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Effects of incorporation of whey protein concentrate on physicochemical, texture, and microbial evaluation of developed cookies

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Abstract: Whey Protein concentrate (WPC) was incorporated into cookies at different levels (0, 2, 4, and 6%). Cookies were analyzed for physicochemical, color, textural, microbial, and sensory attributes. Physicochemical analysis revealed that 6% WPC supplemented cookies shows maximum protein content (13.22%), moisture content (11.33%), fat content (23.08%), and ash content (2.02%) as compared to control. However, control sample shows significantly different ($p \leq 0.05$) value for crude fiber and carbohydrate content. Maximum thickness (9.63 mm), diameter (44.06 mm), and weight (9.10 g) were found for control and these decreased significantly ($p \leq 0.05$) with increase in WPC supplementation level in cookies. Cookie supplemented with 4% WPC showed maximum overall acceptability (4.76). Texture analysis revealed that 6% WPC supplemented cookie shows maximum cutting force (55.3 N). Lightness (L^*) value of cookies decreased from 67.32 to 57.94. Where as a^* and b^* value increased from 0.37 to 3.57 and 25.35 to 27.54, respectively. The total plate count of cookie samples was under acceptable limits.

Subjects: Breads; Cereals & Dough; Food Additives & Ingredients; Sensory Science

Keywords: whey protein concentrate; cookies; physico-chemical properties; texture

1. Introduction

Biscuits and cookies are almost synonymously used in India for the products prepared commercially using refined flour, hydrogenated fats, and sugar along with emulsifiers and other additives.

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PUBLIC INTEREST STATEMENT

Cookies represent the largest category of snack item among baked food products throughout the world. Products like biscuits, cookies, and crackers are widely consumed and have a relatively long shelf life and good eating quality. Whey is rich in calcium, phosphorus, essential amino acids, and water-soluble vitamins, which makes whey a highly nutritious product. Whey can be incorporated advantageously into various food formulations, including cookies, breads, cake, crackers, and pasta. Whey protein has been proven to have positive effect in a number of areas, including immune support, cancer therapy, hypertension/cholesterol control (cardiovascular health), mental health (improve cognitive power and memory power), reduce risk of type 2 diabetes, and infant nutrition.

Commonly, these are made from unleavened dough and are produced from a mixture of flour and water which may contain fat, sugar, and other ingredients mixed together into dough which is rested for a period and then passed between rollers to make a sheet (Okaka & Portter, 1997). Among baked food products, cookies represent the largest category of snack item throughout the world (Akubor, 2005). In many countries, cookies are prepared from fortified or composite flour to increase its nutritive value (Baljeet, Ritika, & Roshan, 2010). Cookies belong to the group of food products which are very popular in daily diet of almost all profiles of consumers (Nassar, Hamied, & El-Naggar, 2008). Variations in the concentration of the main components and huge range of minor ingredients provide a wide variety of different kinds of cookies (Gallagher, Kenny, & Arendt, 2005). Products like biscuits, cookies, and crackers are widely consumed and are having a relatively long shelf life and good eating quality (Warren, Hnat, & Michnowski, 1983). Flour milled from other crops such as maize, millet, sorghum, and rice had been added to wheat flour to extend the use of the local crops (Ramacharitar, Badrie, Beman, Matsuo, & Ridley, 2005). India is recognized to be the second largest producer of biscuits next only to the United States.

Wheat flour is considered as the basic ingredient for bakery products such as: chapattis, rotis, paratha, bread, buns, cookies, cakes, patties, and pan cakes (Awan, Rehman, Rehman, Siddique, & Hashmi, 1995). In India, 70% of the total wheat produced is consumed in the form of chapatti and its variants like tandoori roti, nans, parathas, and poories, while as rest 30% is used for other bakery products such as breads, cookies, cakes, and pastries (Butt, Arshad, Alam, & Nadeem, 2007). The chemical composition of whole wheat flour is: moisture (9.38–10.43%), ash (1.32–1.85%), crude protein (10.13–14.74%), crude fat (1.96–2.52%), crude fiber (2.31–2.99%), nitrogen free extract (78.71–85.37%), wet gluten (23.53–38.71%), and dry gluten (7.51–13.52%) among different wheat varieties (Kamaljit, Baljeet, & Amarjee, 2010).

