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Studies on acceptability and microbial analysis of paneer incorporated with spinach powder

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Abstract
The mean colour and appearance scores of paneer incorporated with spinach powder for treatments T_1, T_2, T_3 and T_4 were 7.75, 8.17, 8.48 and 7.68, respectively. The average flavour scores of paneer incorporated with spinach powder were first increased at treatment T1 (8.06) and then gradually decreased from treatment T_2, T_3 to T_4 with scores 8.16, 8.25 to 7.88. For developed paneer mean score for body and texture were ranged from 7.98 to 8.26 regarding various treatments. Treatments T_1, T_2, T_3 and T_4 with body and texture scores as 7.98, 8.09, 8.26 and 8.17, respectively. The average mouthfeel scores of developed paneer incorporated with spinach powder were 7.54, 7.71, 7.91 and 7.85 for treatment T_1, T_2, T_3 and T_4, respectively. The mean score of overall acceptability for developed paneer with treatments T_1, T_2, T_3 and T_4 were as 7.92, 8.15, 8.35 and 7.88.

Keywords: Incorporated, sensory analysis, overall acceptability, microbial analysis, plate count

Introduction
Paneer is prepared by addition of organic acid into milk which is heating at 90 ºC. Paneer is tremendously used in vegetable dishes in Northwest Pakistan. Paneer is generally prepared by coagulation of buffalo milk. The final formed paneer and its quality depends on the quality of buffalo milk, quality of coagulant and temperature at which organic acid is added to milk for coagulation (Masud *et al.* 2007) [14]. Paneer is a high source of animal protein that is very inexpensive and serves as a significant supply of animal protein for vegetarians. In addition to having a high protein content and digestibility, paneer has a biological value of 80 to 86. Paneer is a low-cost form of animal protein that provides a large amount of animal protein to vegetarians which had higher digestibility (Shrivastava and Goyal, 2007) [18]. Paneer is a South Asian soft cheese made by acid and heat coagulation of milk. It is an unripened cheese that is non-fermentative, non-renneted and non-melting. Paneer is widely consumed in South Asia, either fresh or prepared in a variety of gourmet meals and snacks. Paneer manufacturing is currently widespread over the world. One aspect that has led to increased adoption of paneer in its ability to be deep-fried, making it a favourite for preparing appetisers like *pakoras* or fried paneer pieces. Paneer is a common raw ingredient in South Asia or the creation of a variety of gourmet meals and snacks Paneer manufacturing is currently widespread over the world (Aneja *et al.* 2007) [3]. The spinach leaves extract along with milk helps in the reduction of tooth discolouration which is caused by regular consumption of coffee. Most people feel chalky teeth after they consumed raw spinach leaves as salad this is due to oxalic acid content in spinach which is insoluble and deposits over teeth. Drinking milk will exaggerate spinach teeth sensation because oxalic acid is washed out along with milk and teeth become more whitish (Iskandar *et al.* 2018) [10]. Spinach has a full package of fibers, vitamins (*viz.* vit. A, C, E, K, B_2 and B_6), calcium, magnesium, manganese, iron, folic acid, protein and niacin with other flavonoids due to which it becomes most important leafy vegetable usually consumed after boiling or either fresh raw leaves as a salad. It is also a good source of chlorophyll which is helpful in digestion. Spinach leaves are also helpful in joint pain, inflammation of the lungs, bowel movement, thirst, sore eyes, ringworm scabies and leukoderma (Miri and Roughani 2019) [15]. Spinach leaf powder contains all nutrients such as protein, fiber, antioxidants, and minerals which makes spinach powder an ideal ingredient to be added in the formulation of foods with high nutritional and biological values. In the current experimental study, it is observed that spinach nano powder (0.50 percent, 1 percent, 1.50 percent and 2 percent) was used in the manufacturing of cheese.

The quality of the cheese is raised by measuring its chemical composition, colour and sensory parameters. Cheese containing 0.5 percent and 1percent spinach powder demonstrated high values for sensory parameters than other treatments (El-Sayed, 2019) [5]. In the last two decades of research, it was discovered that there is a paucity of information on the assessment of nutritional, pharmacological, and functional qualities of spinach, as well as the comparative analysis. To assess changes in the characteristics of ambient and cryogenic powdered black pepper, such a complete investigation is necessary.

Material and Methodology

Treatment combinations

Following treatment combinations were considered for preparation of paneer with black pepper.

