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ORIGINAL ARTICLE

Value addition of *Cissus quadrangularis* stem powder in Ethiopian flat bread: Injera and its effect on Nutritional composition, Sensory attributes and Microbial load

Tsegaye kelbore[1] · Belete Yilma[1] · Babuskin Srinivasan[2]

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Abstract Injera is one of the common foods which are highly consumed daily in all parts of Ethiopia. *Cissus quadrangularis* has been documented in Ayurveda for its medicinal uses in gout, syphilis, venereal disease, piles, osteoporosis, anorexia, diarrhoea, scurvy, menstrual disorders, otorrhoea and epistaxis. Despite the health benefit it offers, it is not used in East African cuisine. Considering its good nutritive properties, efforts have been made in this study to determine the effect of *Cissus quadrangularis* stem powder on the nutritional properties of injera. Blends of different ratios of teff flour and Cissus stem powder (98:2, 96:4 and 94:6) were used to prepare injera and 100% teff flour injera was used as control. When compared with control injera, percentages of ash, crude fibre, crude fat and crude protein of the *Cissus quadrangularis* stem powder incorporated injera increased from 3.12 ± 0.06% to 4.79 ± 0.82%, 2.55 ± 0.71% to 3.94 ± 1.52%, 2.43 ± 0.38% to 3.68 ± 0.63% and 10.01 ± 0.17% to 12.58 ± 0.42% respectively; while carbohydrate content decreased with increased level of incorporation from 81.89 ± 0.41% to 76.01 ± 0.63%. The mineral and phytochemical content of injera significantly increased with the incorporation of *Cissus quadrangularis* stem powder, except for minerals potassium and phosphorus. The control sample and 2% Cissus stem powder incorporated injera were rated high by the panelists. However, up to an incorporation level of 4% *Cissus quadrangularis* stem powder, the injera was found to be sensorily acceptable and it also decreased the microbial load of injera during storage.

Keywords *Cissus quadrangularis* · Value addition · Blending ratio · Injera · Sensory

✉ Babuskin Srinivasan
buski87@gmail.com

[1] Department of Chemistry, Arba Minch University, Arba Minch, Ethiopia

[2] Department of Industrial Chemistry, Arba Minch University, Arba Minch, Ethiopia

Introduction

Cissus quadrangularis Linn (Syn. *Vitis quadranglularis* L), is a perennial plant belonging to the grape family generally called veldt grape (or) Devils backbone. This rambling shrub grows normally in warm climatic zones in countries like Malaysia, Africa, India, West Asia and Sri Lanka. The stem of the plant is rigid, glabrous, fleshy, thick and quadranglular at the nodes. The stem part of the plant is fit for human consumption and it is consumed traditionally as curries and chutneys in Southern parts of India. Due to its high ash content, it is being used as a replacement for baking powder in food processing. It is a rich source of calcium, carotene, Vitamin C and Vitamin E (Rao et al. 2011).

The Phytochemical analysis of *Cissus quadrangularis* performed earlier indicated the presence of carotene, ascorbic acid, phenols, phytosterol, terpenoids, β-sitosterol, δ-amyrin, δ- amyrone and calcium (Mishra et al., 2011). Due to the presence of phytochemical components they exhibit very good antioxidant and antimicrobial properties as reported by earlier studies (Murthy et al., 2003; Karadbhajne et al. 2014).

A toxicological safety evaluation of these stem extracts were conducted in a study and it was revealed that it is safe for human consumption (Attawish et al.

2002). Moreover they are approved and listed as an edible vegetable by the USDA (U.S. Department of Agriculture (Attawish et al. 2002). *Cissus quadrangularis* are well recognized for its nutraceutical potential and is currently being explored as a functional ingredient in a wide array of health foods. (Vijayalakshmi et al. 2016).

In Ethiopia teff is a chief food grain used primarily for the production of injera, a popular fermented Ethiopian pancake (Demissie 2001). Since, teff injera is consumed in Ethiopia as a major staple food, the incorporation of *Cissus quadrangularis* stem powder in the former appears to be promising and it may increase the shelf life andnutritional status of injera. Moreover information on the incorporation of cissus in injera is scanty.

