J Food Sci Technol (August 2020) 57(8):2934–2940
https://doi.org/10.1007/s13197-020-04325-5

ORIGINAL ARTICLE

Sensory acceptability of value added cookies incorporated with *Tinospora cordifolia* (TC) stem powder; improvement in nutritional properties and antioxidant potential

Prachi Tyagi[1] · Anil Kumar Chauhan[1] · Som Nath Singh[2]

Revised: 26 December 2019 / Accepted: 5 March 2020 / Published online: 13 March 2020

Abstract *Tinospora cordifolia* (TC) is regarded nature's treasure as it is salutary in various ways to the human health in ayurvedic and vedic scriptures. The TC stem creeping on neem tree (*Azadirachta indica*) are considered best for medicinal use. Present study was carried out to develop functional food as cookies by incorporating the TC stem powder. Functional cookies were prepared by incorporating 2%, 4%, 8%, 10% and 12% of TC stem powder and admissibility was decided on the basis of sensory evaluation to get the optimized cookies (TCC). Further physical parameters (L*, a* and b* color value and spread ratio) were analyzed. TC, TCC and control cookies without TC were evaluated for nutritional composition and antioxidant potential [antioxidant assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing power (FRAP) and nitric oxide (NO), total polyphenolic content and total flavonoid content]. Results showed that with increase in TC addition from 0 to 12% in cookies there was decreases in the sensory parameters and maximum admissible concentration was up to 8% of TC, hence optimized at this level. Incorporation of TC in cookies resulted in increase in b^* value, protein, moisture, total ash, iron, copper, zinc and antioxidant potential, whereas the fat content decreases. Developed cookies proved to be better than standard control cookies with respect to functional properties.

Keywords *Tinospora cordifolia* stem · Cookies · Antioxidant potential · Nutritional composition · Sensory evaluation

✉ Prachi Tyagi
prachiji@gmail.com

Anil Kumar Chauhan
anilchauhancfst@gmail.com

Som Nath Singh
nutrition-dipas@hotmail.com

[1] Centre of Food Science and Technology, IAS, Banaras Hindu University, Varanasi 221005, India

[2] Defence Institute of Physiology and Allied Sciences (DIPAS), DRDO, Timarpur, Delhi 110054, India

Introduction

Since ancient time plants are utmost important for all of us and a good source of medicine. Now a day's attention is rising towards importance of medicinal plants due to their various phytochemicals that are amenable for therapeutic usefulness (Rani et al. 2015). *Tinospora cordifolia* Miers (Willd.) owned by the family of Menispermaceae, called by various names in India such as giloy, guduchi or amrita and very well recognized in Ayurveda and traditional medicine for its admirable therapeutic effectiveness (Sankhala et al. 2012). It is compared as "Nectar of Immortality" due to its potential to bestow juvenility liveliness and long life (Upadhyaya et al. 2011) *T. cordifolia* that creeping on neem tree (*Azadirachta indica*) has a great therapeutic value and considered to have synergistic effect of both, termed as 'Neem giloy' (Mittal et al. 2014). Compared to leaves the stem is extensively used and beneficial part of the plant (Sarala et al. 2012) and its extract is proved to be good source of antioxidant as nutraceutical purpose and provide protection against cardiovascular disease, premature aging and cancer (Ilaiyaraja and Khanum 2011). Diverse ranges of components belonging to different classes such as alkanoids, di-terpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides are

found in giloy and it has no side effect and toxicity if the dose is taken properly (Singh et al. 2003).

As already many researchers worked to described amazing and remarkable beneficial effects of the TC stem but only few studies have been done with food incorporated with giloy stem. Sood (2015) attempted to utilize this in preparation of value added products like RTS, squash and syrup and analyzed for chemical, nutritional and sensory parameters. Cookies reckoned to be a perfect vehicle for conveying nutritionally important helpful constituents with longer shelf life and low cost (Ansari and Kumar 2012). Effect of TC stem powder on sensory, physicochemical and antioxidant properties of the cookies has not been studied so far. With this context the research work was undertaken to utilize stem powder for developing value added cookies and optimizing the concentration of TC by sensory evaluation. Further physicochemical analysis and antioxidant potential of TC powder and cookies were accomplished.

