

# Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts

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**Abstract** *Stevia rebaudiana* is an introduced crop in India. The leaf and its extract although sweet have a bitter after taste that precludes commercial acceptability. The composition of the leaf reflected a high nutritive value and polyphenol concentration averaging 4.15% by weight of dried leaf. Variably processed extracts enriched with polyphenols, pigments and a mixture of both were evaluated for sensory attributes by a semi trained panel when added to coffee and lime juice. Presence of polyphenols influenced the acceptability of the sweeteners marginally, while chlorophyll was found unacceptable in any of the extracts. The antioxidant activity of the extracts was synergistic when it was mixed with coffee and lime juice. Complete purification of stevia leaf extracts to obtain pure glycosides is not necessary for it to become a commercially acceptable sweetener.

**Keywords** Stevia · Composition · Polyphenol · Pigments · Sensory attributes

## Introduction

The South American herb *Stevia rebaudiana* accumulates several sweet tasting diterpene glycosides in its leaves. The leaves are shade dried for storage and processed further to produce purified glycosides (Kalpana and Khan 2008). The commercial importance of stevia stems from the use of these glycosides as sugar substitutes. There are 8 known

glycosides in the plant (Kennelly 2002, Starrat et al. 2002). These glycosides are high-intensity sweeteners ranging from 50 to 300 times sweeter than sugar, with low water solubility and high melting points (Crammer and Ikan 1987). These molecules are also highly stable at broad pH and temperature ranges in solution. Prolonged heating of the stevioside solution resulted in a decrease of the stevioside in the solution by 16.7% after 12 h at 100°C at neutral pH and 46 to 54% after 4 h in acidic solutions of pH 2.4–2.6 (Chang and Cook 1983). Significant decrease in stevioside concentration at 80°C was found only at highly acidic condition (pH 1) in solution and at 140°C as a solid (Kroyer 1999). The instability of the molecule under very low pH and baking has led to doubts regarding its safety. (Virendra and Kalpagam 2008).

The advantages of stevioside as a dietary supplement for human subjects are manifold: it is stable, it is non-caloric, it maintains good dental health by reducing the intake of sugar and opens the possibility for use by diabetic and phenylketonuria patients and obese persons (Geuns 2003). The exclusion of sugar in the food is beneficial to diabetics. Steviol glycosides are also safe for phenylketonuria patients as no aromatic amino acids are involved. Obese persons might lose weight by the fact that excessive sugar in the food is replaced by Stevia extracts or stevioside. Omitting added sucrose in foods increases the relative proportion of polymeric carbohydrates. This has beneficial effect for a balanced food intake for human health. Traditional Indian sweet foods have a very high concentration of sugar, and there is evidence of attempts to replace wholly or a part of this sugar with sugar substitutes (Chetana et al. 2006, Arora et al. 2008).

We studied the viability of the crop in the coastal region of South India, which receives average rainfall of 1200 mm mostly between the months of October and December. Day time temperatures ranged between 23 and 40°C with peak temperatures in May. The crop survived for single seasons

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each year but was unable to survive beyond the summer harvests. Monsoons were pretty well tolerated as long as proper drainage was available. Stevia leaves are rich in tannins and other phytonutrients (Savita et al. 2004, Tadhani and Subhash 2006, Anon 2008). We followed the composition of the leaf through various seasons to assess its nutritive value.

Stevioside is known to have inherent bitterness. Polyphenolics are also known to contribute to bitterness of plant extracts. This study was an attempt to understand the role of polyphenols and chlorophylls in the perceived sensory attributes of stevia extracts. Polyphenols also possess anti oxidant activity that has health benefits. Hence, the study evaluated the need for purification of the extracts of *Stevia rebaudiana*, in terms of the sensory attributes, keeping in mind the benefits of using a crude extract of the leaf and the anti oxidant activity.

### Materials and methods

Fresh stevia (*Stevia rebaudiana*) leaves harvested from test plot were shade dried and stored in sealed plastic bags for further analysis. Harvest times coincided with changing seasons and broadly fell in summer (March–April) and monsoon (October–December). All reagents used were of analytical grade from Sigma, USA or Merck, USA unless otherwise stated. Samples were collected in the years 2004, 2005, 2006 and analysed in duplicate. Average values were taken as reference for further processing.

Moisture, ash, crude protein and fat were analysed in the dried leaf samples (AOAC 1990). Minerals K, Ca, Fe and Na were measured from ash using an Atomic Absorption Spectrophotometer (Varian 220, USA).