Whey is a collective term referring to the serum of watery portion that separates from the curd during conventional cheese making (Chauhan & Chawla, 2011). Main proteins present in milk are whey protein and casein. It is rich in calcium, phosphorus, essential amino acids, and water-soluble vitamins, which makes whey a highly nutritious product (Davis, 2004). Whey can be incorporated advantageously into various food formulations, including cookies, breads, cake, crackers, pasta, confectionary products, ice creams, soups and gravies, frozen desserts beverages infant food formulations, and special dietetic food (Munaza, Prasad, & Gayas, 2012). Whey protein concentrates (WPCs) have found use in biscuits, cookies, cakes, sponges, icings, and glazes to improve texture, flavor, and appearance. There are specific functional properties that are associated with whey proteins incorporated into baked products. They include: solubility, water binding/absorption, viscosity, gelation, cohesion, adhesion elasticity, emulsification, and foaming. Most of these characteristics are important in the processing of baked goods. Malnutrition remains a major problem nowadays. Children receiving complementary foods based on cereals and root crops are especially vulnerable to malnutrition, because these foods are characterized by poor-protein quality, low energy density as well as inadequate micronutrient content (Okaka & Portter, 1997). Protein fortification of bakery products is of current interest because of increasing awareness in consumer towards health and quality of bakery products. Cookies prepared from composite flours have been extensively used as protein fortification vehicles due to their long shelf life and high acceptability (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005). In addition to providing protein, many other health benefits have been attributed to consumption of whey proteins enriched cereal products. Whey protein has been proven to have positive effect in a number of areas, including immune support, cancer therapy, hypertension/cholesterol control (cardio-vascular health), mental health (improve cognitive power and memory power), reduce risk of type 2 diabetes, and infant nutrition. Thus, the present study was aimed to study the effect of incorporation of WPC into physicochemical textural and microbial evaluation of wheat-based cookies.

2. Material and methods

WPC were procured from NDRI (National Dairy Research Institute), Karnal, India. Soft wheat flour, sugar, shortening (Amul Butter) were procured from local market of Awantipora Kashmir (India) and kept at room temperature for further use. All chemicals used were of analytical grade.

2.1. Preparation of cookies

Cookies were prepared using three levels of WPC along with wheat flour. The ingredients were mixed in a mixer (SM-25, SINMAG, Japan) as per formulation given in Table 1. The dough was aged for 30 min and then sheeted manually to a thickness of 5 mm by means of rolling pin. The cookies were cut with a 50-mm diameter cookie cutter. These were baked at 220°C for about 10 min in a baking oven (Model SM-601T, SINMAG, Japan). Then cooled at room temperature for 1 h and packed in air tight polythene bags (LDPE) for further analysis.

2.2. Proximate analysis of cookies

The proximate composition of samples like moisture content, ash content, crude protein, crude fat, and crude fiber was analyzed on dry weight basis according to American Association of Cereal Chemists (2000).

2.3. Color analysis of cookies

The color values of cookies were measured in triplicates using a Hunter's color measurement (USA Virginia Hunter Lab Colorimeter) analyzer. Color readings were expressed by Hunter values for L*, a* and b*. L* values measure black to white (0–100), a* values measure redness when positive and b* values measure yellowness when positive.

2.4. Texture analysis of cookies

Firmness of cookies was measured using Texture analyzer (TA-XT2 Stable Micro systems, UK) equipped with a (HDP/Bs) probe. The following settings were used for measuring firmness: 25-kg load cell, pretest speed 1.5 mm/s, test speed 2.0 mm/s, and distance 5 mm/s, in compression mode (return to start). The highest first peak value was recorded as this value indicated the first rupture of biscuit at one point and this value of force was taken as a measurement for hardness. The four measurements were taken for each sample and their average value was reported.

