International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 12, Issue 14, 2020, pp.-10067-10070.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

MASKING THE BITTERNESS OF *STEVIA REBAUDIANA* BER. EXTRACT USING NATURAL ADJUVANT AND THEIR EFFECT ON RECOVERY OF STEVIOSIDES IN FINAL EXTRACT

SINGH K., TIWARI G.*, NAYAK P.S. AND UPADHYAY A.
Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, Madhya Pradesh, India
*Corresponding Author: Email - drgyanendratiwari@gmail.com

Received: July 01, 2020; Revised: July 26, 2020; Accepted: July 27, 2020; Published: July 30, 2020

Abstract: Stevia is one the most popular natural low calorie sweeteners used extensively in the food and pharmaceutical industries. The leaves of herb have been used to substitute the sugar to counteract the bitter taste of plant based medicine and for treatment of diabetic person. It is 10-15 times sweeter than sucrose, but its metallic bitter aftertaste after use limits its overall acceptability for general consumers. In order to reduce or mask the bitter aftertaste of stevia extract ten adjuvants were used before hot water extraction (first method) and after hot water extraction (second method) and compared with control (hot water extraction without adjuvants).Evaluation of masking effect was done using organoleptic/sensory test along with quantification of sweet principle (stevioside content) in extracts using two methods. Further, seasonal variation in stevioside content in leaves of along with proximate analysis of stevia and adjuvants were done. On the basis of sensory evaluation, the second method was found more effective. In this method stevia leaves were extracted individually and adjuvants were mixed while leaf extract was hot and kept it for 24hrs before filtration. After that it took for sensory evaluation test. On the other hand in first method stevia leaves mixed with adjuvants (10) and extracted and filtered before sensory evaluation test. In both the methods sweet basil, activated charcoal, wood charcoal, lemon grass and mint leaves were found better in overall acceptability in organoleptic test. Maximum stevioside recovery in extracts was found in sole stevia extract. Mixing adjuvants reduced steviosides (the sweet principle) content in final extract. Using sweet basil, activated charcoal, wood charcoal, lemon grass, mint leaves improved sensory properties of extract without much sacrifice in stevioside content. Minimum stevioside recovery was found in extract with wood ash, tulsi leaves, mint and coffee using first method of extraction. Minimum stevioside recovery was found using second method with Tulsi. December picking of stevia leaves gave maximum stevioside in their leaves than August and April picked one.

Keywords: *Stevia rebaudiana, Stevia extract, Stevioside, Rebaudiosides and organoleptic test*

Citation: Singh K., *et al*., (2020) Masking the Bitterness of *Stevia rebaudiana* Ber. Extract Using Natural Adjuvant and their Effect on Recovery of Steviosides in Final Extract. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 12, Issue 14, pp.- 10067-10070.

Introduction

Stevia rebaudiana Bert. (Family- Asteraceae), a natural sweetener with low glycemic index and calorie has recently found widespread use in the food and pharmaceutical industries. Shade dried leaves of Stevia are 10 to 15 times sweeter than sucrose. Glycemic index of sweetening compounds of this plant is zero with no caloric value [1]. The worldwide demand for natural sweeteners is increasing because of consumer awareness about harmful effects of artificial sweeteners. Increased consumption of food products enriched with sugars and artificial sweeteners has resulted in the development of various chronic diseases like obesity and diabetes. Stevia glycosides (200 times sweeter than sugar) from Stevia offer a solution to prevent of complex diabetic problems and obesity in modern mankind. The *Stevia rebaudiana* contains biomolecules mainly of labdanum diterpenes, triterpenes, stigma sterol, tannins, volatile oils and eight diterpenenic glycosidea (stevioside, steviobioside, duleoside and rebaudiosides A, B, C, D and E). Sweet flavour of stevia is due to the presence of glycosides mainly stevioside and rebaudioside A (the sum of both make up from 5 to 10% of drug). The most abundant substance is stevioside and rebaudioside A. Out of the stevia glycosides rebaudiosides A is sweeter and the most stable and is less bitter than stevioside [2]. A post digestive bitter taste is associated to presence of steviosides in high levels and causes certain rejection of Stevia by consumers. Commercial interest and its use by the food and beverage industry have put the species in a prominent position in the international agriculture scene, as described due to the social demand for healthy and natural foods. The highest yields of stevioside and rebaudioside A is found in leaves.

