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RESEARCH ARTICLE

Quality evaluation of innovative chocolate cookies enhanced with rhizome powder of *Curcuma caesia* Roxb. as a value-added functional component

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Abstract

Black Turmeric (*Curcuma caesia* Roxb.) is an herbaceous rhizomatous medicinal plant of family Zingiberaceae. It is popularly used by the tribal communities of North East India due to its several health benefits. This study aims to estimate the effect of the addition of Black Turmeric Powder on the nutrient composition of the functional cookies. In this study Hot-air oven dried Black turmeric powder was used in different concentrations (1%, 2%, and 4% of the total dry weight of the dough) to prepare Chocolate cookies. The results of the sensory evaluation showed that Chocolate cookies with 4% Black turmeric powder variation were well accepted by the panel members. The nutritional composition of the selected cookie sample was contrasted with that of a control chocolate cookie (standard). Results showed that cookies with Black turmeric powder variation had significantly ($p<0.05$) higher ash, total phenolic compound, and total flavonoid content than the control cookies. Shelf-life study of the control and the variation Chocolate cookie also showed that Chocolate cookie with BTP had a longer shelf stability. Thus, it can be observed that, addition of Hot-air oven dried Black turmeric powder to the chocolate cookies has significantly increased the total phenolic content, total flavonoid content and antioxidant properties which are essential nutrients and have great medicinal benefit and consumption of this value-added functional Chocolate cookies may improve health quality in long run.

Keywords: Antioxidant; Black Turmeric; Chocolate Cookies; Functional Food; Health Benefits; Phytochemical Components

1. Introduction

Turmeric is an ancient medicinal herb which is used in different forms such as a spice, flavouring, colouring agent and a principal ingredient in Indian cuisine. Turmeric, also known as "Indian saffron", has been mentioned in the age-old Indian System of Medicine 'Ayurveda' (Arya et al., 2018). Black Turmeric (*Curcuma caesia* Roxb.) is a close ally of yellow turmeric belonging to family Zingiberaceae. In India, this species of turmeric is native to North Eastern States, central India and Bangladesh (Sahu et al., 2018). Though this medicinal plant is popularly used by the tribal communities of North East India due to its several health benefits, still it remains unknown and underutilized by most of the Indian population. Black turmeric is a rich source of numerous phytochemicals such as phenolic compounds, flavonoids, terpenoids etc. which make *Curcuma caesia* a potent source of antioxidants (Kamatou et al., 2008). It also has anti-inflammatory, anti-asthmatic (Arulmozhi et al., 2006), anticarcinogenic (Sahu et al., 2018), Hepatoprotective activities (Kataki et al., 2020) and many more medicinal properties. Since this spice is not in much use, the possible ways to incorporate it in daily food are: it can be used like other spices, in small quantities and in powdered form added to the everyday food; can be used to prepare concoctions to treat flu, cough and cold using its raw extracts. It can also be used as a replacement for the common yellow turmeric in 'Haldi milk', depending upon its taste and smell. Cookies are ready-to-eat, easily accessible and shelf-stable sweet treats, well appreciated by all age groups which can provide instant energy. Recently, Turmeric cookies also referred as 'Golden Cookies' are the whole new way of incorporating this essential ingredient for daily consumption, incorporating the warm, earthy flavour of turmeric into a sweet treat (Renzo et al., 2023). Thus, in this study, Hot air oven dried Black Turmeric powder was used to produce novel formulated cookies to check the effect of Black turmeric powder on the nutritive value of prepared cookies. The shelf stability of these cookies was also checked using microbial analysis.

2. Material and method

2.1. Procurement

Black Turmeric was chosen keeping in mind its high phytochemical content and numerous medicinal properties and related health benefits. For the study, the sample was collected from BCH Organic, a wholesale supplier located near T. Dsarahalli, Bangalore. For this experiments 1 kg grade A medicinal quality Black turmeric was procured for this experimental purpose.

2.2. Pre-processing

After the procured Black turmeric was received, it was washed with water to clean the dart and soil present on the surface. Then the Black turmeric was peeled and sliced into thin to medium slices. Later this sliced turmeric was subjected dehydration (Figure 1).

2.3. Drying methods

Dehydration is one of the oldest techniques of food preservation. Drying follows the principle of 'Heat-mass transfer'. With the application of heat the moisture present in food sample gets evaporated and helps to reduce the water activity (Datta et al., 2024). Here, Hot-air oven was used to dehydrate the Black turmeric. The sliced Black turmeric was spread on petri dish and kept inside the Hot-air Oven at 60 to 80° C for 3 to 4 hours. The Hot-air oven drying process is presented in (Figure 2).