Over the past decades, owing to the change in food habits and increased awareness among people in healthy food consumption, foods loaded additional micronutrients is in demand.Although the plant posses high nutritional value and medicinal properties, it remains an unutilized category among the edible plant in Ethiopia due to the lack of scientific knowledge and awareness related to its value addition in easy consumable forms. Considering the therapeutic potential of the plant, it is believed that their use as nutraceutical supplements is interesting. To explore its utilization, efforts are to be taken for the development of functional food products using *Cissus quadrangularis;* so that this plant can be incorporated in the daily diets of community. In review of the above, the present investigation has been undertaken to incorporate *Cissus quadrangularis* stem powder in Injera. The nutritional profile and phytochemical constituents of *Cissus quadrangularis* stem powder was analyzed. Further, the sensory attributes and microbial quality of the Injera containing *Cissus quadrangularis* stem powder was also analyzed.

Materials and methods

Raw materials collection

Fresh and matured samples of *Cissus quadrangularis* stem were collected from Nechsar National park, Arba Minch town (located at 505 km distance south of Addis Ababa). The collected samples were packaged in clean, dry, polyethylene bags immediately after collection and then stored in refrigerator until the analysis. The teff sample was also purchased from Arba Minch market and packed in polyethylene bags and then stored in desiccator for preparation of Injera.

Preparation of *Cissus quadrangularis* stem powder sample

Fresh *Cissus-quadrangularis* stem collected was washed with tap water and rinsed with distilled and deionized water to wash off extraneous substances, including soil, dust particles and trace metals that may influence analytical results. The stem was then cut into small pieces (10 mm) and the initial weight before drying was taken. The stem pieces were allowed to dry in freeze dryer at −37.4 °C until the moisture content is completely removed. The dried stem pieces were ground into fine powder using the blender. The powder of the sample was then sieved by mesh of size 500 µm. The final weight of the sample was measured and kept appropriately in desiccator for further analysis.

Preparation of *Cissus quadrangularis* stem powder-injera sample

Teff flour was mixed with water (200 g flour + 180 mL water), dough was kneaded by kneader to get optimum consistency and after that dry yeast (5% of flour weight) was added on the top of the dough. The dough was fermented at room temperature for 48 h. After fermentation, 10% of the fermented dough was mixed with water (1:3) and boiled for 4 min. Boiled batter was then cooled to temperature 46 °C and added back to the fermenting dough. After thorough mixing, the batter was fermented at room temperature for 4 h and water was added to the fermented dough to bring optimum batter consistency. Finally, the fermented batter was poured in a circular hot clay griddle, covered with lid to prevent steam from escaping, and was baked for about 3–5 min. *Cissus quadrangularis* stem powder was added in different ratios of 2%, 4%, and 6% to teff flour for the preparation of *Cissus quadrangularis*-injera.

Proximate analysis

The proximate chemical composition of control injera and cissus added injera was performed according to the methods of AOAC (2005): crude fiber, dry weight (DW) by drying in an oven at 105 °C until constant weight; crude protein (Nx6.25) by the Kjeldahl method after acid digestion; crude ash by keeping in muffle furnace at 550° C; fat content by Soxhlet extraction with petroleum ether and moisture by oven dry method. All the analysis was done in triplicates. Finally carbohydrate content was determined by difference, that is, addition of the percentages of moisture, protein, fiber, fat, and ash and then subtracting it from 100%. The energy values were calculated by using factors of 4, 9 and 4 for total carbohydrates, fat and proteins, respectively.

Total alkaloid content determination

40 ml of 10% acetic acid in ethanol was added to 1 g of powdered sample, covered and allowed to stand for 4 h. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total Tannins content determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions.

Total phenol content determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200 µl of the sample extract, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 h incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions.

Estimation of flavonols

1gm of sample was grinded with the help of mortar and pestle in ethanol and the supernatant was collected by centrifugation at 10,000 rpm for 20 min. The supernatant was evaporated to dryness; then the residue was dissolved in a known volume of distilled water (5 ml). 1 ml of extract was pipette out into 25 ml cap, conical flask and 1 ml of distilled water was added. Then 4 ml of vanillin reagent was added from a burette rapidly within 10–15 s to flask A and 4 ml of 70% H_2SO_4 to flask B. A blank flask C was prepared containing 4 ml vanillin reagent and 2 ml water. Both the flasks A and B were shaken in the water bath at temperature below 35 °C. Flasks were kept at room temperature for exactly 15 min. Absorbance was measured at 500 nm of flask A, B, and C against 47% H_2SO_4 (Flask D). The absorbance's of the flasks B and C from that of A. The flavonol content was calculated using a standard curve prepared from phlorogucinol or kaempferol (100 µg/ml).

Determination of ascorbic acid

The method used was as described by AOAC (2005). The values obtained were compared with the standard and used in calculating vitamin C.