The extract is prepared by dynamic maceration, ultrasound and microwave extraction using hot water and ethanol [3]. In human nutrition and food technology application, dried leaves are commonly used with the sweetness is about 10-15 times sweeter than sugar, but with the reduced caloric value of 2.7kcal/g [4,5]. Associated health hazards due to increased sucrose consumption have led to encourage use of low calorie natural sweetners [6]. Stevia is used as a better substitute for sucrose to diabetes mellitus, obesity, high blood pressure, renal protective effect, and promotion of oral health [7]. Aside from the sweet taste, Stevia is popular because of its nutritional and chemical composition that is characterized by a high content of amino acids, minerals, and photochemical, especially polyphenols that contributes to a significant antioxidant activity [8] as compared to the other sweetener (sucrose and artificial sweeteners). stevia. In addition, Stevia leaf has excellent sensory and functional properties than other sweeteners [9, 10].

The major constraint in expansion and acceptability of stevia sweetener is its slightly bitter after taste which limits its expansion as substitute of sugar. Reducing bitter aftertaste to stevia extract by chemical method will make the final produce less acceptable due to the chance of residual effect of applied chemical. Use of physical method along with natural edible sources to mask the bitterness may be more useful and acceptable. This may prove to be new vista in commercialization of product through this technology. Hence present study was undertaken to mask/ reduce the bitterness of stevia extract using various modulators of natural sources.

Materials and Methods

The present investigation was carried out in the Laboratory of Department of Plant Physiology, College of Agriculture, JNKVV, Jabalpur (MP) during the year 2018-19 and 2019-20. Two methods of hot water extraction were used along with ten adjuvants as per treatments and compared with control (extract without adjuvant) replicated thrice.

Treatments

1.Hot water extract with in leaves (control)
2.Hot water extract treated with activated charcoal
3.Hot water extract treated with wood charcoal
4.Hot water extract treated with wood ash
5.Hot water extract treated with coffee seeds
6.Hot water extract treated with sweet basil leaves
7.Hot water extract treated with tulsi leaves
8.Hot water extract treated with lemon grass extract
9.Hot water extract treated with palmarosa grass extract
10.Hot water extract treated with Mushroom (Oyster)
11.Hot water extract with mint leaves

Experimental Design: Randomized Complete Block Design (RCBD)

Hot water extraction of stevia with as per treatment

A. First method

In this method dry Stevia leaves sample (5gm) along with adjuvant as per treatment (2.5gm) was boiled for 8 minutes in 50ml of tap water. Decant the extract and boiled till extract remained to 30ml for each treatment. This extract was used for organoleptic test separately.

B. Second method

In this method dry Stevia leaves sample (5 gm) boil in beaker mixed with 50ml tap water for 8minutes. Decant the extract (by 50 ml) and mix adjuvant as per treatment (2.5 gm) and sealed with aluminum foil. Put this for 24 hours. After that decant the extract again to 30 ml. this extract was used for organoleptic test.

Organoleptic evalution

The organoleptic evolution of freshly prepared stevia extract was done by the panel of 5 judges to assess the acceptability of the product based on the various sensory attributes like colour and appearance, flavor, taste and overall acceptability. The evaluation was done on a nine point hedonic scale [11].

Like extremely	: 9
Like very much	: 8
Like moderately	: 7
Like slightly	: 6
Neither likes nor dislike	: 5
Dislike slightly	: 4
Dislike moderately	: 3
Dislike very much	: 2
Dislike extremely	: 1

The results were expressed as mean scores by taking average of three replications.

Quantification of glycosides Quantification of Stevia glycosides by HPTLC was done for each treatment as per method used previously [12].

Statistical analysis was done using method given by Fisher [13].

Results and Discussion

The difference in SGs composition from the extracts may have an impact on sensory properties that might be relevant for industrial application [14]. The standarsization of processing method for masking bitterness in stevia extract using various adjuvants was the prime objective of the present study. Selection of best performed combination was done by the expert panel of judges. The selection of stevia extract with adjuvant was done on the basis of sensory evaluation and chemical analysis.

The results have been explained with the help of reported values of various parameters given by different researchers as under.

Organoleptic /sensory evaluation of Stevia extract

Organoleptic evaluation of stevia extract after different treatments using two different methods gave different mouth feel, flavour, intensity of bitterness and overall acceptability of stevia [Table-1].

