Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 48 (2012) 9538-9541

ISSN: 2229-712X

Analysis of nutritive value and shelf life of the products prepared from stevia powder

Angeleena D. McCarty[1], Alok Milton Lal[2], S.D. McCarty[3] and Satendra Singh[1,*]
[1]Department of Computational Biology & Bioinformatics, JSBB, SHIATS, Allahabad-211007.
[2]Department of Biochemistry & Bioprocess Technology, JSBB, SHIATS, Allahabad-211007.
[3] Krishi Vigyan Kendra, Allahabad-211007.

ARTICLE INFO

Article history:
Received: 2 May 2012;
Received in revised form:
28 June 2012;
Accepted: 17 July 2012;

Keywords
Stevia,
Nutritive value and
Shelf.

ABSTRACT
Stevia is a natural sweetener. The herb can aid in weight loss by reducing appetite and can be used to suppress tobacco and alcohol cravings. Stevia leaf also contains various vitamins and minerals including vitamins A and C, zinc, , magnesium and iron. Stevia leaves contain numerous natural nutrients important to regulating blood sugar, including chromium, magnesium, manganese, potassium, selenium, zinc, and vitamin B3 (Niacin), which the body converts into niacin amide and nicotinic acid. In the present study the nutritive value and shelf life of the products (Flavoured Milk and Custard Pudding) prepared from stevia powder was estimated and analyzed. In has been found that both products have very high nutritive value and self life indicate that they are also stable preparations under normal domestic storage conditions.

Introduction

Stevia is a small perennial shrub from South America, now grown in a number of locations. The natural sweetener derived from stevia leaves is 30-100 times sweeter than sugar [1]. There is no after-taste and it is safe and non-toxic according to Japanese research. Stevia is high in chromium, (which helps to stabilize blood sugar levels), manganese, potassium, selenium, silicon, sodium and vitamin A. It also contains iron, niacin, phosphorus, riboflavin, thiamine, vitamin C, and zinc. The best quality stevia leaves are usually imported from South America and Mexico, and are about 12 percent to 13 percent stevioside, the active ingredient. The poorest quality, but most ample supply, is currently coming from China, where the leaves contain only about five percent to six percent stevioside. Stevioside is not carcinogenic [2] . The absorption and metabolism have been studied in human volunteers. Stevioside is not absorbed by the gut. Some stevioside is metabolized to steviol by colonic bacteria and absorbed, but this is quickly metabolized to steviol glucuronide, and excreted in the urine.

It's easy to grow, wonderful as a sweetener, contains medicinal properties, is non-caloric, safe to cook with, and has great potential in agriculture [3]. The main use was as a sweetener, particularly in their green tea, known as maté. It was also used in medicine or as a snack. Stevia's leaf is estimated to be 150 to 300 times sweeter than refined sugar [4].

There are three distinct traditions of stevia use. The first is for flavor enhancement; the second is as an herbal tea. The third is medicinal (Hypoglycemic action, Cardiovascular Action, Hypertension an Dental Health). Chromium is necessary for insulin production and the transport of glucose into the body's cells [5]. It improves glucose tolerance in diabetics with impaired glucose tolerance and has been shown to improve the condition referred to as insulin resistance. Magnesium is necessary for every major biological process, including metabolism of glucose and the production of cellular energy. Manganese appears to be involved in lowering blood glucose levels, even in people who are not responsive to insulin. Potassium deficiency increases insulin resistance. Steviosides and Rebaudiosides are the principal constituents of diterpene glucosides with differing sugar molecules attached, as found in the leaves of the stevia plant. Steviosides is a natural sweetener extracted from leaves of Stevia rebaudiana (Bertoni) Bertoni [6].

Materials and methods

Chemical analysis of the products (estimation of nutritive value of the products)

The carbohydrate, protein, crude fibre fat moisture iron calcium, ash and energy of the products were calculated by using the values obtained of stevia powder through biochemical analysis and on the basis of values given in the food table [7] .

Microbial Analysis to study the shelf life of the products

For coliform test McConkey's broth and for Total plate count PDA (potato dextrose agar) &NA (nutrient agar) was prepared and was observed.

Serial dilution

In this technique the sample was serially diluted in sterile (isotonic) saline solution and the basis of obtaining isolated colonies with this technique is the reduction in the number of bacteria per unit volume in the inoculums by dilution [8].