Materials and methods

TC creeping on neem tree was collected from the botanical garden of Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh. Raw materials for preparation of cookies were purchased from the local market. All the required chemicals used were of analytical grade.

Processing of *Tinospora cordifolia* stem

TC stem creeping over neem (Fig. 1a) was washed with tap water, cleaned and sliced into small pieces (Fig. 1b). These small pieces were dried using tray drier (Khera Instruments Pvt. Ltd.) at 45° ± 2 °C for 6 h, further dried pieces as shown in (Fig. 1c) were set into high speed mixer grinder (*Sunflame*) and sieved in order to get the fine powder of TC stem (Fig. 1d). TC was sealed in polythene pouches and stored at room temperature for further use and investigations.

Preparation of cookies

Cookies were prepared as per AACC Method (2000) with some modifications, ingredients used were TC, all-purpose wheat flour (maida), sugar, shortening, baking powder and baking soda. Six blends with replacement of wheat flour by TC, wheat flour: TC at different levels 100:0(control); 98:02; 96:04; 94:06; 92:08, 90:10 and 88:12 were used as shown in Table 1, sugar and shortening was beaten and then further mixed with rest ingredients to obtain dough and then molded into desired shape. Molded cookies were baked in the oven heated at 180 °C for 20 min and cooled for 30 min at ambient temperature. Prepared TC cookies (TCC) and control cookies (CC) were packed and stored for further analysis.

Sensory admissibility of cookies

Optimization of TC in cookies was decided by sensory analysis. Cookies samples were analyzed for sensory characteristics as explained by Nwakalor (2014). Sensory quality characteristics were evaluated by a panel of 15 semi-trained members using a 9-point Hedonic scale. The cookies were evaluated for their flavor, after taste, texture, color, appearance and overall acceptability.

Determination of nutritional composition and trace elements

The proximate composition such as moisture, fat, protein and ash contents was analyzed according to the standard method described by AOAC (2002). Energy value in (Kcal/g) through calorific test of the sample was determined by using bomb calorimeter (Hunan Sundy Science and Technology Development Co. Ltd. China). Trace elements like iron (Fe), zinc (Zn) and copper (Cu) were detected by using FAAS (Flame Atomic Absorption Spectrophotometer (Thermo Electron Corporation, UK M6 Spectro with SOLAAR AA).

Physical properties and color value

The diameter (W, cm) of cookies was measured by laying six cookies edge to edge with the help of a scale, rotating them through 90°, measure again and then average value was obtained. The thickness (T, cm) of cookies was obtained by arranging cookies on top of one another and taking the average value. The spread ratio of cookies was calculated as W/T (Mildner-Szkudlarz et al. 2013). L*, a* and b* values of TC, control cookies (CC) and TC cookies (TCC) were analyzed by ColorFlex, Hunter Lab.

Determination of antioxidant potential

Preparation of sample (TC, TCC and CC) extract

Sample extract was prepared as described by Sharma and Zhou (2011). Accurately weighed 2.5 gm of each (TCS, TC cookies and Control cookies) sample was defatted with 20 mL of hexane and then 0hexane was decanted. Then an aqueous mixture of 25 mL of 70% methanol, 29% water and 0.3% formic acid was added to it, and again kept inside the water bath at 70 °C for 45 min for complete extraction and then aqueous layer was collected and centrifuged at 4500 rpm at 4 °C. Further dried in CentriVap (Vacuum