Extraction of phenolics was carried out by a decoction method. Shade dried leaves (0.5 g) were ground to a fine powder and extracted with 100 ml boiling water for 1 h. This extract was then filtered through Whatman Nr 1 filter paper and analysis was conducted immediately by the Folin-Ciocalteu reagent assay. Absorbance was measured at 650 nm on a U3200 Spectrophotometer (Hitachi, Japan) with a 1 cm cell. All experiments were repeated in triplicate using tannic acid (CDH, Mumbai, India) as standard and values of total polyphenolics are reported as tannic acid equivalents in g/100 g of dried leaf.

Stevioside was analysed using HPLC (Kolb et al. 2001) with the same extracts made for analysis of total phenolics. An 80:20 acetonitrile: water isocratic solvent system with a flow rate of 0.8 ml/min was used on a SP-10A system (Hitachi, Japan) with a Phenomenex amino bonded column (Phenomenex Inc., USA). Detection wavelength was set at 210 nm on a Photo Diode Array detector (SPD-M6A, Hitachi, Japan). Peak areas of standards were compared for quantification. Concentrations were expressed as g/100 g dried leaf.

*Preparation of extracts for sensory analysis:* Extraction procedures were standardized to obtain 3 different extracts

(ET, SC and CO). The ET extract was rich in polyphenols, SC in chlorophyll and CO was a mixture of both these components. Two grams of stevia leaves were extracted repeatedly with ethanol to give a final volume of 75 ml. The ethanol was evaporated and resultant slurry was made up with water. The ethanolic extract of the leaf was labelled ET. For the SC extract ( $\text{Na}_2\text{CO}_3$  precipitated), 2 g of leaf were soaked in sufficient 20%  $\text{Na}_2\text{CO}_3$  solution and heated in an oven at 100°C for 10 min. The soaked leaf was extracted with ethanol as described above. For the CO extract (CaO precipitated), an aqueous extract was made by extracting 50 g leaves with water adjusted to pH 2.0 with phosphoric acid to give 500 ml of extract. This extract was then clarified by the addition of CaO until pH 12 was reached and filtration of solution until it was clear. The pH was adjusted to 7.0 with phosphoric acid again. This aqueous extract of the leaf was labelled CO. Total polyphenol and stevioside content of the extracts were analysed by the methods described above. Chlorophyll was estimated as given below.

The ET, SC and CO extracts were lyophilized on a Christ alpha 1–2 CD Plus lyophilizer (Christ, Germany). 1 ml of 90% acetone was added to lyophilized samples and mixed well. The samples were then centrifuged at 10,000 rpm for 10 min at ambient temperature ( $25 \pm 1^\circ\text{C}$ ). The samples were transferred from centrifuge tubes into cuvettes. A Hitachi SP-10 spectrophotometer (Hitachi, Japan) was used to measure the absorbance of the sample extract at 750, 664, 647, 630 nm against a 90% acetone blank. The concentration of chlorophyll a, b and c were calculated according to the equations (Jeffrey and Humphrey 1975):

$$\text{Chlorophyll a} = (11.85 \times (E_{664} - E_{750}) - 1.54 \times (E_{647} - E_{750}) - 0.08 (E_{630} - E_{750}))$$

$$\text{Chlorophyll b} = (-5.43 \times (E_{664} - E_{750}) + 21.03 \times (E_{647} - E_{750}) - 2.66 (E_{630} - E_{750}))$$

$$\text{Chlorophyll c} = (-1.67 \times (E_{664} - E_{750}) - 7.60 \times (E_{647} - E_{750}) + 24.52 (E_{630} - E_{750}))$$

Concentrations were added to give total chlorophyll expressed in mg/ml of extract.

The antioxidant activity of, ET, SC and CO, was assayed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Fresh DPPH reagent was prepared by dissolving 1.5 mg of DPPH in 25 ml of methanol; 190  $\mu\text{l}$  of DPPH solution were added to 10  $\mu\text{l}$  of samples. Each sample was run in triplicates and appropriate controls were run for each sample. The absorbance of reaction mixture was measured using a Bio-Rad model 680 microplate reader (Bio-Rad laboratories, UK) at 520 nm.

*Sensory studies:* Sensory studies were carried out using discrimination, descriptive and Hedonic scaling tests. The participants for sensory studies consisted of University students of both sexes ( $n=20$ , aged  $21 \pm 1$  years) who volunteered for the study. The subjects were trained adequately in identifying tastes of various test molecules before the tests were carried out. The sensory analysis studies were done

using ET, SC and CO, commercially available stevioside (Nu-naturals Inc., USA), aspartame (Equal, Merisant Inc., USA) and sucrose (SRL, India) in water, coffee and lime juice beverages already familiar to subjects. Stevioside concentration in all the samples served was based on sucrose equivalence ratio as per the labelling in the commercial stevioside. The stevioside concentration in all the extracts was adjusted to 80 mg/100 ml.