2.4. Formulation of enriched Chocolate Cookies using selected black turmeric powder

Cookie is a baked snack item which is commonly consumed by almost every age group as it is a convenient food that gives instant energy.

2.5. Selection criterion for ingredients

All the ingredients were selected based on their availability and keeping in mind how the bitter taste of Black Turmeric can be masked. Hot-air Oven dried BTP was chosen as it retained

Figure 1. Pre-processing of black turmeric (*Curcuma ceasia* Roxb.)

Figure 2. Hot air oven drying of Black turmeric (*Curcuma ceasia* Roxb.) powder

maximum nutrient and phytochemical content after the drying treatment. All the selected ingredient of the chocolate cookies were procured from the local grocery store apart from black turmeric. The ingredient list of the product as follows:

2.5.1. Refined wheat flour: Refined wheat flour was used as the base of the ingredient. Refined flour is lighter flour and gives desirable quality of the bakery product.

2.5.2. Butter: Butter was added as it also plays a critical role in cookie structure; the fat and moisture can enhance or inhibit gluten development, which directly impacts the shape, spread, and texture in the cookies. Butter also acts as shortening agent that gives the desired crispiness to the final product.

2.5.3. Sugar: Sugar was used to provides the sweetness to the product. Sugar slows down the gluten formation, thus, prevents the texture of the cookies from becoming chewy. It also has hygroscopic properties, so, it provides the required moisture to the cookie dough to prevent it from becoming too hard.

2.5.4. Coco powder: Cocoa powder was used for flavouring agent to give the chocolate flavour to the cookies. As well as it also enhances the nutritional properties of the final food product, as research have shown that cocoa powder is rich in various phytochemicals and has high antioxidant properties.

2.5.5. Salt: Salt was added in the cookies to balance the final taste. It is seen that a pinch of salt helps to enhance the sweetness of the food product.

2.5.6. Black turmeric: here black turmeric was added as an addition key ingredient that will further improve the nutritional quality of the final product.

2.5.7. Vanilla essence: Vanilla was added as its essence acts as flavouring agent to give the flavour to the final product.

2.6. Standardization of Chocolate cookies

2.6.1. Composition of the standardized Control Chocolate Cookies

Different variations for the standard chocolate cookies were formulated using several proportion and combinations of butter, sugar and cocoa powder, as described in Table 1. These were evaluated with sensory analysis.

2.6.2. Incorporation of the BTP (Black Turmeric Powder) in standardized control cookie formulation

Trials were conducted to determine the composition of BTP in the chocolate. After several permutation and combination, BTP was incorporated in chocolate cookies in the range of 1% to 4% (0.8 gm to 3.2 gm). This is because, the concentration of BTP higher than 4% was giving very bitter taste due to the presence of Linalool, a terpene alcohol which provides strong aroma and bitter taste (Kamatou et al., 2008). Thus, keeping in mind that a very bitter product might affect the taste perception of other variations and can influence the overall sensory analysis done by the panel member. The final variation of BTP was finalized as 1%, 2% and 4%, as mentioned in the Table 2, for the product development based on acceptance by sensory analysis. The final variations of Chocolate cookies were (as per the formulation mentioned in the Table 3 and developed along with a Control Chocolate cookie (Without BTP). All the Cookie samples were coded as mentioned in the Table 3. These variations were further subjected to sensory analysis and the best accepted variation was statistically analysed. Method of processing is described in Figure 3.

2.6.3. Sensory evaluation of the Chocolate cookie recipe with different Black Turmeric powder variation

Sensory evaluation is a scientific evaluation method that measures, analyses, and interprets the reactions of people to products as perceived by the senses. Sensory evaluation is done for every new chocolate cookie formulation developed, before reaching the market for the consumption purpose. Sensory evaluation is done to understand whether the organoleptic property of the product is accepted by the consumer or not. Sensory evaluation is also done to choose the best product out of a range of similar food product with slight variations. Organoleptic properties of food are perceived by the 5 senses of sight, smell, touch, taste, and sound.

The formulated products were evaluated by panel members based on 7 parameters –appearance, aroma, colour, taste, texture, flavour and overall acceptance. Sensory evaluation was done using '9-point hedonic scale' by 30 semi-trained panel members as shown in Figure 4. Sensory analysis was done with the objective to find the best accepted variation of Black turmeric incorporated Chocolate Cookies.