Mineral analysis of samples

Determination of minerals was carried out by standard methods of AOAC (2005). The samples were wet-digested and used for determination of these minerals Ca, Mg, Na, K, Fe, Cr, Pb and Cd. The standard solutions of these minerals were prepared and used for determining the concentration in samples.

Microbiological analysis of Injera

Isolation of moulds that spoil Injera

The injera samples were kept for a period of 3 days in the laboratory at ambient temperature (10–25 °C) until mould colonies started to appear visually on its surface, then based on their difference in colour and colony morphology the moulds were transferred and grown in potato dextrose agar (PDA) containing Chloramphenicol (60 mg/l) as antibacterial agent. The cultures were then incubated for five days for induction of fungal growth and finally they were isolated.

Characterization and identification of moulds that spoil Injera

Identification to the genus level of the fungal isolates was based on morphological characterization that focussed on colony characteristics, spore size and shape (Barnett and Hunter 1972). The slide cultures were prepared for each one of the isolates.

Effect of *Cissus quadrangularis* on growth of Injera moulds

The moulds (*Aspergillus niger, Penicillium* spp. and *Rhizopus* spp.) were inoculated on Injera Sucrose Agar (ISA) media to assess their growth and sporulation at different temperatures of 20, 28 and 37 °C. ISA media was prepared by drying and powdering fresh injera; then the powder (100 g) was mixed with water (500 ml) and kept in a shaker for 15 min. The injera broth was added with sucrose (2%) and agar (2%), the mixture was filtered through cheese cloth and heated until it boiled. After sterilization (15 min at 121 °C), the media was poured onto Petri dishes. The growth (colony size) of each moulds at different temperatures was observed and recorded as no growth (−), slight mould growth (+), moderate mould growth (+ +) and high mould growth (+ + +) after 4 and 7 days of incubation. A triplicate of each of the fungi on agar plates was used for each temperature treatment.

Total aerobic and yeast-mould counts

Mould and Bacterial growth on the injera is an indicator of length of shelf-life. The samples were drawn from stored injera and they were analyzed for total aerobic and yeast counts. The method as described by AACC (2000) was followed for the analysis of Total aerobic plate count, Yeasts-mould colonies count and Coliforms counts. The final results were expressed as colony forming unit log CFU/g.

Sensory evaluation of prepared Injera

For the sensory evaluation, the product was presented to 20 semi-trained panelists who comprised of 10 males and 10 females from industrial Chemistry department. The age group of participants ranged from 20–35. In this evaluation, the sensory attributes: color, flavor, texture, taste, and overall acceptability of injera with and without *Cissus quadrangularis* stem powder blends was evaluated using a five point hedonic scale, 5 for like extremely, 4 for like moderately, 3 for neither like nor dislike, 2 for dislike moderately, 1 for dislike extremely. Samples were arranged in random order on white plates and served to the sensory panelists. Just before each test, orientation was given to the participants on the procedure of sensory evaluation.

Statistical analysis

All analyses were performed in triplicates. One way analysis of variance (ANOVA) was done to test the statistical significance for nutritional composition, phytochemical analysis, mineral profile and sensory evaluation of each sample. Results were expressed as mean and standard deviation (Mean ± SD) at 95% confident limit. Significant level of $P < 0.05$ was performed using BIM SPSS version 21software to compare the difference between treatment means.

Results and discussion

Percentage yield of Cisuss Quadrangularis stem powder

The percentage yield of 8.4 was obtained by freeze drying method in which the yield of dried powder was 84 g/ 1000 g from fresh stem of *Cisuss quadrangularis* and loss on drying was 916 g/1 kg. The % yield in this study, 8.4% was in good agreement with the previous work 8.3% as reported by Karadbhajne et al. 2014.

Proximate composition of *Cissus quadrangularis* stem powder and Teff Injera (control)

The *Cissus quadrangularis* stem powder and teff injera (control) were tested for different chemical characteristics, i.e. moisture, crude fat, crude protein, crude fiber, total carbohydrates, dietary fiber, and ash content.

The proximate composition of *Cissus quadrangularis* stem powder obtained in this study has carbohydrate content 53.55%, moisture content 8.82%, ash content 7.54%, crude fiber 10.79%, protein 13.88% and fat 5.42% which are closer to values as reported by Nawghare et al 2017.