The tests were conducted as standalone tests, over 6 weeks with a frequency of 2 per week to determine preference of panelists towards taste attributes in the samples. All the tests were repeated twice and results of second test were considered for sensory analysis to adjust the errors in answering questions in the first round of tests. Prior to each test, all subjects confirmed that they were not suffering from common cold or other respiratory infections. All the tests were done between meal times. Each subject was served 20 ml of test sample and was asked to rinse his/her mouth with water at room temperature ( $25 \pm 1^\circ\text{C}$ ) before tasting each test sample. All tests were conducted in an air conditioned room ( $25 \pm 1^\circ\text{C}$ ) with no interference from outside environment.

CO extract, refined stevioside, sucrose and aspartame were presented in 3 sets to evaluate the ability of panelists to distinguish the CO extract from the other three sweeteners, in a triangular distinction test. Each set consisted of 2 samples of sucrose or aspartame or refined stevioside and one sample of CO extract. For descriptive and Hedonic tests, all the extracts were presented with random code numbers.

**Statistical analysis:** All samples were analyzed in triplicate. For the composition analyses, mean and standard deviations were calculated. For all Hedonic tests, sucrose sample was taken as the reference or ideal sample. Rank one was assigned 6, rank two a score of 5 until rank six received 1. Frequency of a particular rank was multiplied by its score and cumulative scores of each rank of the sample were added to give the total score of the sample. Frequency of the ranks 1 and 2, 3 and 4 and 5 and 6 were grouped together and z test for difference in proportions of the ranking frequencies of these groups in different samples was carried out to corroborate ranking results based on the sum of weighted scores. Pearson's correlation coefficient was calculated to determine the relationship between polyphenol concentration and free radical scavenging activity in the different extracts.

## Results and discussion

**Composition of stevia leaves:** Composition of dried leaves is presented in Table 1. Phenolic compounds contribute directly to anti-oxidant activity (Awika et al. 2003). Stevia plants grown under laboratory conditions had 2.5% total polyphenols (Tadhani et al. 2007). This is very different from our result of 4% (Table 1). Stevioside content were 10.8–11.3% with greater yield during summer harvests (Table 1). Glycoside content is known to vary with day

length, with long days preferred for higher concentration (Metivier and Viana 1979). Kohda et al. (1976) reported 2.2% yield. Brandle and Rosa (1992) reported a mean yield of 10.47% in Canada. There have been reports of strains having up to 20% glycoside content (Huang et al. 1995). Since the temperatures of this agro climatic zone ranged on the higher side of the plants tolerance, it can be assumed that the small differences in temperature, humidity and incident sunlight between the seasons did not influence steviol glycoside content.

Moisture levels were lower in samples harvested and processed in summer (Table 1). Protein and fat levels increased in monsoon. Ash content was 8.4–9.5%. But all values correspond to those reported in literature (Savitha et al. 2004). Fe had a consistent pattern of accumulation in leaves across the seasons. Mint, coriander and spinach have been reported to accumulate 50.6, 22.3 and 35.8 mg/100 g Fe on a dry weight basis respectively (Singh et al. 2001). K showed a tendency to accumulate in the leaves in greater concentrations during summer while Na and Ca concentrations increased in monsoon. K may leach from leaves during rain or be withdrawn from leaves which are either senescing or not under active growth. K accumulation during warmer seasons has been reported in diverse species (Yuehua von fircks et al. 2001, Ramirez et al. 2006). Ca has been reported to accumulate in winter in diverse species like olives and rhododendron (Fernandez-escobar et al. 1999, Gary et al. 2006). Na accumulation in monsoon is surprising.

**Composition of stevia extracts:** The ET extract had the highest concentration of polyphenols (Table 2). The SC extract had the highest chlorophyll concentration and the CO extract had the least concentration of both polyphenols and chlorophyll. When adjusted for concentration of stevioside, the CO extract had the equivalent polyphenol concentration of SC extract and chlorophyll concentration of ET extract.