2.6.4. Proximate analysis

Proximate analysis is the quantitative analysis of macromolecule in food sample. It is a system of analysis of nutrients, also termed "conventional analysis" in which the components (carbohydrate, protein, fat, moisture, and ash content) of the food material are determined rather than individual nutrients (monosaccharides, amino acid, fatty acids) (Sritama, 2024). The selected Chocolate

Table 1. Standardization of Control Chocolate Cookies

Ingredient	Control 1	Control 2	Control 3	Control 4
Refined wheat flour	40 gm	40 gm	40 gm	40 gm
Sugar	20 gm	20 gm	20 gm	25 gm
Cocoa powder	20 gm	15 gm	10 gm	10 gm
Butter	30 gm	25 gm	20 gm	20 gm
Milk	2 tbsp	2 tbsp	2 tbsp	2 tbsp
Baking powder	½ tsp	½ tsp	½ tsp	½ tsp
Vanilla essence	½ tsp	½ tsp	½ tsp	½ tsp
Salt	2.5 gm	2.5 gm	2.5 gm	2.5 gm

cookie with BTP variation (Code 110) and the control Chocolate cookie (code106) samples were utilized for estimation of ash content using muffle furnace (AOAC 2016), moisture content using desiccator (AOAC 2016), protein by Kjeldahl method (AOAC 2016), fat by Soxhlet extraction method (AOAC 2016), carbohydrate by Anthrone reaction (AOAC 2016).

2.6.5. Phytochemical analysis

Phytochemicals are the secondary plant metabolites with various medicinal properties and have several health benefits such as anticarcinogenic, anti-inflammatory, antioxidant properties and many more (Datta et al., 2024). Black turmeric is high in bioactive compounds including phenolic compounds, flavonoids. These compounds have essential antioxidant properties capable of producing beneficial effects in the body (Sahu et al., 2018). The Chocolate cookie with BTP variation (Code 110) and the control Chocolate cookie (code 106) samples were used for estimation of total phenolic content using Folin-ciocalteu method (Sánchez-Rangel et al., 2013), total flavonoid content using Aluminium Chloride colorimetric technique (Sahu and Saxena, 2013) and alkaloid content using acid-base titration method (Debnath et al., 2015), and antioxidant activity using DPPH method (Kalita et al., 2013).