T_1= Paneer from Buffalo milk (control)

T_2= Paneer with 0.5 percent of spinach powder by weight of buffalo milk

T_3= Paneer with 1 percent of spinach powder by weight of buffalo milk

T_4= Paneer with 1.5 percent of spinach powder by weight of buffalo milk

Experimental Methodology

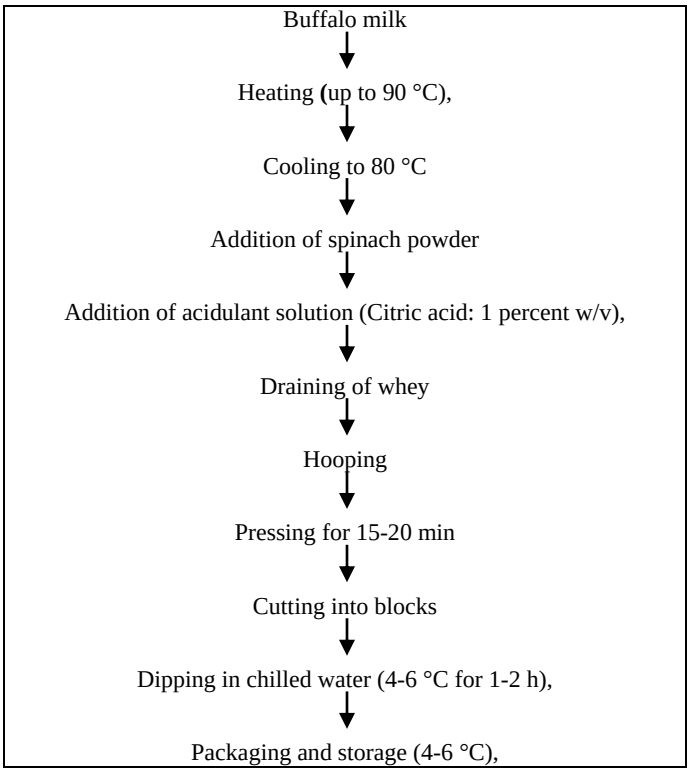

Fig 1: Flow chart for preparation of paneer. (Ref: Guruditta *et al.* 2019) [7]

Sensory analysis

A panel of five judges used a nine-point Hedonic scale to rate the paneer incorporated with spinach powder for colour and appearance, flavour, body and smoothness. The mean was calculated using the numerical scores given by judges for each quality aspect and this mean was then statistically analyzed. The scoring was done on a "9-point hedonic scale" developed by Quarter Master, Food and Container Institute, (Gupta, 1976) USA.

Microbial analysis

The freshly prepared product was microbiologically analysed for yeast, mould, coliform and standard plate count according to Ranganna (1986) [16].

Coliform count

The coliform count was carried out on dehydrated violet red bile agar (VRBA) Medium (IS: 1224: part I and II, 1981). Enumeration of a coliform count of paneer incorporated with spinach powder was determined by using the pour plate method described by Hought *et al*., (1992), employing violet red bile agar (VRBA) of pH 7.4 ± 0.1. The prepared plates were incubated at 37 °C for 48 hr. Colonies with dark red colouration were counted and they were expressed as log CFU/gm of sample.

Yeast and mould count

Yeast and mould count were determined using a Potato Dextrose Agar (PDA) medium using the procedure given in IS: 1224 (part I and II, 1981). Paneer was tested for yeast and mold counts as per suggested by Marshall (1993) using potato dextrose agar (PDA) and pH of media adjusted to 3.5 ± 0.1 using tartaric acid solution. The prepared plates were incubated at 30 °C for 3-5 days and counts were expressed as log CFU per ml of sample. The pour plate technique was followed and the results were expressed in CFU/gm.

Standard plate Count

The microbiological analysis of paneer samples held at room temperature and refrigeration temperature was performed and examined at one-day intervals up to four days at room temperature and seven-day intervals followed by two days at refrigeration temperature for estimation of standard plate count (SPC), The results expressed in log of 103 x CFU/gm. Serial dilutions were prepared in 0.1 percent saline water. 1 ml of each serial dilution were inoculated in petri plates

containing 15-20 ml sterilized nutrient agar and incubated at 37 °C to 24 to 48 hrs. The results expressed in CFU/gm and the pour plate technique was followed.