The proximate composition of teff injera (control) without the addition of *Cissus quadrangularis* has moisture content 52.23%, ,carbohaydrate 81.89%, protein 10.01%, fat 2.43%, crude fiber 2.55% and ash 3.12%. These results were in accordance with the values reported by Gebrekidan 2016. The ash and crude fiber content of *Cissus quadrangularis* is higher because naturally the plant has high amount of minerals and fiber content (Veerabahu and Chinnamadasamy 2010).

Phytochemicals and minerals composition of *Cissus quadrangularis* stem powder

The phytochemicals analyzed in the stem powder of *Cissus quadrangularis* has alkaloid content of 99.5 mg/ 100 g, total phenol content of 547.45 mg/100 g, tannin content of 58.74 mg/100 g and flavonol content of 53.53 mg/100 g (shown in Table. 1). The values reported in the present study correlated with the values reported by Nawghare et al 2017. The ascorbic acid content of 205.62 mg/100 g was observed for dried *Cissus quadrangularis* stem powder, when compared with fresh stem (353 mg/100 g) there was significant loss of acorbic acid and this may be due to loss during drying of stem.

The present study revealed that calcium content found in the stem powder was 153.62 mg/100 g which plays an important role in living cells as an intracellular regulator or messenger. Phosphorus level of 88.15 mg/100 g was

Table 1 Phytochemicals and mineral contents of *Cissus quadrangularis* stem powder

Minerals	Ca	Mg	Na	K	P	Fe	Cr	Pb	Cd
Value in mg/100 g	153.62 ± 1.14	129.43 ± 1.71	33.32 ± 0.67	126.4 ± 1.06	88.15 ± 0.74	23.20 ± 0.33	0.04 ± 0.07	ND	0.01 ± 0.02
Phytochemicals	Alkaloid	Phenol	Tannin	Flavonols	Ascorbic acid				
Value in mg/100 g	99.5 ± 0.34	547.45 ± 0.65	58.74 ± 0.29	53.53 ± 0.41	205.62 ± 0.33				

Values are expressed as mean ± SD

observed. The iron content was observed to be 23.20 mg/100 g, and it is well known that iron deficiency causes anaemia. Potassium content of 126.4 mg/100 g, sodium and magnesium content of 33.32 mg/100 g and 129.43 mg/100 g respectively; along with chromium 0.04 mg/100 g, and cadmium 0.01 mg/100 g were observed. The result obtained is in close relation with the mineral profile for *Cissus quadrangularis* stem powder in which sodium, potassium, calcium, magnesium, phosphorus, iron, were 44.2 ± 0.11, 143 ± 1.2, 94.0 ± 0.62, 150.0 ± 1.18, 118.1 ± 0.12, 34.00 ± 0.11 (mg/100 g) respectively as reported by Veerabahu and Chinnamadasamy 2010.

Phytochemicals and mineral composition of Teff Injera

The white teff injera has no alkaloid content present and other phytochemicals like phenol, tannin, flavonols were present in much lesser amount when compared with *Cissus quadrangularis* stem powder. The value of ascorbic acid in prepared injera was 2.45 ± 0.23 and this was higher when compared with the value observed by Santosh Khokhar et al. 2012.

Mineral content in mg/100 g of control or raw injera has sodium 10.82±0.26, potassium 131.27±0.86, phosphorus 90.43±1.07, calcium 58.28±0.95, iron 12.56±0.31 and magnesium 103.54±1.44 (shown in Table 2). Among the minerals, higher value was observed for potassium followed by magnesium and then phosphorus which is in line with the result obtained by Abebe et al. 2007.

Interaction effect of *Cissus quadrangularis* and blending ratios on proximate composition of Cissus stem powder incorporated Injera

The *Cissus quadrangularis* stem powder 2, 4 and 6% were incorporated in teff flour for the preparation of injera. The results obtained were tabulated and subjected to statistical analysis using analysis of variance techniques (shown in Table 3).

Blending ratio had no significant ($P > 0.05$) effect on the moisture content of the injera produced. The moisture content was observed in the same range for control and composite injera samples. This has shown that there were no interaction effects of cissus stem powder on moisture content of injera blends. As the *Cissus quadrangularis* stem powder has no or negligible moisture content, it had no effect on the moisture content of injera.

The significant differences ($P < 0.05$) were observed for the ash content of *Cissus quadrangularis* stem powder-teff injera product. Ash content of injera was affected by blending ratios. The ash content of the control injera was lower 3.12% than those of the composite injera samples.