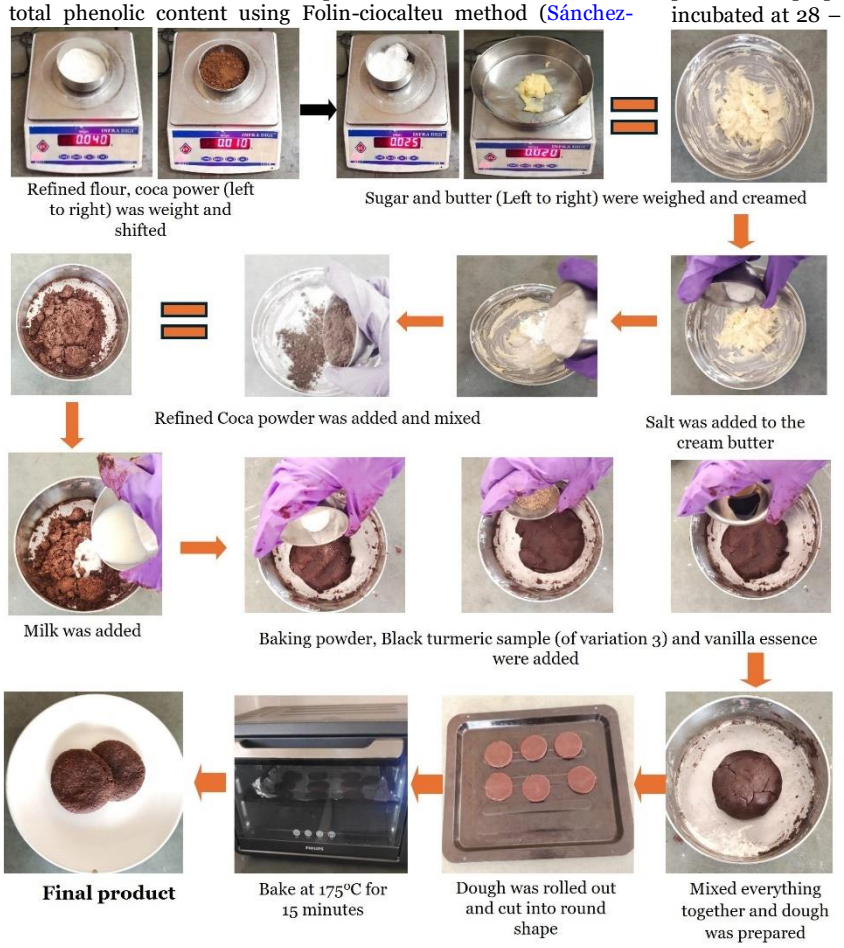

Figure 3. Development of Chocolate cookies with control and variations

2.6.6. Shelf-life study of Control (code 106) and selected Chocolate Cookie variation (code 110)

Shelf-life refers to the limited duration during which a product, maintained within particular packaging and environmental parameters, remains suitable for use before it becomes unfit for human consumption (Torrieri, 2016). It ensures till what time the food will remain palatable, wholesome, nutritious and retains its desired organoleptic, chemical, physical, and microbiological characteristics. In this study, shelf-life study was done to understand for Control and Variation 3 (code 106, code 110 respectively) chocolate cookies. The shelf-life study was limited to 14 days (2 weeks) due to the insufficient time obtain the storage stability. The cookies were sealed and stored in airtight containers and were opened only during the time of microbial testing. Consequent serial-dilution was performed for Chocolate cookies (Control and variation 3). The petri plates and test tubes were autoclaved before initiating the process of shelf-life study to make sure all the glass wares are sterilized. Shelf-life study was done using 'Spread Plate technique' which was conducted at an interval of every 5 days i.e., Day 0, Day 07, Day 14.

Nutrient agar was used to check the bacterial growth. Bacterial plates were prepared by taking 1 ml of dilution 10^{-8} and were incubated at 28 – 30°C for 24 hours. Potato Dextrose Agar (PDA Agar) was used to check the fungal growth. Fungal plates were prepared by taking 1 ml of dilution 10-4 and were incubated at 28 – 30°C for 24 hours.

The number of CFU in each plate were counted on next day using the given formula:

$$\text{No of microorganisms (g/ml)} = \frac{\text{No of colonies X Dilution factor}}{\text{Weight/ volume of aliquots taken (g/ml)}}$$

Table 2. Variation of Black turmeric

BTP variations	
Variation	Amount
BTP variation 1 (1% of dry weight)	0.8 gm
BTP variation 2 (2% of dry weight)	1.6 gm
BTP variation 3 (4% of dry weight)	3.2 gm

3. Result

3.1. Sensory evaluation of Control and Variation Chocolate cookies

Panel members choose the Control 4 as the standard Chocolate cookie (Table 1). Table 4 and Figure 5 represents the mean sensory score given by the 30 semi trained panel members. Product coded 106 had a higher mean score of 8.13 ± 0.57 with respect to appearance, followed by product coded 101 (7.97 ± 0.72). Whereas product coded 112 (7.57 ± 0.90) had a lowest mean score. Both product coded 106 and 101 got same highest mean value (7.80 ±0.81) with respect to aroma, followed by product coded 110 (7.87 ± 0.56). Product coded 112 got the lowest mean as it had a strong turmeric aroma to it. No significance difference was seen in the perception of colour of the product by the panel members ($p > 0.05$). Though there was a slight difference in the mean values of the products. The high mean value was seen in product coded 106 (8.07 ±0.64), whereas lowest was seen in product coded 112 (7.27±0.58). Taste is important for acceptability of the Chocolate cookies by the consumer. Product coded 106 (7.87 ± 0.9) and product coded 110 (7.83 ± 0.99) were more acceptable. Overall acceptability score revealed that product coded 112 (6.13 ±0.78) was liked less due to the bitter taste of BTP due to incorporation of higher percentage of BTP. With respect to texture, product coded 101 (7.77±1.04) had the best texture followed by 106 (7.37±1.07). Product coded 112 had the least mean score (6.17±1.21) as it was perceived a little too soft by the panel members. Flavour is another important factor on which the acceptability of the product by the consumer depends. From the mean sensory score, it was found the product coded 106 got the highest mean (7.77±0.9), followed by product coded 110 (7.67±0.66). lowest mean was seen in product coded 112 (5.83±1.02), as it was having a strong and bitter flavour which was overpowering the chocolate flavour. Taking into consideration the overall acceptability, product coded 106 (7.87 ±1.25) was the most acceptable and liked, whereas among the variations, product coded 110 (7.8±0.61) was the most liked amongst all panel members. Hence, chocolate cookies with 4% BTP incorporation (Code 110) was the most liked variation.