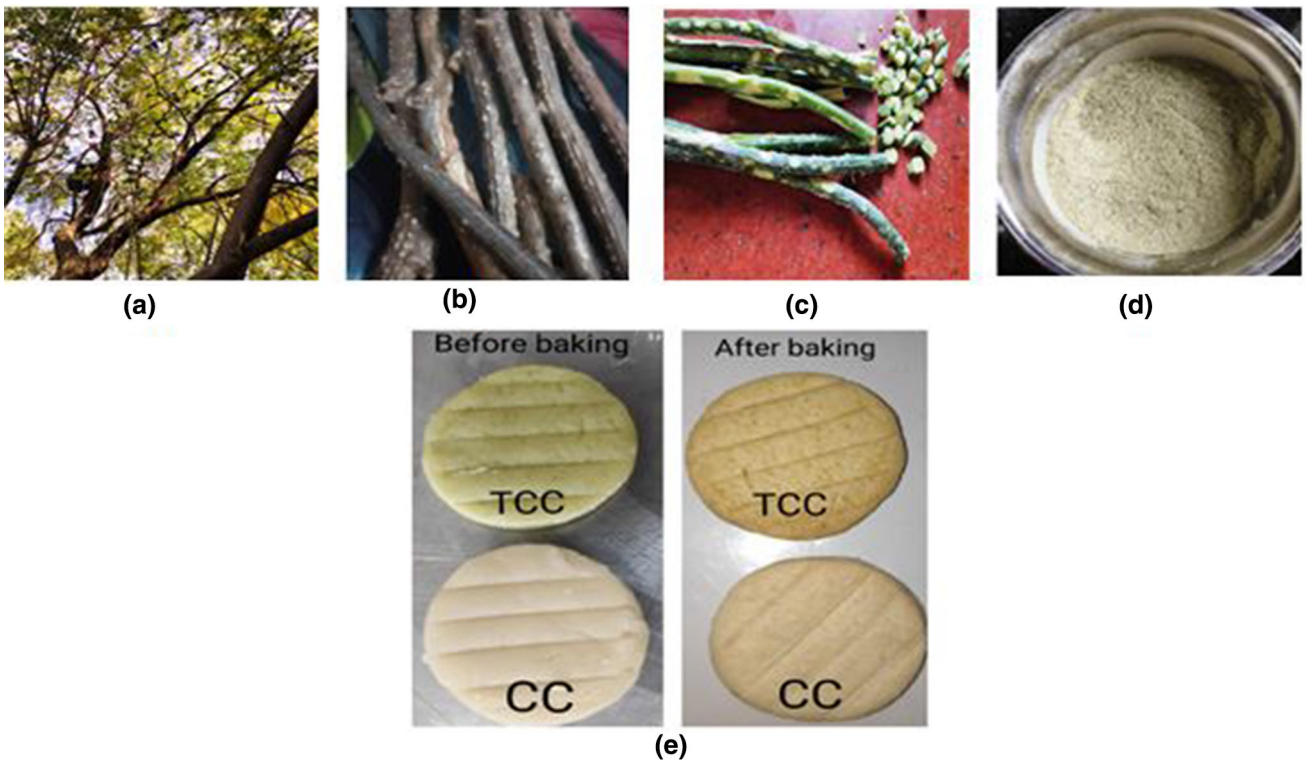

Fig. 1 **a** *T. cordifolia* creeping on neem tree, **b** *T. cordifolia* stem, **c** peeled stem and pieces, **d** *T. cordifolia* (TC) stem powder and **e** optimized TC cookies and control cookies

Table 1 Ingredients used for preparation of control cookies CC and TCC (2%, 4%, 6%, 8%, 10% and 12%)

Ingredients (g)	(Control)	2%	4%	6%	8%	10%	12%
Wheat flour(Maida)	100	98	96	94	92	90	88
Sugar	60.00	60	60	60	60	60	60
Fat	40	40	40	40	40	40	40
Baking soda	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Baking powder	1.25	1.25	1.25	1.25	1.25	1.25	1.25
TC stem powder	0	2	4	6	8	10	12
Water (mL)	5	5	5	5	5	5	5

concentrator, LABCONCO, 300) and dried sample was collected, and 1 mg/mL aqueous solution was prepared for each test sample.