2.5. Microbial evaluation of the cookies (total plate count)

The cookie samples (1 g) were powdered and dissolved in 10-ml sterile saline solution and homogenated for 1 min. Serial dilutions using 1 ml of homogenates were made in 9-ml sterile saline, dispensed in test tubes, and 0.1 ml of the dilution (10^{-1} , 10^{-2} and 10^{-3}) was spread on sterile Petri plates containing nutrient agar media. The plates were incubated at 37°C for 24 h. The plates were observed for number of colonies produced on each plate of different dilutions and colonies were counted by colony counter (Digital Colony Counter). Counts of visible colonies were made and expressed as CFU/g as given below:

$$\text{CFU/g} = \frac{\text{No. of colonies (Mean)} \times \text{Dilution factor}}{\text{Volume of sample used (0.1 ml)}}$$

2.6. Sensory properties of cookies

Sensory properties of cookies were analyzed using five-point Hedonic scale (5-Excellent, 4-Good, 3-Average, 2-Fair, 1-Poor). Cookie samples prepared from each four blend were presented in coded form. The order of presentation of samples to the panel was randomized. Tap water was provided to rinse the mouth between evaluations. The panelists were instructed to evaluate the coded samples for color, taste, texture, appearance, and overall acceptability.

2.7. Statistical analysis

All the experiments were conducted in triplicate for each sample. The data generated were analyzed using Minitab for calculation of least significance difference (LSD). Significance was accepted at $p \leq 0.05$ level.

Table 1. Effect of whey protein concentrate on proximate composition of cookies

WPC supplement (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
T ₀ (0%)	6.1 ± 0.36 ^a	9.08 ± 0.13 ^a	22.25 ± 0.24 ^a	0.96 ± 0.12 ^a	1.92 ± 0.10 ^c	59.68 ± 0.48 ^d
T ₁ (2%)	8.15 ± 0.18 ^b	10.48 ± 0.13 ^b	22.55 ± 0.05 ^a	1.51 ± 0.09 ^a	1.03 ± 0.15 ^b	56.26 ± 0.23 ^c
T ₂ (4%)	9.94 ± 0.12 ^c	11.88 ± 0.13 ^c	22.86 ± 0.14 ^b	1.88 ± 0.12 ^b	0.59 ± 0.13 ^a	52.84 ± 0.21 ^b
T ₃ (6%)	11.33 ± 0.19 ^d	13.22 ± 0.15 ^d	23.08 ± 0.09 ^b	2.02 ± 0.04 ^b	0.21 ± 0.10 ^a	50.12 ± 0.32 ^a
p ≤ 0.05	0.35	0.20	0.22	0.15	0.18	0.50