Figure 4. Sensory evaluation of Chocolate cookies with Black Turmeric variation by the panel members

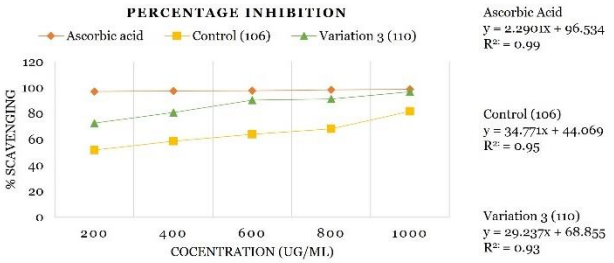

Figure 6. Antioxidant activity content of Chocolate cookie samples

3.2. Proximate analysis

The consolidated result of proximate analysis of the product codded 106 and 110 is given in the Table 5. Ash content was found to be significantly higher in product coded 110 (10.5% ± 0.5) than product coded 106 (8.5% ± 0.5). The mean value of moisture content was slightly higher in product coded 106 (11.5% ± 2.7) than 110 (10.1% ± 5.8) with no significance difference. With respect to the macro nutrients, the mean values of protein of product coded 110 was slightly higher than product coded 106, which was 8.1 ± 0.5 g/100 gm. Fat and carbohydrate content were higher in product coded 106 (10.6 ± 0.7 gm/100 gm and 63.02 ± 3.76 gm/100 gm respectively).

3.3. Phytochemical analysis

Table 6 depicts the consolidated mean values of phytochemical properties of product coded 106 and 110 with significant difference. Total Phenolic and Total Flavonoid content was significantly high ($p < 0.05$) in product coded 110 which was 23.22 ± 1.3 mg/100 gm and 3.99 ± 0.3 mg/100 gm respectively. Whereas the Total Phenolic and Total Flavonoid content was significantly low in product coded 106 which was 20.6 ± 0.6 gm/100gm and 1.57 ± 0.2 gm/100gm respectively. The alkaloid content was significantly low in product coded 110 (2.04 ± 0.18) than product coded 106 (2.5 ± 0.2) ($p < 0.05$).

3.4. Antioxidant activity

Table 7 and Figure 6 represents the free radical scavenging activity of the developed food product with ascorbic acid as the standard. The concentration range was between 200 ug/ml to 1000 ug/ml. From the Figure 6 it can be observed that, at lowest concentration (200 mcg), the percentage inhibition of product coded 110 was found to be 72.67% followed by product 106 (51.83%). Whereas, at highest concentration (1000 mcg), the percentage inhibition of product coded 110 was found to be 96.72% followed by product 106 (81.76%).

3.5. Shelf-life study of Chocolate cookies

The findings of shelf-life study are shown in the Table 8 and Table 9. The shelf-life assay was carried on for 14 days after the preparation of both product coded 106 and 110, during which the Colony Forming Units (CFU) were checked on Day 1, day 7 and day 14 with a 7-day interval. According to Table 8, no Colony Forming Units (CFU) of bacterial growth was observed in product coded 106

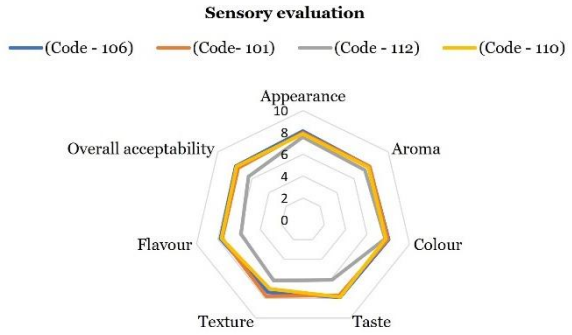

Figure 5. Sensory evaluation of Chocolate cookies (Control + Variations)

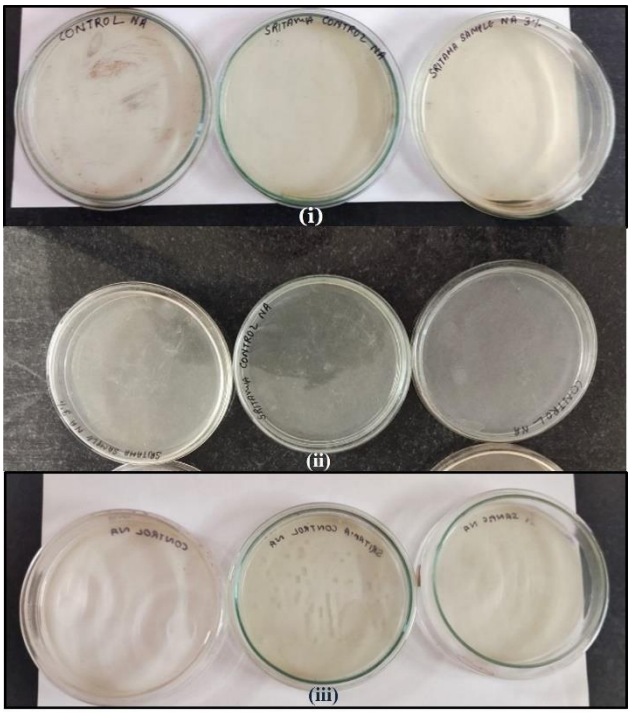

Figure 7. Bacterial Isolation (i) Day 1 (ii) Day 7 and (iii) Day 14.