Table 2 Phytochemicals and mineral content of Teff injera

Minerals	Ca	Mg	Na	K	P	Fe	Cr	Pb	Cd
Value in mg/100 g	58.28 ± 0.95	103.54 ± 1.44	10.82 ± 0.26	131.27 ± 0.86	90.43 ± 1.07	12.56 ± 0.31	ND	ND	ND
Phytochemicals	Alkaloid	Phenol	Tannin	Flavonols	Ascorbic acid				
Value in mg/100 g	ND	131.27 ± 0.86	0.014 ± 0.002	5.23 ± 0.09	2.45 ± 0.23				

Values are expressed as mean ± SD

Table 3 Nutritional composition of injera prepared with varying proportions of *Cissus quadrangularis* stem powder

Treatments	Proximate composition (percent)					
	Moisture	Ash	Crude fiber	Fat	Protein	Carbohydrate
T_0	$52.23^a \pm 0.34$	$3.12^a \pm 0.06$	$2.55^a \pm 0.71$	$2.43^a \pm 0.38$	$10.01^a \pm 0.17$	$81.89^a \pm 0.41$
T_1	$52.25^a \pm 0.51$	$3.46^b \pm 0.72$	$2.97^b \pm 1.20$	$3.02^b \pm 1.50$	$11.52^b \pm 0.40$	$79.03^b \pm 0.32$
T_2	$52.38^a \pm 0.20$	$3.57^c \pm 0.14$	$3.64^c \pm 0.32$	$3.14^b \pm 1.12$	$11.91^b \pm 1.02$	$77.74^c \pm 0.91$
T_3	$52.28^a \pm 1.41$	$4.79^d \pm 0.82$	$4.04^d \pm 1.52$	$3.68^c \pm 0.63$	$12.58^c \pm 0.42$	$76.01^d \pm 0.63$

Values are expressed as mean ± SD

Different letters (a,b,c) in each column represent significant difference ($P < 0.05$)

Where, T_0 = control (injera made of 100 g teff flour), T_1, T_2, T_3 = injera made with 2, 4 & 6% *Cissus quadrangularis* stem powder respectively

The highest 4.79% and lowest 3.12% ash contents of injera were recorded for those with 6% *Cissus quadrangularis* stem powder and control injera respectively. The highest ash content was observed in samples with 6% *Cissus quadrangularis* stem powder which directly indicated that increasing the blending ratio increased the ash content. The reason for this is that the *Cissus quadrangularis* stem powder is rich in mineral contents when compared with Teff.

Blending ratio resulted in significant difference ($P < 0.05$) in fiber contents of composite injera blends. The highest fiber content of 4.04% observed in teff blended with 6% *Cissus quadrangularis* stem powder and the lowest 2.55% in control injera. As *Cissus quadrangularis* stem powder contains much higher crude fiber than teff, so it lead to increase in fiber contents of blended injera samples.

The effect of blending showed significant differences ($P < 0.05$) in fat content between control and 6% cissus blended injera. But there were no significant differences ($P > 0.05$) observed between 2 and 4% cissus-injera blends. The highest fat content 3.68% was observed in injera made of teff blended with 6% *Cissus quadrangularis* stem powder and the lowest 2.43% in teff injera without *Cissu quadrangularis* stem powder. The increasing amount of *Cissus quadrangularis* stem powder content in injera increased the percentage of fat content.

Blending ratio brought significant differences ($P < 0.05$) in protein contents. The highest protein content 12.58% was observed in teff blended with 6% *Cissus quadrangularis* stem powder and the lowest 10.01% in control teff injera. The reason for this was that the protein content of *Cissus quadrangularis* stem powder used was higher than the protein content of the teff and thus incorporation of teff with *Cissus quadrangularis* stem powder increased the protein content of injera.

The interaction of cissus with injera had showed significant effect ($P < 0.05$) on carbohydrate content. But when compared with other parameters like ash, fiber, fat and protein, the carbohydrate levels decreased with increasing cissus powder addition. The highest total carbohydrate content, 81.89% was observed in teff injera without *Cissus quadrangularis* stem powder and the lowest, 76.01% in teff blended with 6% *Cissus quadrangularis* stem powder. The value of total carbohydrate was reduced from 81.89% to 76.01% due to blending which indicated that *Cissus quadrangularis* stem powder contained lower total carbohydrate content, compared to teff.

Energy value of Injera prepared by varying proportions of *Cissus Quadrangularis* stem powder

Energy value of a product is important in assessing its impact on human health. The energy value of the prepared injera incorporated with *cissus quadrangularis* stem powder were analyzed and slight differences existed in the energy values of injera. The T_0 sample had the highest energy value of 389.47 kcal/100 g followed by T_1 sample having energy value of 388.38 kcal/100 g. T_2 and T_3

samples had energy values of 386.86 kcal/100 g and 385.48 kcal/100 g respectively.