**Sensory studies:** Triangular discrimination tests were done to evaluate the panelist's ability to distinguish the purified samples from CO extract. The CO extract was used

**Table 1** Composition of *Stevia rebaudiana* leaves

Components	Summer	Monsoon
Total polyphenols <sup>1</sup>	4.2 ± 1.00	4.1 ± 0.64
Stevioside <sup>1</sup>	11.3 ± 0.73	11.0 ± 0.68
Moisture <sup>1</sup>	7.7 ± 0.62	8.6 ± 1.08
Protein <sup>1</sup>	12.0 ± 1.57	12.9 ± 0.74
Fat <sup>1</sup>	2.7 ± 0.64	3.7 ± 0.51
Ash <sup>2</sup>	8.4 ± 2.40	9.5 ± 1.08
Na <sup>2</sup>	32.7 ± 3.42	63.4 ± 2.90
K <sup>2</sup>	839.0 ± 8.55	730.3 ± 38.03
Fe <sup>2</sup>	31.1 ± 15.33	31.3 ± 12.41
Ca <sup>2</sup>	722.0 ± 20.7	807.7 ± 8.91

<sup>1</sup>g/100g dry leaf,

<sup>2</sup>mg/100g dry leaf (n=3)

for this test since it had median concentration of polyphenols and chlorophyll. All panelists were able to distinguish CO from other sweeteners including purified stevioside. Among the 3 sets, CO extract had greater intensity of sweetness. Only 33% of participants felt less intense sweetness of CO extract than either sucrose or aspartame and 22% felt that it was less intensely sweet when compared to commercial steviosides. The remaining participants felt the intensity of sweetness to be equal or more. The CO extract was described to be bitter, medicinal and minty. The difference between purified stevioside and CO extract was described to be medicinal. This is indicative of the influence of phytochemicals extracted along with steviol glycosides in the perception of taste. This test was conducted in water and hence the influence of the polyphenols and pigments were felt by the panelists without the interference of other food components. The awareness of traditional medicines and their taste could have caused this description, since medicinal invariably indicates bitterness or astringency (Masumi et al. 2008).

The descriptive test was done to analyze the sensory attributes of different sweeteners in beverage samples. This

**Table 2** Composition (mg/ml) of different extracts of *Stevia rebaudiana* leaves prepared for sensory analyses

Extract	Total polyphenol	Total chlorophyll	Stevioside
ET	2.0 ± 0.02	0.8 ± 0.01	8 ± 0.01
SC	0.7 ± 0.01	1.6 ± 0.04	8 ± 0.01
CO	0.8 ± 0.02	0.9 ± 0.02	8 ± 0.01

All values are expressed as mg/ml of the extract after dilution to a standard 8 mg/ml concentration of stevioside (n = 3)

ET = ethanolic extract, SC = Na<sub>2</sub>CO<sub>3</sub> precipitated, CO = CaO precipitated

**Table 3** Descriptive test of sweeteners in coffee and in lime juice

Extract	Colour		Distinct flavour		After taste		Acceptability	
	Light	Dark	Chemical	Fruity	Bitter	Sweet	Sweet	Better
<b>Coffee</b>								
S	-	13	6	6	1	12	9	5
A	11	2	1	2	5	7	4	9
CS	11	-	-	-	9	3	-	-
CO	2	9	2	1	12	-	-	-
ET	6	3	1	1	8	4	1	3
SC	4	8	-	-	10	-	-	1
<b>Lime juice</b>								
S	7	1	4	7	1	6	6	1
A	8	-	5	4	2	5	6	-
CS	8	-	6	3	2	8	2	-
CO	15	1	3	4	3	8	5	-
ET	-	14	7	3	10	2	-	-
SC	-	19	5	5	13	-	-	-

A: Aspartame, S: Sucrose, CS: Commercial Stevioside, ET, CO, SC: As in Table 2.

was done following the discrimination test, to ascertain if the description of the CO extract provided in the first test was applicable to all 3 extracts and their influence on different beverages in which they were mixed. Acceptability, colour, flavour and after taste descriptors were analysed in the first round of descriptive tests. The response of the panelists is given in Table 3. In terms of colour, the CO extract did not influence the colour of lime juice, while ET and SC extracts made the lime juice darker. Similarly coffee colour was not adversely affected by CO and SC extracts, while coffee with ET extract was perceived to be lighter. In terms of distinct flavour, both chemical and fruity flavours were ascribed to lime juice by all the sweeteners including sucrose. In contrast, in coffee these were not perceived as distinctly except with sucrose. The aftertaste was mostly described as sweet in lime juice sweetened with CO extract, CS (Commercial Stevioside), aspartame and sucrose. The ET and SC extracts, on the other hand, gave a distinct bitter after taste to the juice. In coffee, a bitter after taste was perceived with all 3 extracts as well as CS. With sucrose alone a distinct sweet taste was perceived. Overall acceptability was poor for all 3 extracts in coffee. However, in lime juice, CO extract was considered acceptable.