as well as product coded 110 till day 7. After 7th day product coded 106 showed microbial growth (4.7 x10 $^{-7}$ CFU/ml) (shown in Figure 7) whereas product coded 110 showed no bacterial growth till the end of the shelf-life study. According to Table 9, no fungal growth was observed in both product coded 106 and 110 up to day 14 (Figure 8).

4. Discussion

For Sensory evaluation, Control 4 was selected by the panel members as standard recipe because the taste, texture, aroma, appearance and flavour of Control 4 cookies were liked by the panel members most. According to Table 4, product coded 110 was chosen as most liked sample by the panel member in overall category. According to the panel members, this variation of chocolate cookie has an appealing chocolaty colour to it. The fragrance of the cookies was not over-powder by the aroma of Black turmeric powder. In this variation, the bitterness of the black turmeric was well masked by the dark chocolate flavour. Along with that, the texture of the cookies was also acceptable. Thus, Chocolate Cookie variation coded 110 (with 4% BTP) was selected for further nutrient analysis.

Table 5 depicts the consolidated mean value of proximate evaluation of product coded 106 and 110 with significant difference. From the above-mentioned table, the ash content of the product coded 110 was found to be higher than the product code 106. This higher mineral content might be because the BTP contributed to the mineral content of final product coded 110. The common minerals present in Curcuma species are sodium, potassium, calcium, and phosphorus (Taoheed et al., 2017). The moisture content was slightly low in the product code 110. This

Table 3. Formulation of Chocolate cookies with BTP variation

Ingredient	Control 1 (Code 106)	Variation 1 (Code 101)	Control 2 (Code 112)	Control 4 (Code 110)
Refined wheat flour	40 gm	40 gm	40 gm	40 gm
Sugar	25 gm	25 gm	25 gm	25 gm
Cocoa powder	10 gm	10 gm	10 gm	10 gm
Butter	20 gm	20 gm	20 gm	20 gm
Milk	2 tbsp	2 tbsp	2 tbsp	2 tbsp
Baking powder	½ tsp	½ tsp	½ tsp	½ tsp
Vanilla essence	½ tsp	½ tsp	½ tsp	½ tsp
BTP	-	0.8 gm	1.6 gm	3.2 gm
Salt	2.5 gm	2.5 gm	2.5 gm	2.5 gm

Table 4. Mean sensory score of Chocolate cookies (Control and with Black Turmeric variations)

Parameters	(Code - 106)	(Code- 101)	(Code - 112)	(Code - 110)	p-value
Appearance	8.13 ± 0.57	7.97 ± 0.72	7.57 ± 0.90	7.87 ± 0.56	0.01*
Aroma	7.80 ±0.81	7.80 ±0.61	7.27±0.58	7.70±0.60	0.004*
Color	8.07 ±0.64	8.00±0.69	7.73±0.64	7.80±0.61	0.1 NS
Taste	7.87±0.9	7.67±1.06	6.13 ±0.78	7.83±0.99	< 0.001*
Texture	7.37±1.07	7.77±1.04	6.17±1.21	7.03±0.85	0.002*
Flavor	7.77±0.9	7.60±0.72	5.83±1.02	7.67±0.66	<0.001*
Overall acceptability	7.87 ±1.25	7.60 ± 0.77	6.37±0.81	7.8±0.61	<0.001*

Data expressed as Mean ±Standard deviation
*Significant at 5% level
NS - Non-significant
106 – Control: Standard Chocolate cookies
101 – Variation 1: Chocolate cookies with 0.8 gm oven dried Black turmeric powder
112 – Variation 2: Chocolate cookies with 1.6 gm oven dried Black turmeric powder
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