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

3. Results and discussion

3.1. Effect of WPC on proximate composition of cookies

As shown in Table 1, the moisture content of cookies increased from 6.1 to 11.33%. Highest moisture content (11.33%) was observed in T₃ (6% WPC supplemented cookies), while the lowest moisture content of 6.1% was found for T₀ (control sample). Significant difference (p ≤ 0.05) in moisture content was found for all samples. Increase in moisture content with increasing WPC supplementation level may be due to more bound water in the system (O'Brien, Chapman, Neville, Keogh, & Arendt, 2003). These results are in accordance with Gallagher et al. (2005) who reported that biscuits with WPC were higher in moisture content compared with control sample. Protein content in cookies also increased from 9.08 to 13.22% with an increase in the WPC supplementation level. The highest value for protein content (13.22%) was observed in T₃ (6% WPC supplemented cookies) while lowest value of 9.08% was reported in T₀ (control sample). Results revealed that the protein content in all samples differed significantly. Similar results were observed by Munaza et al. (2012) in WPC enriched biscuits. Fat content of cookies increased slightly from 22.25 to 23.08%. The highest value of fat (23.08%) was observed in T₃ (6% WPC supplemented cookies) while lowest value (22.25%) was observed in T₀ (control sample). Results showed that fat content of T₃ and T₂ (6 and 4% WPC supplemented cookies respectively) does not differ significantly, while as sample T₁ and T₀ (2 and 0% WPC supplemented cookies respectively) differed significantly. Mahmood, Butt, Anjum, and Nawaz (2008) also reported same variations in fat content of soy fortified cookies. Ash content of cookies increased from 0.96 to 2.02%. The highest value of ash content (2.02%) was reported in T₃ (6% WPC supplemented cookies) followed by T₂ (1.88%) and T₁ (1.51%), while lowest value for ash content (0.96%) was observed in T₀ (control samples). Gallagher et al. (2005) also found increase in ash content in biscuits enriched with WPC and casein. Fiber content of cookies decreased from 1.92 to 0.21%. Maximum fiber content of 1.92% was observed in T₀ (control sample) followed by T₁ (1.03%) and T₂ (0.59%). However, sample T₃ showed lowest fiber content of 0.21%. Gayas, Shukla, and Khan (2012) also reported decrease in fiber content in biscuits fortified with soya. Carbohydrate content of all cookies decreased from 59.68 to 50.12%. It was observed that increase in moisture, protein, ash and fat content results decrease in total carbohydrate. Results also showed that with an increase in the WPC supplementation level, there was increase in moisture, protein, ash and fat content, and total carbohydrate decreases. Similarly, Singh and Mohamed (2007) also reported decrease in carbohydrate content in soy protein supplemented cookies.

3.2. Effect of WPC on dimensional characteristics of cookies

It is evident from Table 2 that the mean values for the thickness of cookies decreased with an increase in the WPC supplementation level in wheat flour. Significant difference in thickness was observed for samples T₂ and T₃. Gallagher et al. (2005) also reported decrease in thickness value of biscuits fortified with WPC. As shown in Table 2, diameter and weight of cookies also decreased with an increase in the level of WPC supplementation. Mishra and Chandra (2012) also reported decrease in diameter in rice bran and soy fortified biscuits. As shown in Table 2, significant difference in spread

Table 2. Effect of whey protein concentrate on dimensions of cookies

WPC supplement (%)	Thickness (mm)	Diameter (mm)	Weight (g)	Spread ratio	Spread factor
T ₀ (0%)	9.63 ± 0.15 ^c	44.06 ± 0.15 ^d	9.10 ± 0.13 ^c	45.74 ± 0.76 ^a	0.94 ± 0.02 ^a
T ₁ (2%)	8.83 ± 0.15 ^c	42.96 ± 0.15 ^c	8.30 ± 0.10 ^b	48.65 ± 1.01 ^b	0.94 ± 0.02 ^a
T ₂ (4%)	6.90 ± 0.1 ^b	40.09 ± 0.1 ^b	7.75 ± 0.21 ^a	59.28 ± 0.94 ^c	1.12 ± 0.02 ^b
T ₃ (6%)	5.73 ± 0.05 ^a	39.06 ± 0.15 ^a	7.18 ± 0.07 ^a	68.14 ± 0.93 ^d	1.25 ± 0.02 ^b
p ≤ 0.05	0.18	0.21	0.21	0.39	0.89

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

ratio was observed for all cookies. Increase in spread ratio in WPC supplemented cookies may be due to significant decrease in thickness of WPC supplemented cookies. Similar results in spread ratio were observed by Kumari and Grewal (2007) in carrot powder supplemented cookies. Spread factor value of all cookies varied from 0.94 to 1.25. Significant differences in spread factor were observed for all cookies. Alobo (2001) also observed increase in spread factor value in millet flour supplemented biscuits.