Hedonic ranking results for aroma, flavour and holistic preference of different sweeteners in the chosen beverages are given in Table 4. In terms of aroma in lime juice, aspartame ranked highest followed by sucrose. ET extract of stevia leaf had the highest score among the stevioside containing samples. SC extract was least preferred while the commercial stevioside sample had a score similar to that of CO extract. The proportion of top 4 ranks received by the CO extract for aroma was significantly different from that of sucrose, and hence the ranking pattern confirmed the data from weighted scores (Fig. 1). With reference to

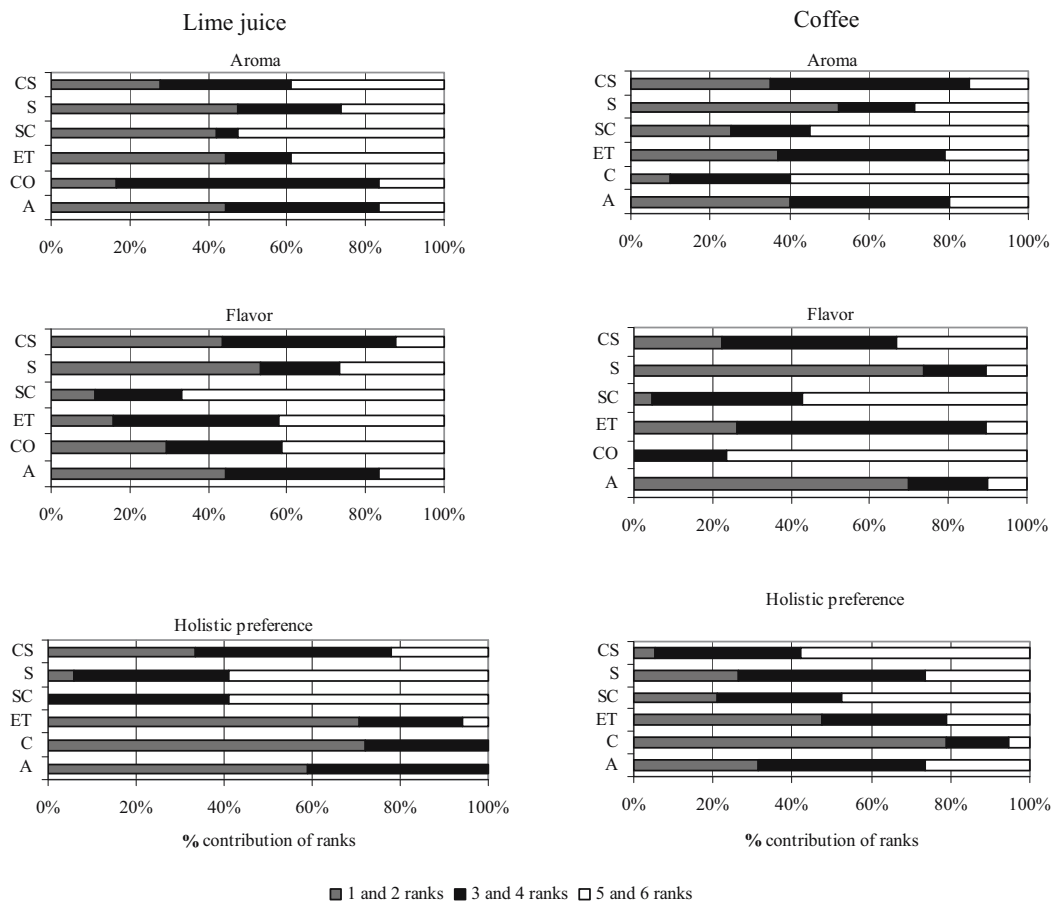
flavour preference, the scores showed a pattern similar to that of aroma, with aspartame and sucrose scoring higher than stevioside samples. Commercial stevioside scored higher than CO extract and SC extract scored the worst. The proportion of panelist ranking 1 and 2 and 5 and 6 for the SC sample was the reverse of the sucrose sample and proved to be significantly different (Fig. 1). For holistic preference, surprisingly the pattern was completely reversed, with CO and ET extracts scoring significantly higher than all other sweeteners. SC extract scored the worst again. Aspartame, CO and ET definitely scored a greater proportion of the first and second ranks than sucrose (Fig. 1).