Interaction effect of *Cissus quadrangularis* and blending ratios on Phytochemicals and Mineral Profile of blended Injera

The interaction effect of *Cissus quadrangularis* stem powder on injera's phytochemical and Mineral contents are shown in Table 4. Significant changes were observed in the phytochemical and mineral contents of injera blends.

The blending ratios at 2, 4 and 6% have shown significant differences ($P < 0.05$) in the mineral contents (Na, Ca, Mg, Fe) among cissus stem powder blended injeras. The mineral contents (mg/100 g) of sodium (10.82 -13.67), Calcium (58.25–64.56), Magnesium (103.54–110.29) and Iron (12.56–14.05) increased with inclusion of cissus stem powder. The reason for this is due the higher amount of minerals present in stem powder when compared with Teff injera. The addition of *Cissus quadrangularis* stem powder at 2% and 4% has not shown significant differences ($P > 0.05$) in potassium and phosphorus levels, whereas the maximum level (6%) of cissus stem incorporation has shown significant difference ($P < 0.05$) between them and control.

Cissus quadrangularis stem powder and blending ratio had significant effect ($P < 0.05$) on phyto-chemicals content in injera. Alkaloid content was not detected in control injera, but it increased to a minimal level of 5.89 mg/100 g with 6% substitution of cissus stem powder. The contents of other phytochemicals like phenol, tannin and flavonol increased from 131.27 to 165.12, 0.014 to 1.94, 5.23 to 10.30 mg/100 g respectively in stem powder blended injeras. The increase in phytochemical levels may be attributed due to presence of high amount of phytochemicals naturally in *Cissus quadrangularis*. The increase in phytochemical contents in this study correlated with the study conducted by Tigist et al. 2019 where they have incorporated Fenugreek greek flours in injera.

Vitamins are vital organic compounds that are needed in smaller amounts for proper metabolic growth and development. As most of the vitamins are not synthesized in our body, it should be obtained through diet. The blending ratio had significant effect ($P < 0.05$) on Vitamin-C content of injera. The amount of Vitamin-C increased from 2.45 to 14.79 mg/100 g with the cissus stem powder incorporation on injera. The increase in Vitamin-C levels helps in effective absorption of minerals present in injera (Stavin and Lloyd 2012).

Sensory evaluation of *Cissus quadrangularis* stem powder incorporated Injera

Sensory analysis has been carried out to select the best possible combinations and the data is been represented on the Table 5. The tastes of the blends were rated by panelists from like extremely to dislike extremely. The sensory analyses of samples were performed by students of Industrial Chemistry by using a five point hedonic scale (Urala and Lahteenmaki 2003).

The panelists were explained very well about the analysis and a report was generated based on the scores given by panelists. The initial sense of the quality and acceptability of any food is judged by its color. Colour is an important sensory attribute of any food, because of its influence on quality. Good-looking colour of a product is a compulsory component in fast moving consumer goods to appeal consumers. The control sample of injera got the highest sensory score for colour followed by T_1. T_3 sample of injera got the lowest sensory score of 3.7 for colour. The colour of treated samples T_1 and T_2 has not shown significant difference ($P > 0.05$) between the samples except for 6% cissus incorporated injera which differed ($P < 0.05$) with other samples. The sensory score for colour gets decreased with maximum level incorporation of *Cissus quadrangularis* stem powder in injera.

The sensory score for flavour and taste gets decreased on increased incorporation of *Cissus quadrangularis* stem powder in teff injera. Flavour is the main criteria that make the product to be liked or disliked. Taste and the flavour are undoubtedly the most important attributes determining the quality of injera or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Hansen and Schieberle 2005). The significant difference ($P < 0.05$) in terms of taste and flavor were observed for 6% cissus stem powder incorporated injera.

T_1 sample of injera got highest sensory score of 4.8 for texture and T_3 got the lowest sensory score of 3.5. In terms of overall acceptance, it can be arranged in the order of $T_1 > T_2 > T_3$ and it can be concluded that T1 and T2 are best accepted next to control T_0. The sensory attributes in this study matches with the study conducted by Karadbhajne et al. 2014, where they incorporated Cissus dried extract in cookies and biscuits and it has been observed that until 5% of cissus incorporated foods are favored by consumers whereas increase in concentrations above 10 and 15% has decreased the sensory acceptability of the product. The decrease in sensory acceptability with *Cissus quadrangularis* incorporation in foods was due to its contribution of bitter taste and darker color when it was added at higher concentrations.