Total polyphenol content (TPC)

TPC was determined by using Folin–Ciocalteu phenol reagent according to the method explained by Stoilova et al. (2007). The sample extracts 1 mL or solution of standard (30–150 mg/L) was taken and 10 mL of distilled water was added followed by addition of 1 mL Folin–Ciocalteu phenol reagent. After 6 min incubation at room temperature, 2 mL of 20% sodium carbonate (Na_2CO_3.) was added to the mixture. Further kept for 60 min in the dark without disturbance and absorbance was measured at 750 nm using UV–visible spectrophotometer (Jenway 6700 UK). Phenolic concentration expressed as gallic acid (GA) equivalents GAE/g of dry weight.

Total flavonoid content (TFC)

TFC was estimated by using aluminum chloride method as described by Zhishen et al. (1999). An aliquote of 1 mL of sample extract or standard solutions (60–300 mg/L) was mixed with 4 mL of distilled water, 0.3 mL 5% sodium nitrite, 0.3 mL of 10% aluminium chloride and 2 mL of 1 M sodium hydroxide were added and the solution mixture was diluted to 10 mL with distilled water Absorbance was measured at 510 nm on UV–visible spectrophotometer

against reagent. Flavonoid contents were measured using Rutin Trihydrate (RT) equivalents RTE/g of dry weight.

Antioxidant assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The effect of the extract on DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined according to the method of Uddin et al. (2008). An aliquote of 0.2 mL of extract(1 mg/mL) was added to 3 mL of methanolic solution of DPPH (0.004%). In blank, 0.2 mL distilled water was taken instead of sample extract. The mixture was shaken vigorously and allowed to stand for 30 min in the dark at ambient temperature. The absorbance (nm) of the solution was measured at 517 nm with spectrophotometer using methanol for baseline correction. The quercetin dihydtrate were used as standards. DPPH values was measured using quercetin dihydtrate (QD) standard curve equation and expressed DPPH expressed as QD equivalents QDE/g of dry weight.

NO (nitric oxide) scavenging assay

The NO potential was evaluated by the method adopted by Hazra et al. (2008). Sample extract were added to 0.8 mL, 10 mM sodium nitroprusside made in phosphate buffer saline solution having pH 7.4, further kept for 150 min at ambient temperature. Afterwards sulfanilamide (1 mL, 0.33% in 20% glacial acetic acid) was mixed and again kept the mixture for 5 min. further 1 mL of 0.1% solution of *N*-(1-napthyl)ethylenediaminedihydrochloride was added and kept for 30 min. Lastly chromophore of pink color created was measured at 540 nm on UV–visible spectrophotometer. For positive control, quercetin dihydtrate (Q) were used. Values of NOs was measured by using quercetin dihydtrate standard curve equation and expressed as QD equivalents QDE/g of dry weight.

FRAP (ferric ion reducing antioxidant power) assay

The FRAP was done by method of Benzie and Strain (1996). FRAP solution was prepared by mixing 25 mL, 300 mM (acetate buffer), 2.5 mL, 10 mM (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 2.5 mL, 20 mM $FeCl_3$. Sample extract 0.2 mL or standards (60–300 mg/L) were mixed with 2.8 mL FRAP solution and was shaken vigorously and allowed to stand for 30 min in the dark at ambient temperature and absorbance was measured at 593 nm using UV–visible spectrophotometer. Ferrous sulphate (FS) was used as standard and was expressed as FSE/g of dry weight.

Statistical analysis

Data of physicochemical analysis and antioxidant potential were taken in triplicate and expressed as mean with standard deviation (Mean ± SD). One-way ANOVA with Bonferroni test were used to analyze sensory data using Graph pad prism, p value < 0.05 was considered significant difference.

Results and discussion

Sensory acceptability

The data related to sensory analysis in terms of sensory attributes shown in (Table 2) indicated that sensory attributes decreased with increase in the concentration of TC from 0 to 12% replacing wheat flour in cookies. At 10% and 12% level, the cookies showed unacceptable significant change from control cookies in sensory attributes (flavor, color and appearance, after taste, texture and overall acceptability) and undesirable bitterness as after taste was also observed. It means that additional 2% TC in cookies beyond 8% level decreased the liking. Sensory values at 8%, significantly different from control but not significant with 4% and 6%, was in acceptable range. Up to 8%, cookies were accepted by the consumers and hence TC at the level of 8% without causing any adverse effect on the sensory attributes and acceptable by the consumers was considered or decided as optimized cookies (Fig. 1e). Ajila et al. (2008) studied the effect of mango peel powder (MPP: 0%, 5%, 7.5%, 10%, 15%, 20%) on the sensory properties of biscuits and found 10% MPP in biscuit as optimum level.