3.3. Effect of WPC on calorific value of cookies

Calorific values were calculated on the basis that fat (9 kcal/g), protein (4 kcal/g), and carbohydrates (4 kcal/g). Akubor (2003) used term Atwater factor (4 × protein, 9 × fat, 4 × carbohydrate) for calculation of calorific value in cookies. As evident from the Table 3, the highest calorific value of 475.36 kcal/g was observed in T₀ (control sample), followed by T₁ (2% WPC supplemented cookies) 469.98 kcal/g and T₂ (4% WPC supplemented cookies) 463.33 kcal/g, while lowest calorific value (461.13 kcal/g) was observed in T₃ (6% WPC supplemented cookies). On evaluation of results, it was found that the calorific value of T₂ and T₃ is found at par but differed significantly with T₀ and T₁. Results revealed that increase in WPC supplementation level results decrease in calorific value of cookies.

3.4. Effect of WPC sensory perception of cookies

The results for the sensory evaluation of cookies are given in Table 4 and it is revealed that WPC results in different impact on sensory attributes like color, texture, taste, flavor, and overall acceptability. The results indicate that the mean score for the color had been increased from 3.77 to 4.83. Highest score (4.83) was showed by T₃ (6% WPC supplemented cookies) while lowest mean score (3.5) was observed in T₀ (control). Mean score for texture had been decreased from 4.64 to 2.93 with increasing level of WPC supplementation. Mean score for texture revealed that the T₀ (control cookies) had highest score of 4.64, while lowest mean score (2.93) was noticed in T₃ (6% WPC supplemented cookies). The quality score in response to taste of the cookies depicted that the maximum score of 4.93 was showed by T₀ (control cookies). While lowest mean score of 3.21 was observed in T₃ (6% WPC supplemented cookies). As the WPC supplementation level increases in cookies, it

Table 3. Effect of whey protein concentrate on calorific value of cookies

WPC supplement (%)	Fat (9 kcal/g)	Protein (4 kcal/g)	Carbohydrate (4 kcal/g)	Calorific value (kcal/g)
T ₀ (0%)	200.31 ± 2.17 ^a	36.33 ± 0.52 ^a	238.72 ± 1.94 ^d	475.36 ± 1.65 ^c
T ₁ (2%)	203.01 ± 0.511 ^b	41.93 ± 0.52 ^b	225.04 ± 0.94 ^c	469.98 ± 1.71 ^b
T ₂ (4%)	205.77 ± 1.31 ^c	47.53 ± 0.52 ^c	210.02 ± 1.50 ^b	463.33 ± 3.17 ^a
T ₃ (6%)	207.75 ± 0.86 ^d	52.90 ± 0.60 ^d	200.48 ± 1.30 ^a	461.13 ± 0.76 ^a
p ≤ 0.05	2.07	0.82	2.22	3.06

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

Table 4. Effect of whey protein concentrate on sensory perception of cookies

WPC supplement (%)	Color	Texture	Taste	Flavor	Overall acceptability
T ₀ (0%)	3.5 ± 0.30 ^a	4.64 ± 0.04 ^b	4.93 ± 0.05 ^b	4.48 ± 0.02 ^b	4.6 ± 0.02 ^c
T ₁ (2%)	4.03 ± 0.15 ^b	4.43 ± 0.02 ^b	4.27 ± 0.04 ^b	4.27 ± 0.03 ^b	3.81 ± 0.02 ^b
T ₂ (4%)	4.27 ± 0.02 ^b	4.31 ± 0.03 ^b	4.47 ± 0.04 ^b	4.85 ± 0.05 ^b	4.76 ± 0.04 ^c
T ₃ (6%)	4.83 ± 0.15 ^c	2.93 ± 0.05 ^a	3.21 ± 0.03 ^a	3.71 ± 0.03 ^a	3.38 ± 0.12 ^a
p ≤ 0.05	0.27	0.05	0.06	0.05	0.04