Table 4 Phytochemicals and mineral profile of injera prepared by varying the proportions of *Cisus quadrangularis* stem powder

Treatments	Contents in (mg/100 g)										
	Na	K	P	Ca	Fe	Mg	Alkaloid	Phenol	Tannin	Flavonols	Vit-C
T_0	$10.82^a \pm 0.26$	$131.27^a \pm 0.02$	$90.43^a \pm 0.6$	$58.25^a \pm 0.92$	$12.56^a \pm 0.25$	$103.54^a \pm 0.41$	ND	$131.27^a \pm 0.86$	$0.014^a \pm 0.002$	$5.23^a \pm 0.09$	$2.45^a \pm 0.23$
T_1	$11.96^b \pm 0.5$	$131.17^a \pm 0.20$	$90.33^a \pm 0.94$	$61.66^b \pm 0.27$	$13.02^a \pm 0.11$	$104.27^b \pm 0.72$	$1.97^a \pm 0.02$	$139.74^b \pm 0.75$	$0.76^b \pm 0.10$	$7.45^b \pm 0.14$	$6.55^b \pm 0.09$
T_2	$12.39^c \pm 0.90$	$131.67^a \pm 0.02$	$90.58^a \pm 0.08$	$63.44^c \pm 0.81$	$13.92^b \pm 0.44$	$106.68^c \pm 0.04$	$3.97^b \pm 0.17$	$153.18^c \pm 0.56$	$1.37^c \pm 0.16$	$9.24^c \pm 0.14$	$10.67^c \pm 0.17$
T_3	$13.67^d \pm 1.05$	$133.74^a \pm 0.03$	$92.67^b \pm 0.16$	$64.56^d \pm 0.92$	$14.05^b \pm 1.07$	$110.29^d \pm 0.32$	$5.89^c \pm 0.21$	$165.12^d \pm 0.45$	$1.94^d \pm 0.15$	$10.30^d \pm 0.13$	$14.79^d \pm 0.16$

Values are expressed as mean ± SD

Different letters (a,b,c,d) in each column represent significant difference ($P < 0.05$)

Microbiological analysis of blended Injera

Normally, freshly prepared injera is free of fungus, but due to larger surface of the flat bread the probability of it getting spoiled by fungi is high. Temperature is one of the significant environmental factors that badly affect not only the growth and germination of spoilage moulds in *vitro*, but also in *vivo* (Gebrekidan and Gebrehiwot 1982).

The growth rate of moulds (Aspergillus niger, *Penicillium* spp.and *Rhizopus*spp.*)*were studied on injera sample blended with *Cissus quadrangularis* stem powder (4 g) as natural additives. This study was performed at three different temperatures of 20 °C, 28 °C and 37 °C upto 10 days. Blended injera was considered as safe up to 7 days because there were no moulds or only slight mould formation, but after7 days fungi colonies appeared, each variety displayed different color. Heavy mould growth was observed on the samples on the 8th day of fermentation. The moulds were then isolated and identified through microscopic evaluation and morphological studies to be *Aspergillus niger, Penicillium* spp. *and Rhizopus* spp.

Aspergillus niger preferred warmer temperature and they became more dominant and visible to the naked eye at these temperature (shown in Table 6). *Rhizopus* which appears white to dark grey commonly called bread moulds were observed as injera ages. As stated by Gock et al. 2003, *rhizopus* is a non-septate mycelium with root like rhizoids; black columellate, sporangiophores, in clusters. *Penicillium* species are xerophilic fungi that frequently bring about spoilage of baked goods/foods.

The results revealed that *Penicillium* sp. and *Rhizopus* sp. growth appeared on the 5th day at 20 °C, and on the 8th day, the growth was found to be moderate at the same temperature. The slight growths of these moulds were noted after 7 days for other temperatures 28 and 37 °C.

In the case of *A.niger,* no growth was noticed up to the 5thday at 20 °C, but it grew slightly at 28 °C whereas moderate growth was noticed at 37 °C at 8th day of fermentation The results obtained correlated with the study conducted earlier by Ashagrie and Abate 2012 it was revealed that *Penicillium* and *Rhizopus* were more dominant in spoiling injera at lower temperatures (16–20 °C), while *A.niger* grew much faster at higher temperature (25–32 °C).