In lime juice, aroma perception was aided by the high concentration of polyphenols present in the ET extract. Flavour on the other hand was preferred equally in both CO and ET extracts showing that polyphenols did not interfere with flavour perception. When individual parameters were discounted and holistic preference calculated, the pattern got completely reversed and CO and ET extract got a higher score than aspartame, commercial stevioside or sucrose sample. This was also confirmed by ranking proportions. SC extract remaining at the bottom of the scale in the Hedonic scaling helped to conclude that the presence of high concentrations of chlorophyll in that extract was adversely

**Table 4** Hedonic ranking of samples

Rank	Lime juice aroma		Lime juice flavour		HP of lime juice		Aroma of coffee		Flavour of coffee		HP of coffee	
	Sample	Score	Sample	Score	Sample	Score	Sample	Score	Sample	Score	Sample	Score
1	A	76	A	75	CO	92	S	85	S	97	CO	97
2	S	75	S	65	ET	81	A	78	A	93	ET	78
3	ET	66	CS	62	A	78	CS	76	ET	72	A	66
4	CO	62	CO	55	CS	68	ET	75	CS	59	S	65
5	CS	62	ET	54	S	40	SC	57	SC	48	SC	58
6	SC	60	SC	42	SC	37	CO	49	CO	31	CS	42

A, S, ET, CO, CS, SC: As in Table 3, HP: Holistic preference



**Fig. 1** Ranking frequency of sensory analysis of lime juice and coffee containing different stevia extracts. A, CO, ET, SC, S, CS – see Table 3



affecting its acceptability. This indicates that partially purified CO and ET extracts favourably influenced acceptability in lime juice.

Coffee aroma was significantly affected by the addition of SC and CO extracts of stevia leaves, which scored the lowest (Fig. 1). The first 2 and last 2 ranks assigned to by these samples were disproportionate to that of the sucrose sample. The ET extract containing high concentrations of polyphenols did not affect the aroma perception of coffee since it scored close to aspartame and commercial stevioside. The SC and CO extract also seemed to be affecting acceptability in terms of flavour (Fig. 1). All the stevioside containing samples got significantly low proportion of top 4 ranks in terms of flavour. Continuing with the pattern seen in lime juice, there was a complete reversal of ranks based on holistic preference (Fig. 1). CO scored the highest and showed a significantly higher preference than all other sweeteners. SC remained at the bottom of the ranking chart along with commercial stevioside.

There have been many attempts to improve the sweetness of steviol glycosides. One such strategy is enzymatic addition of glucose units to the 13th carbon causing a marked decrease in bitterness (Fukunaga et al. 1989, Lobov et al. 1991, Kochkiyan et al. 2006). There have been attempts to modify the plants for changing the ratio of stevioside and rebaudioside since rebaudioside has better sensory properties compared to stevioside (Jim Brandle 2001 US Patent No: 6255557). Many patents and processes that have been standardized for purification of stevioside, work under the assumption that removal of color and odor causing impurities would substantially improve the sensory properties of stevia based sweeteners. Polyphenols are responsible for some important sensory properties associated with wine. This complex and large family of molecules is responsible for production of taste sensations ranging from bitter to astringent and pungent depending upon the composition of the polyphenol in the food (Andrewes et al. 2003, Preys et al. 2006). It has been reported that higher concentrations of polyphenols, tannins and -epicatechins affect the perceived flavour of cocoa favorably (Bonvehi and Coll 1997, Misnawi et al. 2004). From our sensory studies, it was apparent that the sweetness perception of stevia based sweeteners was increased by presence of polyphenols. In coffee and lemon juice, the property of bitterness and astringency are preferred respectively and hence the ET and CO extract scored well in those samples respectively.

**Antioxidant property:** While in coffee, the increase in free radical scavenging activity was substantial, only marginal rise was visible in lime juice (Table 5). The SC extract showed lower activity than the other samples tested. This could be due to the lower polyphenol concentration in this sample as compared to others. The ET extract has the highest free radical scavenging activity. The *r* value calculated reflects a direct correlation between the antioxidant activity and polyphenol concentration of different extracts, while there is

**Table 5** Antioxidant property of stevia extracts\*

Sample	Water	Coffee	Lime Juice
CO	17.6 ± 1.73	39.5 ± 1.96	22.5 ± 2.39
SC	14.3 ± 2.21	29.3 ± 1.54	19.4 ± 4.31
ET	40.4 ± 0.75	59.3 ± 1.93	45.3 ± 1.22
CS	2.0 ± 0.13	18.8 ± 3.31	17.5 ± 1.56

(n=3)

ET, CO, CS, SC : As in Table 3

\*CS also served as a control for the antioxidant activity of foods since it did not have inherent antioxidant activity.

no proof of correlation between antioxidant activity and chlorophyll concentration. There is a direct correlation between the antioxidant activity and polyphenol concentration ( $r = 0.954$ ,  $p \leq 0.05$ ) based on analysis of different extracts.