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

imparts bitterness in taste of cookies. Results pertaining to flavor of cookies are presented in the Table 4, and revealed that the highest score for flavor (4.85). While lowest mean score of 3.71 was observed in T₃ (6% WPC supplemented cookies). On evaluation of results, it was found that T₃ (6% WPC supplemented cookies) differed significantly with rest of the treatments. Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, texture, taste, and flavor of the cookies. The mean score regarding overall acceptability of cookies revealed that T₂ (4% WPC supplemented cookies) got the maximum score of 4.76, while T₃ (6% WPC supplemented cookies) was at bottom obtained minimum score of 3.38. The decrease in overall acceptability was due to decrease in quality score in response to texture, taste, and flavor of the 6% WPC supplemented cookies. T₂ (4% WPC supplemented cookies) got maximum scores for entire sensory attributes than other treatments. The results obtained are in close agreement with Singh, Bajaj, Kaur, Sharma, and Sidhu (1993) who reported similar results regarding overall acceptability score of sensory evaluation in soy flour fortified biscuits.

3.5. Effect of WPC on texture of cookies

As indicated in Table 5, the highest peak force was observed in 6 (%) WPC supplemented cookies (55.3 N), followed by control cookies (50.2 N) and 2% WPC supplemented cookies (48.96 N), while mean lowest peak force was observed in 4% WPC supplemented cookies (47.16 N). Results revealed that mean peak force value decreased from control sample with incorporation of WPC till a level of 4% and then there was an abrupt rise at 6% WPC incorporation level. On evaluation of results, it was found that T₃ (6% WPC supplemented cookies) differed significantly with rest of the treatments. Cookies with 2 and 4% WPC supplementation level (T₁ and T₂, respectively) produced results most similar to the T₀ (control sample). Such similar results to the control cookies may be due to the fact that whey proteins are soluble and do not actively bind water unless they are highly denatured (Gallagher et al., 2005). Our results are also in accordance with the observations made by Singh et al. (1993) in terms of hardness of biscuits prepared from soy flour and wheat flour.

Table 5. Effect of whey protein concentrate on texture of cookies

WPC supplement (%)	Color	Texture	Taste	Flavor	Overall acceptability
T ₀ (0%)	3.5 ± 0.30 ^a	4.64 ± 0.04 ^b	4.93 ± 0.05 ^b	4.48 ± 0.02 ^b	4.6 ± 0.02 ^c
T ₁ (2%)	4.03 ± 0.15 ^b	4.43 ± 0.02 ^b	4.27 ± 0.04 ^b	4.27 ± 0.03 ^b	3.81 ± 0.02
T ₂ (4%)	4.27 ± 0.02 ^b	4.31 ± 0.03 ^b	4.47 ± 0.04 ^b	4.85 ± 0.05 ^b	4.76 ± 0.04 ^c
T ₃ (6%)	4.83 ± 0.15 ^c	2.93 ± 0.05 ^a	3.21 ± 0.03 ^a	3.71 ± 0.03 ^a	3.38 ± 0.12 ^a
p ≤ 0.05	0.27	0.05	0.06	0.05	0.04

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

Table 6. Effect of whey protein concentrate on the color (L*, a*, b*) of cookies

WPC supplement (%)	L*	a*	b*
T ₀ (0%)	67.32 ± 0.01 ^d	0.35 ± 0.01 ^a	25.35 ± 0.01 ^a
T ₁ (2%)	60.97 ± 0.02 ^c	2.38 ± 0.02 ^b	25.75 ± 0.03 ^a
T ₂ (4%)	58.65 ± 0.02 ^{ab}	2.56 ± 0.01 ^{bc}	26.77 ± 0.03 ^b
T ₃ (6%)	57.94 ± 0.03 ^a	3.55 ± 0.02 ^d	27.54 ± 0.02 ^c
p ≤ 0.05	0.03	0.02	0.03

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (*p < 0.05).