Table 1: Sensory evaluation of paneer incorporated with spinach powder

Colour and appearance					
Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	8.00	8.00	7.00	8.00	7.75^{b}
T_2	8.15	8.20	8.10	8.22	8.17^{a}
T_3	8.53	8.50	8.56	8.48	8.52^{a}
T_4	7.60	7.80	7.55	7.78	7.68^{b}
SE ± 0.1299 CD at 5% 0.4002					
Flavour score					
Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	8.03	8.05	8.08	8.06	8.06^{c}
T_2	8.18	8.14	8.16	8.16	8.16^{b}
T_3	8.25	8.27	8.22	8.24	8.25^{a}
T_4	7.85	7.92	7.87	7.89	7.88^{d}
SE ± 0.0112 CD at 5% 0.0346					
Body and texture					
Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	8.00	8.00	8.00	7.90	7.98^{c}
T_2	8.02	8.10	8.20	8.05	8.09^{b}
T_3	8.15	8.25	8.45	8.20	8.26^{a}
T_4	8.10	8.15	8.28	8.15	8.17^{ab}
SE ± 0.0446 CD at 5% 0.1376					
Mouth feel					
Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	7.50	7.55	7.50	7.60	7.54^{d}
T_2	7.80	7.64	7.60	7.80	7.71^{c}
T_3	8.00	7.80	7.85	8.00	7.91^{a}
T_4	7.95	7.77	7.74	7.92	7.85^{b}
SE ± 0.0468 CD at 5% 0.1442					
Overall acceptability					
Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	7.75	8.06	7.98	7.90	7.92^{c}
T_2	8.17	8.16	8.09	8.16	8.15^{b}
T_3	8.52	8.25	8.26	8.38	8.35^{a}
T_4	7.68	7.88	8.17	7.78	7.88^{d}
SE ± 0.0699 CD at 5% 0.2154					

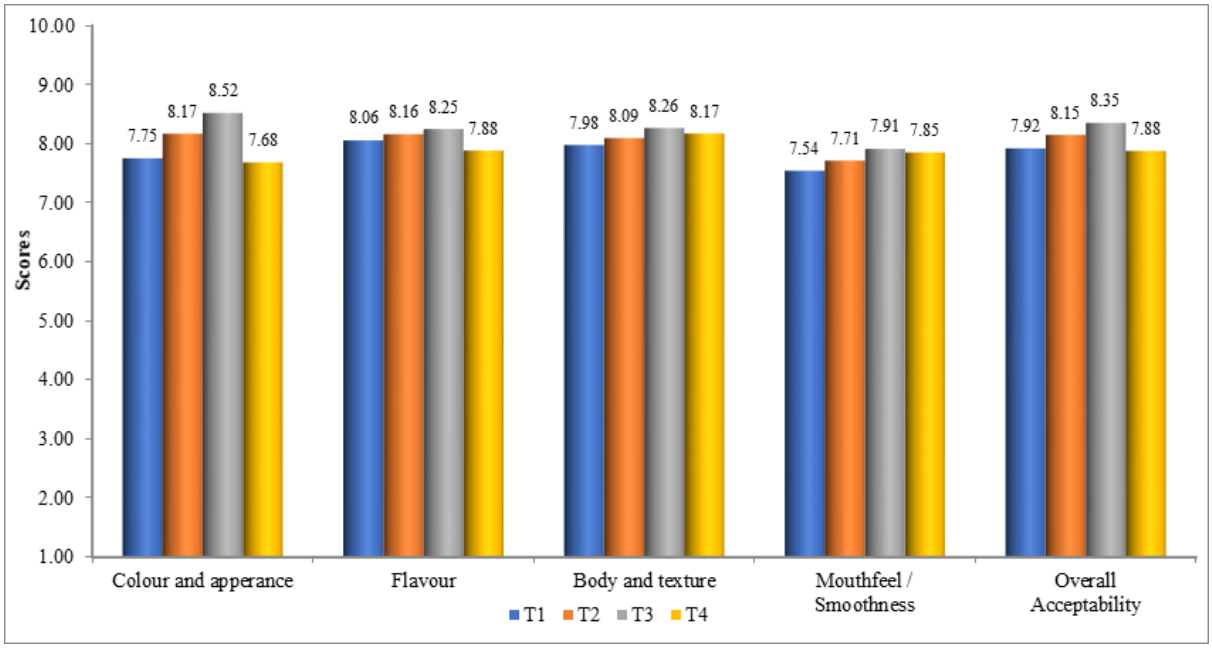

Fig 2: Sensory qualities of paneer incorporated with spinach powder

Table 2: Yeast and mould count of paneer incorporated with spinach powder (CFU/gm

Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	1.00	1.02	1.00	1.03	1.01^c
T_2	1.05	1.06	1.08	1.10	1.07^b
T_3	1.09	1.10	1.11	1.12	1.11^a
T_4	1.11	1.12	1.12	1.13	1.12^a
SE ± 0.0076 CD at 5% 0.0236					

Table 3: Coliform count of paneer incorporated with spinach powder

Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	N.D.	N.D.	N.D.	N.D.	N.D.
T_2	N.D.	N.D.	N.D.	N.D.	N.D.
T_3	N.D.	N.D.	N.D.	N.D.	N.D.
T_4	N.D.	N.D.	N.D.	N.D.	N.D.
SE ± 0.00 CD at 5% 0.00					

(CFU/gm)

Table 4: Standard plate count of paneer incorporated with spinach powder (CFU x 10^3 /gm)

Replication Treatment	R_1	R_2	R_3	R_4	Mean
T_1	3.04	3.05	3.00	3.07	3.04^d
T_2	3.15	3.11	3.14	3.17	3.14^c
T_3	3.26	3.22	3.28	3.24	3.25^b
T_4	3.34	3.37	3.33	3.39	3.36^a
SE ± 0.01350 CD at 5% 0.0416					

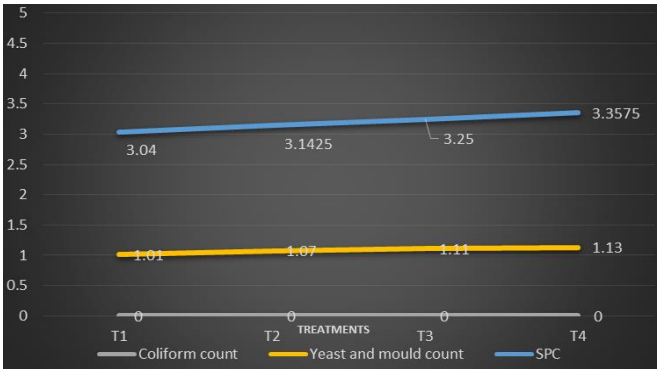

Fig 3: Coliform counts, yeast and mould counts and standard plate counts of paneer incorporated with spinach powder

Results

Colour and appearance

In present study T_2 (8.17) and T_3 (8.52) were found at par with each other and significantly ($p < 0.05$) different from T_1 (7.75) and T_4 (7.68). It indicates that the colour and appearance score is increased by up to 0.5 and 1 percent of spinach powder, respectively as shown in table 1. Galla *et al.* (2017) [6] studied that percent spinach leaves powder. In terms of organoleptic acceptability, increasing the integration level resulted in a considerable reduction in the colour score of burfi added with spinach powder. Sarnaik *et al.* (2020) [17] found that there were substantial changes that occurred regarding colour and appearance mean scores of the black pepper powder added to paneer. Ahmed and Bajwa (2022) [2] reported that the light reddish/brownish hue provided by the phenolic compounds of amla was diminishing the colour and appearance score of amla paneer because paneer absorbs the colour of the additional Components.

Flavour score

Paneer incorporated with spinach powder content had a significantly ($p<0.05$) different mean flavour score. The flavour rating of the different treatments under consideration ranged from 7.88 to 8.25. In this current study it was found that T_1, T_2, T_3 and T_4 were significantly ($p<0.05$) different from each other. Obtained flavour scores indicated that spinach incorporated paneer had grade distribution as 'Like very much' as shown in table 1. Galla *et al.* (2017) [6] reported that as the amount of spinach leaves powder in the composite flour-based burfi grew, so did the bitterness as an aftertaste in burfi with 15 percent spinach leaves powder. Joseph and Rao (2019) [12] Studied that as the percentage of lemongrass extract increased, the flavour rating of the paneer with lemongrass in it continued to rise. Sarnaik *et al.* (2020) [17] found that there were significant variations in mean flavour scores of the black pepper powder added to paneer.

Body and texture score

As shown in the table 1 average body and texture ratings of the paneer incorporated with spinach powder continued to rise. The body and texture score of paneer incorporated with spinach powder for various treatments increased from treatment T_1 (7.98), T_2 (8.09) to T_3 (8.26)) and declined at treatment T4 (8.17). Dwivedi *et al.* (2014) [4] stated that the mean score of paneer spread regarding body and texture were different from each other during storage of paneer spread. Maske *et al.* (2018) [13] reported that the body and texture score of sago powder incorporated paneer were varies from each other and according to percent amount of sago powder added to paneer. Sarnaik *et al.* (2020) [17] found that the body and texture score initially increased and declined later.