Nutritional composition and trace elements

The results of the nutritional analysis are represented in Table 3. These results revealed moisture, fat, ash, protein, iron, zinc and copper for TC to be 9.12%, 3.49%, 6.26%, 6.8(%), 1.72(μg/g), 10.35 (μg/g) and 10.01 (μg/g) respectively, the gross energy value measured by Bomb calorimeter for TC was 4.09 kcal/g. When results shown in Table 3 for TCC and control were compared with each other, there was significant increase in the values of moisture, protein, ash, iron and zinc, whereas the values of copper was non-significant and fat % was significantly decreases. The mean values of total energy estimated by bomb calorimeter for TC cookies and control cookies were 5.03 and 4.98 kcal/g respectively. Galla et al. (2017), found augmentation in total ash, mineral content and protein content together with depletion in fat percentage with

Table 2 Sensory acceptability of CC and TCC

Sensory attributes	CC 0%	TCC 2%	TCC 4%	TCC 6%	TCC 8%	TCC 10%	TCC 12%
Flavor	8.13 ± 0.74	7.87 ± 0.83	7.73 ± 0.70	7.40 ± 0.73	7.20 ± 0.67*	6.13 ± 0.74	5.07 ± 0.70
After taste	8.20 ± 0.67	7.87 ± 0.64	7.33 ± 0.72	7.13 ± 0.64	6.60 ± 0.50***	4.93 ± 0.96	3.87 ± 0.64
Texture	7.67 ± 0.61	7.60 ± 0.63	7.40 ± 0.73	7.13 ± 0.64	7.13 ± 0.64	6.07 ± 0.59	5.53 ± 0.51
Color and appearance	8.07 ± 0.59	7.53 ± 0.74	7.40 ± 0.73	7.13 ± 0.74	7.07 ± 0.79**	6.40 ± 0.82	5.87 ± 0.64
Overall acceptability	7.87 ± 0.74	7.73 ± 0.70	7.60 ± 0.63	7.47 ± 0.83	7.20 ± 0.86	6.20 ± 1.01	5.13 ± 1.13

Values are (mean ± SD), $n = 15$, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; TCC compared with CC

Table 3 Nutritional composition and trace element values of *T. cordifolia* (TC) cookies, control cookies (CC) and optimized TC cookies (TCC)

Parameter	TC	CC	TCC
Moisture (%)	9.12 ± 0.37	1.51 ± 0.04	1.81 ± 0.03***
Protein (%)	6.80 ± 0.05	5.42 ± 0.07	5.98 ± 0.01***
Fat (%)	3.49 ± 0.22	17.42 ± 0.13	16.64 ± 0.16**
Ash (%)	6.26 ± 0.09	0.64 ± 0.04	0.84 ± 0.02
Iron (µg/g)	1.72 ± 0.377	1.03 ± 0.095	1.28 ± .043**
Zinc (µg/g)	10.35 ± 0.25	3.93 ± 0.15	8.71 ± .055***
Copper (µg/g)	10.01 ± 0.05	1.14 ± 0.21	1.51 ± 0.25
Energy value (kcal/g)	4.09.10 ± 0.29	4.97 ± 0.63	5.03 ± 0.11

Values are mean ± standard deviation (SD) of triplicates, (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; TCC compared with CC

incorporation of spinach powder at 5%, 10% and 15% in cookies.

Physical properties and color value

Color value such as L*, a* and b* value indicates the lightness, redness and yellowness respectively are depicted in Table 4. Negative value of a* (− 2.22) denotes to greenness in TC powder. L* and a* value of cookies significantly decreased while b* value increased with addition of TC in cookies. Resembled decrement in L* and a* value and increment in b* value in cookies prepared with addition of mango peel powder in comparison with control were observed by Ajila et al. (2008). Value of spread ratio was significantly decreased with incorporation of TC. Similarly, Sharma et al. (2013) reported spread ratio to be lower with addition of TC leaf power.