Total yeast-mould and bacterial count

Total aerbic count (TAC) and yeast-moulds counts were performed for control injera and *Cissus quadrangularis* (4%) stem powder injera. The significant difference in both TAC and yeast-mould counts were observed between control and blended injera samples. The TAC of control sample was 3.07 ± 0.15 log CFU/g after 4 days and it rose

Table 5 Sensory analysis of *Cissus quadrangularis*-Injera blends

Treatments	Color	Flavor	Texture	Taste	Overall acceptability
T_0	$4.9^a \pm 0.24$	$4.8^a \pm 0.12$	$4.8^a \pm 0.23$	$4.8^a \pm 0.31$	$4.82^a \pm 0.32$
T_1	$4.7^a \pm 0.32$	$4.7^a \pm 0.21$	$4.7^a \pm 0.22$	$4.7^a \pm 0.37$	$4.72^a \pm 0.21$
T_2	$4.3^a \pm 0.21$	$4.4^a \pm 0.32$	$4.3^a \pm 0.25$	$4.5^a \pm 0.27$	$4.35^a \pm 0.43$
T_3	$3.7^b \pm 0.14$	$3.8^b \pm 0.18$	$3.5^b \pm 0.27$	$3.7^b \pm 0.22$	$3.67^b \pm 0.45$

Values are expressed as mean ± SD

Different letters (a,b,c) in each column represent significant difference ($P < 0.05$)

5- like extremely, 4- like moderately, 3- neither like nor dislike, 2- dislike moderately,1- dislike extremely

Table 6 Mould growth observation in control and blended injera at different fermentation temperature and time

Moulds observed	Growth rate of moulds at different temperature								
	20 °C			28 °C			37 °C		
	Control at 4th day)	on T_2 at 5th day	on T_2at 8th day	Control (at 4th day)	on T_2 at 5th day	on T_2at 8th day	Control (at 4th day)	on T_2 at 5th day	on T_2at 8th day
Penicilliun spp.	+	+	+ +	+	−	+	+	−	+
Rhizopus spp.	+	+	+ +	+	−	+	+	−	+
A.niger	−	−	+	+	+	+	+	+	+ +

− : no mould growth; + : slight mould growth; + + : moderate mould growth

to 3.89 ± 0.17 log CFU/g after 6 days of storage, whereas for cissus incorporated injera TAC of 2.52 ± 0.1 and 3.28 ± 0.12 log CFU/g were observed after 4 and 6 days of storage respectively. The TAC levels were significantly lower in blended injera after 6 days when compared to control sample. The yeast-mould count of control was 2.89 ± 0.18 log CFU/g after 4 days and it rose to 3.67 ± 0.15 log CFU/g after 6 days of storage. In Cissus blended injera significant difference were observed in yeast-mould counts, it was 2.43 ± 0.13 log CFU/g after 4 days and 2.96 ± 0.17 log CFU/g after 6 days of storage. Both TAC and Yeast-mould counts were low in blended injera when compared with control, this is because of the presence of more amount phytochemicals present in *Cissus quadrangularis* than Teff. There were no coliforms detected in injera during the storage period.

Reda Nemo et al. 2015 reported that the yeast-mould & total aerobic count of street vended foods were 3.4 and 4.4 for injera. The counts of TAC and yeast-mould in this study were lower when compared with other studies. The study carried out by Girma et al. 2013 shows that during 2–6 days of injera storage, yeast-mould and total aerobic plate counts were around 2.85–4.08 log CFU/g and 3.70–4.30log CFU/g for control injera. However, on 9% flaxseed substituted injera yeast-mould and total aerobic plate counts were around 2.27–3.93 log CFU/g and ND-3.77 log CFU/g. The extent of flaxseed incorporation in injera has a positive effect on controlling the mould and bacterial growth; similarly incorporation of cissus stem powder has reduced the bacterial and mould counts in this study. The positive effect was due to the effect of active antimicrobial and phytochemical constituents present in the cissus stem powder (Murthy et al., 2003).

Conclusion

It can be understood from the present study that incorporation of *Cissus quadrangularis* stem powder has significantly influenced mineral and phytochemical content of injera. However there were no significant difference observed for few minerals like potassium and phosphorus. In terms of sensory up to 4% of cissus incorporation has been well accepted by consumers. The inclusion of cissus has also significantly reduced the microbial load when compared with control. It is recommended that *Cissus quadrangularis* stem powder can be incorporated in injera for enhancing their nutritional value and reduction of microbial load. More new products from *Cissus quadrangularis,* beneficial for the community are to be developed and research in this direction should be encouraged.

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Declarations

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