Sweetness/ Mouth feel of extract

Stevia extract obtained of pre-mixed stevia leaves with adjuvants (activated charcoal, wood charcoal, wood ash, coffee powder, sweet basil leaves, tulsi leaves, lemon grass, palmarosa grass, mushroom and mint) differentially mitigated or masked the metallic bitter aftertaste as compared to control, in which no adjuvant was added. Results of sweetness score of final extract as per treatments by organoleptic taste revealed that stevia extract without any adjuvant found sweetest mouth feel. Similar sweetness mouth feel was sensed in treatments having sweet basil leaves, activated charcoal, lemon grass leaves, mint leaves, wood charcoal and palmarosa grass leaves. This may be due to the reason that above adjuvants somewhat mask inherent bitter aftertaste of stevia and thereby feel more sweet in the extract. Extract obtained when pre-mixed with coffee, tulsi and mushroom felt minimum sweetness as they may absorb sweetness on one hand and increased bitterness on the other hand. Leaf extract obtained without adding any adjuvant was found most sweet as added adjuvant could hinder or absorb sweetness to some extent.

The results were similar when second method of extraction was applied. In this method adjuvants were mixed after hot extraction of only sole stevia leaves immediately while hot and kept for 24 hrs and then filtered the extract separately for sensory evaluation. Results felt somewhat similar sweetness as that of first method. In this method also maximum sweetness was felt in the extract without any adjuvant.

Flavour of stevia extract

Using first method of extraction best flavour of stevia extract was found when mixed with sweet basil leaves, mint leaves, activated charcoal, wood charcoal, lemon grass leaves, tulsi leaves and without any adjuvant as per sensory evaluation. Poor sensory flavour was found in extract with coffee, mushroom and wood ash. These results might be due to the fact that sweet basil, mint and other adjuvants imparted addition in flavor and masked the unpleasant flavor of stevia. Flavour of coffee and mushroom is disagreeable in extract which further negatively affected the flavor of final extract.

Flavour of stevia extract in various treatments gave slightly different results under second method of making extract. The best flavour of combined stevia extract was found when sweet basil was used as adjuvant. The similar good flavour was noted with extract using tulsi, lemon grass, palmarosa grass, and mint. The difference in results from first method may possibly be due to the fact that when adjuvants mixed with stevia before extraction will evaporate the aromatic oil from extract due to heat. This will give inferior aroma than the second method. In the second method, adjuvants were added after hot extraction of stevia leaves. Hence aroma of adjuvants will intact in this method of extraction which finally gave better flavour of stevia extract as compared to stevia extract without adjuvants. Inferior flavor of stevia extract using other adjuvants like wood ash, coffee and others was due to inferior flavour properties of adjuvants.

Intensity of bitterness of stevia

Minimum bitterness in extract using first method was found with activated charcoal, lemon grass, wood charcoal and sweet basil leaves. Activated and wood charcoal may be active in absorbing or masking the bitterness of stevia when mixed with stevia leaves before extraction. This may be the possible reason of reduction of bitterness feel in stevia extract. On the other hand, coffee and tulsi as adjuvant gave the most bitter taste in stevia extract. It is obvious that coffee is itself bitter and tulsi full of flavonoid contents in its leaves which felt bitter in extract.

Using second method of extraction, the minimum bitterness was felt in extract using sweet basil, wood charcoal, activated charcoal and others and in extract without any adjuvant. However maximum bitterness was found with coffee and wood ash.

Table-1 *Effect of various treatments using first and second method on sweetness / mouth feel, flovour and intensity of bitterness in Stevia extracts*

Treatments	Sweetness		Flavour		Intensity of bitterness		Over all acceptability	
	I	II	I	II	I	II	I	II
T_1	8.33	8.33	6.00	6.00	5.00	6.33	6.44	6.88
T_2	7.66	7.66	7.00	6.33	7.66	5.33	7.44	6.44
T_3	6.66	7.00	7.00	5.66	6.66	6.33	6.77	6.33
T_4	6.00	5.66	5.00	5.33	4.66	4.33	5.22	5.08
T_5	3.00	2.66	4.00	4.66	2.33	5.00	3.11	4.10
T_6	8.33	8.33	7.66	8.00	6.66	6.33	7.55	7.55
T_7	3.66	4.66	6.66	7.66	4.00	5.33	4.77	5.88
T_8	7.33	7.66	7.00	7.33	7.00	5.66	7.11	6.88
T_9	6.66	7.33	6.00	7.00	4.33	5.66	5.66	6.99
T_{10}	4.33	4.00	4.33	5.66	5.66	5.33	4.77	4.99
T_{11}	7.33	6.66	7.33	7.66	4.66	6.33	6.44	6.88
SEm ±	0.7471	0.6424	0.5932	0.4425	0.5819	0.6727	0.5497	0.5711
CD (at 5%)	2.3021	1.9759	1.8277	1.3633	1.7931	1.9845	1.6217	1.6848