Red wine has more antioxidant activity than white wine due to its polyphenol content (Pignatelli et al. 2006). Polyphenols are also known to prevent oxidative rancidity and thus enhance the shelf life of foods (Eriksson 1982). The addition of polyphenolic compounds to dairy products is known to cause an increase in the functional property of food, including health benefits and sensory properties (O'Connell and Fox 2001). Since partially purified extracts synergise the anti-oxidant property of foods when added, it is necessary to improve purification methods that keep this property intact.

## Conclusion

The data on composition indicates that summer harvest of stevia plants was the best for overall quality. Although the leaves are rich in minerals, the quantity of intake of leaves is not enough to meet requirement. The different qualities of extracts containing different concentrations of polyphenols and chlorophyll were able to elicit a similar or better sensory response from participants than the purified stevioside. The presence of plant pigments (largely chlorophyll) influenced the acceptability of extracts of leaf adversely. In contrast, the presence of polyphenols enhanced overall preference of the beverages. The overall quality of beverages is favourably affected by using partially purified extracts containing polyphenols. The high concentration of polyphenols may play a beneficial role when incorporated as part of sweetener since they contribute to anti-oxidant activity inherent in foods that they are added to. Hence purification processes for steviol glycosides may be rationalized for suitable processing to retain the polyphenols and remove chlorophylls.

## References

- Andrewes P, Johannecke LHC Busch, Teun D J, Anneke Groenewegen, Helene A (2003) Sensory properties of virgin olive oil polyphenols: Identification of Deacetoxy-ligstroside aglycon as a key contributor to pungency. *J Agric Food Chem* 51:1415–1420
- Anon (2008) Dr.Duke's phytochemical and ethnobotanical databases – [www.ars-grin.gov/duke](http://www.ars-grin.gov/duke). Accessed on 05.09.2008

