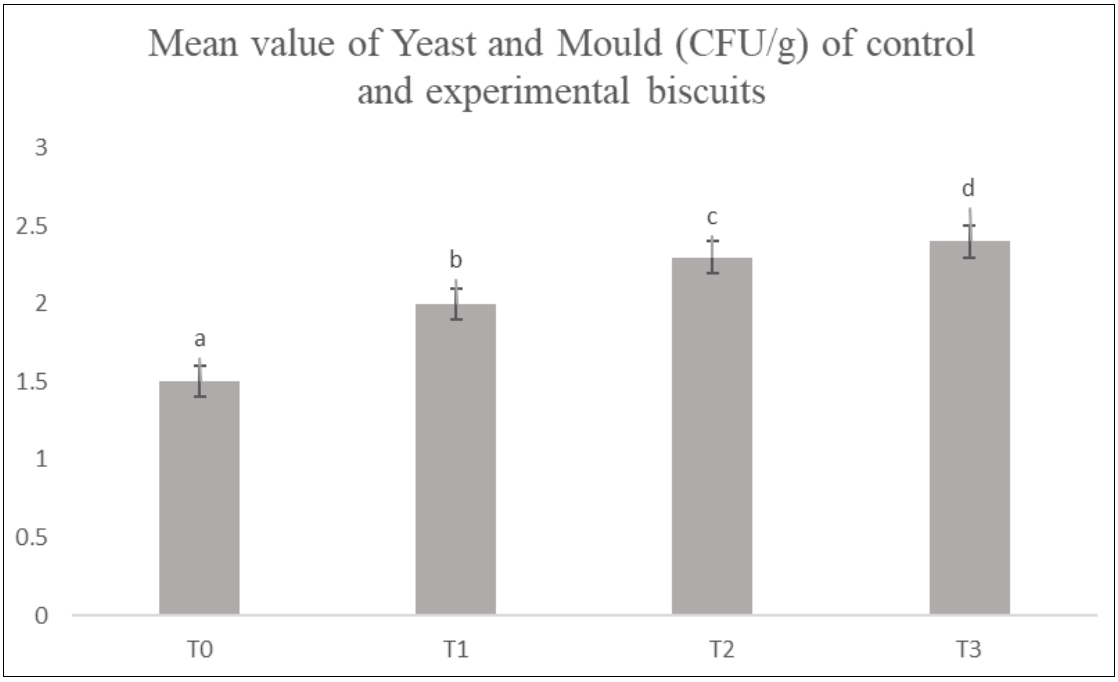

Fig 2: graphical representation of yeast and mould of newly prepared biscuit

Conclusion

It was discovered that the mean SPC (CFU/g) of the control (T_0) biscuit was 1.40 CFU/g while the mean SPC (CFU/g) of the experimental (T_1, T_2 and T_3) biscuit was 1.63, 2.00, and 2.20 (CFU/g), respectively. The mean value of the control (T_0) biscuit was 1.50 CFU/g, and the mean value of the experimental (T_1, T_2 and T_3) biscuit was 2.0, 2.30, and 2.40 (CFU/g), respectively, according to descriptive statistical analysis of yeast and mould (CFU/g).

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