3.6. Effect of WPC on color (L*, a*, b* value) of cookies

Color is an important attribute of cookies. As shown in Table 6, the lightness (L*) value of cookies decreased from 67.32 to 57.97. The highest L* value (67.32) was observed in control cookies (T₀) and lowest L* value (57.97) was observed in 6% WPC supplemented cookies (T₃), which is due to the development of colored compounds through the Maillard reaction between the WPC lactose and the free amino groups from the lysine incorporated with the protein ingredients. The addition of WPC reduced lysine availability and increased color development. Results revealed that the T₀, T₁, and T₂ were differed significantly but T₃ was found at par with T₂ for L* value. Redness/greenness of cookies shows an increasing trend from lowest value (0.35) observed in control cookies (T₀) to highest value (3.55) found in 6% WPC supplemented cookies (T₃). The increase in redness of cookies could be attributed to caramelization of sugar and darkening of WPC during baking at high temperature. Also it was revealed that the yellowness of cookies (b*-value) increases with an increase in the WPC level. The b* value of the samples ranges between 25.35 and 27.54. It was observed that the yellowness of cookies increases from control sample (T₀) to 6% WPC supplemented sample (T₃) which may be attributed to degradation of compounds during baking. On evaluation of results, for a*-value T₁ and T₂ were not differed significantly but T₀ and T₃ differed significantly while for b*-value T₀ and T₁ are found at par but significantly differ with T₂ and T₃. Similar results were observed by Kumar and Sinha (2010) for soy fortified Kutki biscuits substituted by millet flour.

3.7. Microbiological analysis of WPC supplemented cookies

The data given in Table 7 reflect the mean total plate count of the microbial load in cookies on Nutrient Agar and Fungi count on Potatoes Dextrose Agar. The maximum value for total microbial load on nutrient agar (4.62×10^2) was observed in T₃ (6% WPC supplemented cookies) and minimum value of (3.05×10^2) was observed in T₀ (control cookies), while maximum value for fungal count (0.13×10^3) was observed in T₃ (6% WPC supplemented cookies) while lowest value of (0.5×10^1) was found in T₀ (control cookies). Results revealed that the total microbial load of all samples does not differ significantly. On evaluation of results in case of fungal count, T₀ and T₃ differed significantly and T₁ and T₂ do not differ significantly. The increase in microbial load of WPC supplemented cookies as compared to control may be due to increase in moisture content with increasing WPC supplementation level. The microbial load of given cookie samples were compared with microbiological

Table 7. Microbiological evaluation of whey protein concentrate supplemented cookies

WPC supplement (%)	TPC	Fungal count
T ₀ (0%)	$3.05 \times 10^2 \pm 37.33^d$	$0.5 \times 10^1 \pm 9.33^a$
T ₁ (2%)	$3.55 \times 10^2 \pm 37.33^c$	$0.51 \times 10^2 \pm 37.33^a$
T ₂ (4%)	$4.12 \times 10^2 \pm 16.0^b$	$0.71 \times 10^2 \pm 17.33^a$
T ₃ (6%)	$4.62 \times 10^2 \pm 16.0^a$	$0.13 \times 10^3 \pm 9.33^b$
p ≤ 0.05	7.74	6.44

Notes: Results are expressed as mean ± standard deviation. Standard deviation with same superscripts in a column are not significantly different (p < 0.05).

standards of fortified blended products and were found that total plate count less than 10^5 cfu/g. It was observed that the value is still within acceptable limit.

4. Conclusion

From the present study, it can be concluded that the 6% WPC supplemented cookie samples were nutritionally rich. Sensory evaluation results revealed that 4% WPC supplemented cookie sample scored highest in most of the attributes. Diameter, thickness, and weight of the cookie samples decreased with an increase in the WPC level. The textural characteristics of cookie samples indicated that control, 2 and 4% WPC supplemented cookies did not show significant difference. The Total Plate Count of cookie samples was found within the acceptable limits. This study concluded that the supplementation level of 4% WPC results in acceptable sensory, textural and nutritional characteristics.

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Competing interests

The authors declare no competing interest.

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