Table 5. Proximate analysis of Chocolate cookies

Parameters	Code 106	Code 110	p-value
Moisture %	11.5 ± 2.7	10.1 ± 5.8	0.7 NS
Ash %	8.5 ± 0.5	10.5 ± 0.5	0.008*
Protein (g/100g)	7.7 ± 0.4	8.1 ± 0.5	0.4NS
Fat (g/100g)	10.6 ± 0.7	9.8 ± 0.7	0.25NS
Carbohydrates (g/100g)	63.02 ± 3.76	61.50 ± 2.86	0.6 NS
Energy (kcal/100g)	378.28 ± 4.9	366.5 ± 4.3	0.4NS

Data expressed as Mean ±Standard deviation of triplicates
*Significant at 5% level
NS - Non-significant
106 – Control: Standard Chocolate cookies
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

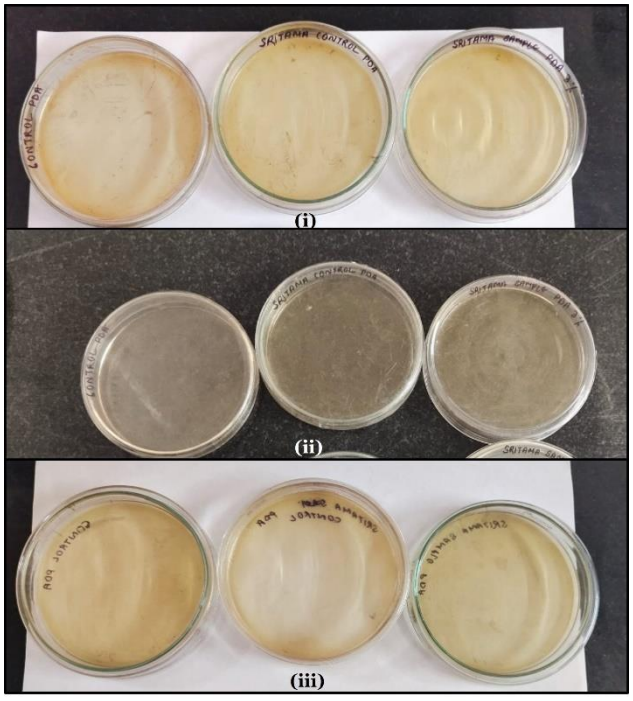

Figure 8. Fungal isolation (i) Day 1 (ii) Day 7 and (iii) Day 14.

might be because the BTP increases the dry ingredient content of the cookie dough and higher the amount of dry ingredient lesser is the moisture content. Other parameters such as moisture, Protein, Fat and Carbohydrate showed almost similar results. Though no significance difference in macronutrient content was seen between both products, but the addition of BTP have slightly increased protein content in the product coded 110 which might be contributed by the black turmeric powder.

The result of the phytochemical analysis shows that the product coded 110 had a high total flavonoid and phenolic content than the control chocolate cookie. This might be due to the addition of BTP in the cookies as well as cocoa powder itself is a potent source of phenolic compound. Whereas Product coded 106 showed a lower phytochemical concentration might be due to the absence of additional BTP. Similar study done by Ayo et al (2018) stated that addition of turmeric flour in biscuits increased the phytochemical properties of the final product. Alkaloid content was Significantly lower in product coded 110. This could be attributed to the adherence to the correct baking duration for product code 110. Various research studies indicates that extended exposure to heat can lead to a decrease in alkaloid content, as alkaloids undergo structural decomposition over time. Alkaloid acts as an antinutrient and high level of alkaloids exerts toxicity and adverse effects to humans, especially in physiological and neurological activities (Egbuna and Ifemeje, 2015). Thus, reduction in the alkaloid content reduces the toxicity level of food.

The result of the antioxidant activity depicts that the DPPH scavenging activity shows an increase in percent inhibition as the concentration increases. Figure 6 shows that addition of BTP in the product coded 110 has increased its antioxidant activity. Product 110 exhibits a greater capacity for scavenging free radicals compared to product 106. The finding of present study is in per with a study done by Lim et al (2011), where yellow turmeric was

incorporated in the bread and antioxidant properties were checked. The result showed that addition of turmeric in bread had increased antioxidant properties by 5 times.

The shelf-life study for the product coded 106 and selected variation product coded 110 was done through microbial analysis which included both bacterial and fungal isolation. The shelf-life assay was carried on for 14 days. 'Nutrient agar' was used for the bacterial growth and 1ml of 10^{-8} serial dilution was inoculated and spread plate method was done. 'Potato dextrose agar' was used for fungal growth and 1 ml of 10^{-4} was inoculated by spread plate method. Shelf life refers to the duration during which a specific food product can be stored without becoming unsuitable for consumption. It determines how long the food will remain tasty, safe, nutritious, and maintains its intended sensory, chemical, physical, and microbiological qualities (Torrieri, 2016).