Mouthfeel/smoothness

As shown in the table 1 average mouthfeel scores, i.e. the smoothness of spinach powder integrated paneer, initially increased from treatment T_1 (7.54), T_2 (7.71), to treatment T_3 (7.91) but declined again at treatment T_4 (7.85). Maske *et al.* (2018) [13] estimated that amount of sago powder to be added to paneer affects the mouthfeel of paneer. All mean mouthfeel scores of sago powder incorporated paneer showed the variation and par to each other. Sarnaik *et al.* (2020) [17] found that mouth feel score mean initially inclined from treatments T_1 to T_3 as 7.00 to 8.06, respectively. But at treatment T_4 mouthfeel score declined to 7.31 which was superior to treatment T_1.

Overall acceptability

As shown in table the 1average overall acceptability score for spinach incorporated paneer gradually increased from treatment T_1 (7.92), T_2 (8.15) and T_3 (8.35) and declined to (7.88) at treatment T_4. Dwivedi *et al.* (2014) [4] stated that the ratings for overall acceptability of paneer spread based on appearance, flavor and texture. The highest overall acceptability score (8.70) was felt in samples made with 1.5 percent salt and potassium metabisulphite as a preservative on 0th day storage (x2, y2, z1). Galla *et al.* (2017) [6] also reported that the panelists approved of biscuits containing 10 percent spinach leaf powder. verall acceptability. Maske *et al.* (2018) [13] reported that the mean scores of general acceptability decreased as the level of sago powder increased. There was a drop in the end product's acceptability. Sarnaik *et al.* (2020) [17] that reported the overall acceptability score mean initially inclined up to treatments T_1, T_2 and T_3 as (6.90, (7.53) and (8.31), respectively but declined at treatment T_4 as (7.28).

Microbial

Yeast and mould count

As shown in the table 2 yeast and mould count of paneer made from spinach powder and buffalo milk. The range was determined to be between 1.01CFU/gm to 1.12 CFU/gm. Agnihotri and Pal (1995) [1] studied the freshly formed paneer, the pH and moisture content of the initial standard plate count (3.94 + 0.41 CFU/gm) did not improve significantly. Jadhav *et al* (2016) [11] stated that the extension of storage time at each storage period and temperature, SPC, yeast and mould count grew continuously and progressively.

Coliform Count

From table 3 it was ndicated that the paneer and spinach powder mixture's *E. coli* type count. *E. coli* was absent in all treatments. *E. coli* count for paneer incorporated with spinach powder was zero. Both treatments had been properly prepared and were at par with each another. Agnihotri and Pal (1995) [1] reported that noticed that newly made paneer and discovered E. coli type counts reached most probable number presumptive (MPN) 1445.40 ± 584.59 CFU/gm on day 7 of storage, from its starting value of 22.01 ± 3.75 CFU/gm.

Standard plate count

From table 4 it was cleared that the standard plate count of paneer made from spinach powder and buffalo milk. The range was determined to be between 3.04 x 10^3 CFU/gm and 3.36x 10^3 CFU/gm. Treatment T_4 had a higher standard plate count (3.36x 10^3 CFU/gm while treatment T_1 had a lower count (3.04 x 10^3 CFU/gm). Agnihotri and Pal (1995) [1] found that, the standard plate counts of fresh sample of market paneer were ranged from 2.5 x 10^4 to 3.5 x 10^5 CFU/gm.

Conclusion

The mean colour and appearance scores of paneer incorporated with spinach powder for treatments T_1, T_2, T_3 and T_4 were 7.75, 8.17, 8.48 and 7.68, respectively. Obtained average flavour scores for paneer incorporated with spinach powder indicated that it had grade as 'Like very much.' Treatment T_4 had highest score for body and texture while treatment T_1 had lowest body and texture score. T_3 had highest score for mouthfeel while lowest mouthfeel score observed at treatment T_1. In present study mouth feel score of paneer incorporated with spinach powder was higher than control paneer. This revealed that treatment T_3 had highest score for overall acceptability than other treatments. The score of overall acceptability for spinach powder incorporated paneer were declines after treatment T_3. Treatment T_4 had highest yeast and mould count as 1.13 x 10^3 CFU/gm than other treatments. The coli form count was absent in all treatments T_1, T_2, T_3 and T_4. The fresh samples of developed paneer had standard plate count as 3.04 x 10^3 CFU/gm, 3.14 x 10^3 CFU/gm, 3.25x 10^3 CFU/gm and 3.36x 10^3 CFU/gm for treatments T_1, T_2, T_3 and T_4, respectively.

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