Overall acceptability of stevia extract

Under first method of extraction the most acceptable extracts were found when extracted with sweet basil leaves, activated charcoal, lemon grass, wood charcoal, mint leaves along with extract without adjuvant. The stevia extracted with coffee was found again most disagreeable in sensory/organoleptic test.

Using second method of extraction, the best overall acceptability of extract was found again when adjuvants like sweet basil leaves, palmarosa grass, lemon grass, mint, activated charcoal, wood charcoal were used. These were similarly effective with stevia extract without any added adjuvants. Katja *et al.* [15] also done sensory evaluation taste for stevia extract and found the relative sweetness of stevia extract compared to sucrose of different low concentration.

Quantification of stevioside (%) in final extract

Extract obtained using first method

HPTLC analysis revealed variation in steviose content (%) in each final extract due to different adjuvant using both methods of extraction separately [Table-2]. Stevioside (the main sweetness component) was found maximum in extract when no adjuvant mixed before the extraction of stevia leaves. Use of adjuvant before extraction of stevia leaves differentially reduced the stevioside content in final extract. Some adjuvant like wood charcoal, sweet basil leaves, lemon grass leaves, palmarosa grass leaves and activated charcoal were effective in reducing stevioside content (%) in final extract in descending order. On the other hand, use of wood ash, mint leaves, tulsi leaves, coffee and mushroom drastically reduced sweet principle (stevioside) in final extract. Mixing of adjuvants reduced stevioside in extract depending on adjuvants. This result clearly indicates the possibility of adsorbing of glycosides by various adjuvants. These results also confirm the organoleptic evaluation under study, in which sweet basil, lemon grass, activated charcoal and wood charcoal clearly found effective in masking bitterness with moderate reduction in sweetness and thereby increase in pleasant flavour. Sweet basil was very effective in producing good flavor and overall acceptability. However, sweetness alone is always found maximum with plain stevia extract.

Table-2 *Effect of various treatments using first and second method of extraction on stevioside content (%) in Stevia extract*

Treatment	Stevioside content (%)	
	First Method	Second Method
T_1	1.29	1.29
T_2	0.19	0.85
T_3	0.57	1.16
T_4	0.02	0.83
T_5	0.12	0.71
T_6	0.23	0.91
T_7	0.05	0.00
T_8	0.21	0.85
T_9	0.20	0.83
T_{10}	0.15	0.57
T_{11}	0.024	0.98
SEm ±	0.0142	0.0263
CD (at 5%)	0.042	0.0782

Extract obtained using second method

Entirely different results were found regarding recovery of steviooside content (%) in extract using second method than that of extracts using first method for all the treatments [Table-2]. This method was found very effective in maximum recovery of stevioside content. Maximum stevioside was recovered in stevia extract when no adjuvant was mixed. Wood charcoal mixing in hot stevia extract kept for 24 hrs was very effective among all adjuvants as it resulted in maximum stevioside recovery (1.16%) against steviosides (1.29 %) in plain leaf extract. Mint, activated charcoal, wood ash, sweet basil leaves, lemon grass and palmarosa grass leaves were also effective in recovering maximum stevioside in the extract. Adding tulsi as adjuvants drastically reduced the stevioside to the extent that it could not be detected during HPTLC estimation.

These results also explained the sensory evaluation results as sweet basil, activated charcoal, wood charcoal, lemon grass, palmarosa grass and mint leaves were outstanding in overall acceptability of extract. They partially effective in masking the bitterness of stevia extract without much compromising in stevioside recovery from stevia leaves. Although adding adjuvants always reduce the stevioside recovery but at the same time mask the bitterness by some adjuvants resulted in better overall acceptability. This justifies the results of organoleptic test with results of quantified stevioside using both the methods under study.