Antioxidant potential of TC, CC and TCC

The values depicted in Table 5 of TPC, TFC, DPPH scavenging, NO scavenging and FRAP of fat free extract of TC was found to be 3.883 ± 0.009, 67.532 ± 0.009, 127.33 ± 0.01, 281.5 ± 0.015 and 197.5 ± .041 respectively. Polu et al. (2017) found antioxidant activity of aqueous fractions of stems powder of *T. cordifolia* as 92.46 ± 2.06, 74.25 ± 2.14, 46.23 ± 0.34 and 182.14 ± 1.03 (µg/mL) by ABTS, DPPH, Iron chelating activities and Nitric oxide respectively and Total Phenol and Total Flavonoids content to be 1.8 ± 0.16 (GAE/g of dry weight) and 0.17 ± 0.08 (QE/g of dry weight).

Table 4 Physical properties and color value of the prepared *T. cordifolia* (TC) cookies, control cookies (CC) and optimized TC cookies (TCC)

Parameters	TC	CC	TCC
Color measurements value			
L*	77.62	72.56 ± 0.04	64.61 ± 0.06***
a*	− 2.22 ± 0.01	5.07 ± 0.08	3.64 ± 0.03***
b*	22.83 ± 0.01	30.05 ± 0.21	34.89 ± 0.12***
Spread ratio	–	6.57 ± 0.09	6.29 ± 0.07*

Values are mean ± standard deviation (SD) of triplicates, (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; TCC compared with CC

Table 5 Total polyphenolic content, total flavonoids content and antioxidant potential of TC, CC and TCC

Extract	TPC (GAE/g of dw)	TFC (RTE/g of dw)	DPPH (QDE/g of dw)	NO (QDE/g of dw)	FRAP (FSE/g of dw)
TC	3.883 ± 0.009	67.532 ± 0.009	127.33 ± .001	281.5 ± 0.015	197.5 ± .041
TCC	1.37 ± 0.048	22.06 ± 0.025	69 ± 0.001	146 ± 0.009	52.5 ± .004
CC	1.23 ± 0.158	6.4 ± 0.004	24 ± 0.001	114.5 ± 0.007	9.15 ± .009

Values are mean ± standard deviation (SD) of triplicates

Nakagawa and Yokozawa (2002), suggested that elimination of NO responsible for the therapeutic value, protective action and lipid peroxidation chain reaction.

When comparing TCC and CC for these values in Table 5, results revealed that incorporation of TC to the cookies significantly increased the TFC, NO scavenging, DPPH scavenging and FRAP values of TCC than CC. Matching trend of TPC, TFC and antioxidant activity in the cookies incorporated with pomegranate peel powder, pomegranate juice (Paul and Bhattacharyya 2015), *Moringa oleifera* leave and cocoa powder (Ajibola et al. 2015) were found higher than control cookies.

Conclusion

The outcomes of the present study support the fact that TC is a good source of nutrients and trace elements such as iron, zinc, copper and proved to hold promising antioxidant potential. TC addition in cookies was admissible up to 8% without creating nasty sensory attributes. Moreover, TCC found to be better in terms of nutritional properties with antioxidant potential than the Control cookies. As it owned antioxidant potential, that have great role in human physiology and proven to be good for health. And one should take proper micro nutrients and antioxidants along with proper diet. Therefore, cookies incorporated with TC with ayurvedic goodness boost nutraceutical potential and advantageous to all age group people. Further research with respect to phytochemical analysis to recognize the active principles compound present after baking and toxicity of TCC is under the investigation.

Acknowledgements The author is grateful to Banaras Hindu University for providing fellowship. The author is also grateful to Dr. Bhuvanesh Kumar (Director) and Dr. Somnath Singh, (Scientist 'F' and Head of Food and Nutrition division), (DIPAS), DRDO, Delhi, for providing lab facilities.

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