- AOAC (1990) Official methods of analysis. 15th edn, Association of Official Analytical Chemists, Washington DC
- Arora S, Gawande H, Narendra K, Yarrakula S, Singh VP, Sharma V, Wadhwa BK, Tomer SK, Sharma GS (2008) Textural and structural properties of kalakand made with artificial sweeteners - saccharin, acesulfame-k, sucralose and aspartame. *J Food Sci Technol* 45:263–266
- Awika JM, Rooney LW, Wu X, Prior RL, Zevallos LC (2003) Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *J Agric Food Chem* 51:6657–6662
- Bonvehi JS, Coll FV (1997) Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder. *Food Chem* 60(3):365–370
- Brandle JE, Rosa N (1992) Heritability for yield, leaf-stem ratio and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*. *Can J Plant Sci* 72:1263–1266
- Chang SS, Cook JM (1983) Stability studies of stevioside and rebaudioside A in carbonated beverages. *J Agric Food Chem* 31:409–412
- Chetana R, Srinivasa PC, Yella Reddy S (2006) Effect of sugar substitutes on moisture sorption characteristics of *laddu*, an Indian traditional sweet. *J Food Sci Technol* 43:634–638
- Crammer B, Ikan R (1987) Progress in the chemistry and progress of the rebaudiosides. In: *Developments in Sweeteners – 3*, Grenby TH (ed), Elsevier Applied Science, London, p 45–64
- Eriksson CE (1982) Lipid oxidation catalysts and inhibitors in raw materials and processed foods. *Food Chem* 9:3–19
- Fernandez-Escobar R, Moreno R, Garcia-Creus M (1999) Seasonal changes of mineral nutrients in olive leaves during the alternate-bearing cycle. *Sci Hort* 82:25–45
- Fukunaga Y, Miyata T, Nakayasu N, Mizutani K, Kasai R, Tanaka O (1989) Enzymatic transglucosylation products of stevioside: Separation and sweetness evaluation. *Agric Biol Chem* 48:1831–1834
- Gary CH, Valerie Antoine, Margi Chan, Daiva Nevidomskyte, Martina Koniger (2006) Seasonal changes in photosynthesis, protein composition and mineral content in *Rhododendron* leaves. *Plant Sci* 170:314–325
- Geuns JMC (2003) Molecules of interest: Stevioside. *Phytochem* 64:913–921
- Harry BW Jr, Allerton R, Harry WD, Hewitt GF (1955) Stevioside I. The structure of the Glucose moieties. *J Org Chem* 20:875–883
- Huang YS, Guo AG, Qian Y, Chen LY, Gu HF (1995) Studies on the variation of steviosides content and selection of type R-A in *Stevia rebaudiana*. *J Plant Res Environ* 4:28–32
- Jeffrey SW, Humphrey GF (1975) New Spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae, and natural phytoplankton. *Biochem Physiol Pflanz* 167:191–194
- Kalpna R, Khan Md K (2008) Post-harvest management of stevia leaves: A review. *J Food Sci Technol* 45:391–397
- Kennelly EJ (2002) Sweet and non-sweet constituents of *Stevia rebaudiana* (Bertoni) Bertoni. In: *Stevia, the Genus Stevia. Medicinal and Aromatic Plants—Industrial Profiles*, Kinghorn AD (ed), Vol 19, Taylor and Francis, London and NY, p 68–85
- Kochkiyan V, Markosyan A, Abelyan L, Balayan A, Abelyan V (2006) Combined enzymatic modification of stevioside and rebaudioside A. *Appl Biochem Microbiol* 42(1):31–37
- Kohda H, Kasai R, Yamasaki K, Murakami K, Tanaka O (1976) New sweet diterpeneg Glucosides from *Stevia rebaudiana*. *Phytochem* 15:981–983
- Kolb N, Herrera JL, Ferreyra DJ, Uliana RF (2001) Analysis of sweet diterpene glycosides from *Stevia rebaudiana*: Improved HPLC Method. *J Agric Food Chem* 49 (10):4538–4541
- Kroyer G. Th (1999) The low calorie sweetener Stevioside: Stability and interaction with food ingredients. *Lebensm Wiss Technol* 32:509–512
- Lobov SV, Kasai R, Ohtani K, Tanaka O, Yamasaki K (1991) Enzymic production of sweet stevioside derivatives: Transglucosylation by glucosidases. *Agric Biol Chem* 55:2959–2965
- Masumi K, Emi T, Yohko M, Takahiro U (2008) The taste sensory evaluation of medicinal plants and Chinese medicines. *Int J Pharm* 351:36–44
- Metivier J, Viana AM (1979) The effect of long and short day length upon the growth of whole plants and the level of soluble proteins, sugars and stevioside in leaves of *Stevia rebaudiana* Bert. *J Exp Bot* 30:1211–1222
- Misnawi JS, Jamilah B, Nazamid S (2004) Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting. *Food Qual Prefer* 15:403–409
- O’Connell JE, Fox PF (2001) Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *Int Dairy J* 11:103–120
- Pignatelli P, Ghiselli A, Buchetti B, Carnevale R, Natella F, Germano G, Fimognari F, Di Santo S, Lenti L, Violi F (2006) Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis* 188:78–83
- Preys S, Mazerolles G, Courcoux P, Samson A, Fischer U, Hanafi M, Bertrand D, Cheynier V (2006) Relationship between polyphenol composition and some sensory properties in red wines using multiway analysis. *Anal Chim Acta* 563:126–136
- Ramirez RG, Gonzalez-Rodríguez H, Ramírez-Orduna R, Cerrillo-Soto MA, Juárez-Reyes AS (2006) Seasonal trends of macro and micro minerals in 10 browse species that grow in northeastern Mexico. *Anim Feed Sci Technol* 128:155–164
- Savita SM, Sheela K, Sharan Sunanda, Shankar AG, Parama Ramakrishna (2004) *Stevia rebaudiana* – A functional component for food industry. *J Hum Ecol* 15(4):261–264
- Singh G, Asha Kawatra, Sehgal S (2001) Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Food Hum Nutr* 56:359–364
- Starrat AN, Kirby CW, Pocs R, Brandle JE (2002) Rebaudioside F, a diterpene glycoside from *Stevia rebaudiana*. *Phytochem* 59:367–370
- Tadhani MB, Patel VH, Rema Subhash (2007) In vitro antioxidant activity of *Stevia rebaudiana* leaves and callus. *J Food Comp Anal* 20(3–4):323–329
- Tadhani M Subhash R (2006) Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. *J Med Sci* 6(3):321–326
- Virendra VP, Kalpagam P (2008) Assessment of stevia (*Stevia rebaudiana*)-natural sweetener: A review. *J Food Sci Technol* 45:467–473
- Yuehua von Fircks, Tom Ericsson, Lisa Sennerby-Forsse (2001) Seasonal variation of macronutrients in leaves, stems and roots of *Salix dasyclados* Wimm. grown at two nutrient levels. *Bio-mass Bioenergy* 21:321–334