Seasonal variation in stevia content in stevia crop

Maximum stevioside content in leaves was found in winter picked stevia (Picked in December) under Jabalpur conditions, while the minimum stevioside content was found in rainy season picked one (August) while moderate stevioside in summer (April) picked stevia [Table-3]. These results may be due to the difference in physiological and developmental stage, because in late winter stevia starts flowering and at this stage maximum stevioside was found. It is established fact applicable for all medicinal plants where active ingredients were generally at their maximum at the onset of flowering of crop. These active ingredients gradually reduced after start of reproductive phase in their life-cycle.

Table-3 *Stevioside content (%) of various seasons in stevia crop*

SN	Season of picking leaves	Stevioside content (%)
1	Apr-19	1.29
2	Aug-18	0.75
3	Dec-18	0.59

Conclusion

It is concluded from the present study that adding adjuvants like sweet basil leaves, activated charcoal, lemon grass, palmarosa grass, wood charcoal and mint leaves are effective in masking the bitter aftertaste of stevia to some extent. But adding such adjuvants resulted in reducing in sweet principle of stevia (stevioside content).:

Application of research: Adjuvants like activated charcoal, wood charcoal, sweet basil leaves, lemon grass and palmaroasa grass can be used improve taste, flavor, reducing bitterness and overall acceptability with much sacrificing the content of stevioside, while mint leaves enhance sensory acceptability but drastically reduce sweet principle. Stevia picked in December (at the onset of flowering) had highest stevioside content.

Research Category: Plant Physiology

Acknowledgement / Funding: Authors are thankful to Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, Madhya Pradesh, India

****Research Guide or Chairperson of research: Dr Gyanendra Tiwari**
University: Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, India
Research project name or number: PhD Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Plant Physiology, College of Agriculture, Jabalpur, 482004, Madhya Pradesh, India

Cultivar / Variety / Breed name: *Stevia rebaudiana* Ber.

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Puri M., Sharma D., Tiwari A.K. (2011) *Biotechnology Advances*, 29, 781-791.
[2] Crammer B. and Ikan R. (1986) *Chemistry in Britain*, 22(10), 915-917.
[3] Mohammed Abdalbasit A., Gasmalla Ruijin, Yang Abubakr, Musa Xiao. Hua, Fayin, Ye. (2017) *Arabian Journal of Chemistry*, 10(5), 726-731.
[4] Kaubaa M., Roselolo S.E., Sic Zlabur J., Jambrak R.A., Brncis M., Grimi N. and Barba F.J. (2015) *Journal of Agricultural and Food Chemistry*, 63 (31), 6835-6846.
[5] Savita S.M., Sheela K., Sunanda S., Shankar A.G. and Ramakrishnan P. (2010) *Journal of Human Ecology*, 15(4), 261-264.
[6] Lemus M.R., Vega G.A., Zura B.L. and Ah-Hen K. (2012) *Food Chemistry*, 132 (3),1121-1132.
[7] Abau-Arab A.E., Abau-Arab A.A. and Abu Salim M.F. (2010) *African Journal of Food Science*, 4, 269-281.
[8] Zlabur J.S., Voca S., Cevi N., Jezek D. and Brncic M. (2013) *Journal of Agriculturae Conspectus Scientificus*, 78 (1), 25-30.
[9] Goyal S.K., Goyal R.K. and Samsher (2010) *International Journal of Food Sciences and Nutrition,* 61(1), 1-10.
[10] Reis F., Alcaire F., Deliza R. and Ares G. (2017) *Quality and Preference*, 111, 579-589.
[11] Amerin M.A., Pongborn R.M. and Roessler E.B. (1965) *Principle of sensory evolution of food. Academic Press, New York.*
[12] Karishma C., Ennus T., Tamboli, Singh M. and Ahmad S. (2012) *J Pharm Bioallied Sci*, 4(4), 276-281.
[13] Fisher R.A. (1992) *Statistical methods for research workers. In, Kotz S., Johnson N. L. (eds) Breakthroughs in Statistics. Springer Series in Statistics (perspectives in Statistics). Springer, New York, NY*
[14] Epinoza M.I., Vincken J.P., Sauders M., Castro C., Stieger M. and Agrosin E. (2014) *Journals of Agricultural and Food Chemistry*, 62(49), 11797-11804.
[15] Katja O.B.S., Jakob P.L., Katharina V.R. and Krammer G. (2010) Proceeding of 4th stevia symposium 2010) *Publisher, Euprint, heverlee, Belgium, Ed (s), J.M.C. Genus*, 121-134.