According to Table 8, product coded 106 showed microbial growth post 7th day whereas the product coded 110 showed no microbial growth till the end of the shelf-life study (14th day). The growth of bacteria requires nutrition and most importantly moisture. On day 14, product 110 demonstrated resistance to microbial growth, likely due to its moisture content being within the safe threshold (10.1%). According to Rezaei et al (2010), maintaining moisture content below 15-16% is commonly regarded as safe to inhibit microbial growth and prevent product contamination. Additionally, storing the products in airtight containers and only opening them during the shelf-life study minimized the likelihood of food contamination.

According to Table 9, no fungal growth was observed in both product coded 106 and 110 up to day 14. Spoilage from mould growth is more likely to occur in moist product. Since the texture of both chocolate cookies were firm enough, pathogenic fungal spoilage was inhibited. The products had low moisture content and stored in air-tight container; thus, environmental moisture could not affect the products. Another possible reason could be that BTP has shown to have antimicrobial properties. Pandey et al (2014) did a study to explore the antibacterial efficacy of *Curcuma caesia* against bacterial cultures. The results indicated that the active crude methanol root extracts exhibited the most significant antibacterial activity, resulting in the maximum zone of inhibition against *B. cereus* and *K. pneumoniae*.

5. Conclusion

The current study studied the effect of addition of Black Turmeric Powder on the nutrient composition of the functional cookies. Chocolate cookies were prepared by adding Black Turmeric powder in 3 different concentration which were 1% (code = 101, 0.8 gm), 2% (code = 112, 1.6 gm) and 4% (code = 110, 3.2 gm) of the total dry weight of the dough, along with a Control cookie (code = 106) without the any BTP. Upon sensory evaluation, 4% BTP Variation was found to be significantly highly accepted by the panel ($p<0.05$). Proximate analysis between Control and variation cookie showed no significant difference in almost all parameters except ash content (Ash content higher in product coded 110). During the phytochemical analysis, it was observed that the total flavonoid compound and total phenolic content were notably higher in product code 110 ($p<0.05$). The % DPPH scavenging properties of the product coded 110 was found to be closer to the % scavenging capacity of ascorbic acid. Presence of higher phytochemical components in the Chocolate cookie with BTP showed a comparatively higher result in the shelf-life that the control Chocolate cookie. Thus, it can be observed that, addition of Hot-air oven dried BTP to the chocolate cookies has significantly increased the total flavonoid content, total phenolic content and antioxidant activities which are essential nutrients and have great medicinal benefit. Hence the chocolate cookie with 4% hot-air oven dried BTP has the potential to reduce various heath related disorders. With the increase in the awareness about the health-related concerns in the consumers, the developed value-added chocolate cookies will provide with more healthier choices.

Table 6. Phytochemical analysis of Chocolate cookies

Parameters	Code 106	Code 110	p-value
Total Phenolic content (mg/100g)	20.6 ± 0.6	23.22 ± 1.3	0.04*
Total Flavonoid content (mg/100g)	1.57 ± 0.2	3.99 ± 0.3	< 0.001*
Alkaloids (mg/100g)	2.5 ± 0.2	2.04 ± 0.18	0.03*

Data expressed as Mean ±Standard deviation of triplicates
*Significant at 5% level
NS - Non-significant
106 – Control: Standard Chocolate cookies
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

Table 7. DPPH radical scavenging activity for Chocolate cookies

Concentration (ug/ml)	Percentage of inhibition Ascorbic acid	Code 106	Code 110
200	97.02	51.83	72.67
400	97.48	58.63	80.92
600	97.79	64.12	90.38
800	98.40	68.32	91.30
1000	98.85	81.76	96.72

106 – Control: Standard Chocolate cookies
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

Table 8. Bacterial growth in Nutrient agar media

Variations	CFU- Bacteria		
	Day 1	Day 7	Day 14
106	0	0	4.7 x10 $^{-7}$ CFU/ml
110	0	0	0

106 – Control: Standard Chocolate cookies
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

Table 9. Fungal growth in Potato Dextrose Agar media

Variations	CFU- Fungi		
	Day 1	Day 7	Day 14
106	0	0	0
110	0	0	0

106 – Control: Standard Chocolate cookies
110 – Variation 3: Chocolate cookies with 3.2 gm oven dried Black turmeric powder

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Authors' contribution:
The first author designed, conducted the experiment and developed the manuscript. The second author supervised throughout the study and the manuscript development.

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Authors have no conflict of interests.

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