Components required for Ringer Solution

NaCl	9 gm
KCL	0.42 gm
$CaCl_2$	0.24gm
$NaHCo_3$	0.2 gm
Distilled water	1000 ml

Preparation of Ringer Solution

Take 1000 ml clean and dry conical flask. Weigh 9 gm of NaCl, 0.42 gm of KCL, 0.24gm of $CaCl_2$ and 0.2 gm of $NaHCo_3$ pour them into clean and dry conical flask. Measure 1000 ml of Distilled water in a measuring cylinder and pour down distilled water to make up the volume 1000 ml shake it well to dissolve

Tele: +91 9565448367
E-mail addresses: Satendra.singh@shiats.edu.in

all the components [9].

Serial dilution

Take 100 ml x (5) clean and dry Conical flask add 99 ml of Ringer Solution followed by putting cotton plugs on the top along with 15 test tubes containing 9 ml of Ringer Solution each plugged with cotton (3 Test tubes for a sample) Autoclave at 121 psi for 45 min. Add 1 gm of the sample in a conical flask followed by marking the sample code in laminar flow to make up the stalk solution for Serial dilution. Vortex the Stalk Solution and pour 1 ml of the Stalk solution in test

Tube followed by marking (10^{-1}) and Sample Code. Vortex the tube and pour 1 ml from the first tube into the second followed by marking (10^{-2}) and the sample code. Vortexes the test tube second pour 1 ml of the solution into the third test tube followed by marking (10^{-3}) and the Sample code. The process is been followed up for the rest of the samples in sterilized condition (laminar flow) [10].

Test tube showing serial dilution

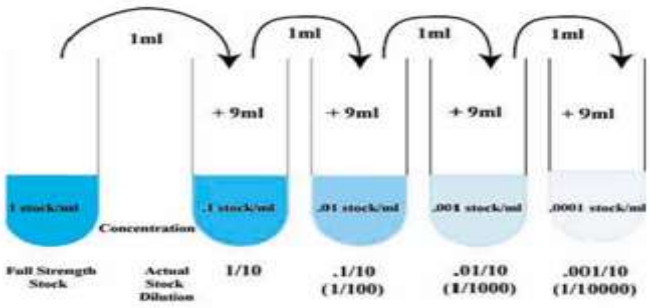

Figure 1. Serial dilution

Mac'Conkey Broth

Components required for Mac'Conkey Broth

Peptone	20 gm
Lactose	10 gm
NaCl	5 gm
Bile Salt	5 gm
Neutral red	10 ml (sol 1 % aques solution)
Distilled Water	1000 ml
pH -7.5	

Preparation of the Mac'Conkey Broth

Take 1000 ml clean and dry conical flask. Weigh 5 gm of NaCl, 10 gm of Lactose, 20 gm of Peptone and 5 gm of Bile salt along with 10 ml of Neutral red pour them into clean and dry conical flask.

Measure 1000 ml of Distilled water in a measuring cylinder and pour down distilled water to make up the volume 1000 ml shake it well to dissolve all the components and maintain the pH. Plugged with cotton plugs and autoclaved at 121 psi for 45 min.

Mac'Conkeys Broth microbial Analysis (Coliform Test)

Take 31 Test tubes with inverted Durhum tubes plugged with cotton plugs and autoclave along with the Broth at 121 psi for 45 min. Pour 5 ml of the Broth in the test tubes which contains inverted durhum tubes. 1 ml of the Sample is been taken from the Serial Dilution test tubes tagged with the sample code and the Dilution Number and poured in the broth containing tubes along with inverted Durhum tubes [11] .

There Should be 2 set of Microcial analysis for each dilution and a control which must contains broth along with inverted Durhum tubes Except the sample. Later all the tubes were plugged with the cotton plugs in an sterilized condition and incubated in an incubator for 48 hr at 35°C.

Potato Dextrose Agar

Components required for Potato Dextrose Agar

Unpeeled potato	150 gm
Dextrose	10 gm
Agar powder	10 gm
Distilled Water	500 ml
pH -5.5	

Preparation of the Potato Dextrose Agar

Take 500 ml clean and dry conical flask. Weigh 150 gm of unpeeled potato, 10 gm of Dextrose, maintain the pH and add 10 gm of Agar powder pour them into clean and dry conical flask. Measure 500 ml of Distilled water in a measuring cylinder and pour down distilled water to make up the volume 500 ml shake it well to dissolve all the components. Plugged with cotton plugs and autoclaved at 121 psi for 45 min.

Potato dextrose Agar Analysis

Take 31 clean and dry Petri plates pre autoclaved at 121 psi for 15 min. Pour 1 ml of the sample from the dilution test tubes which are tagged with the sample code and the dilution number on to the plates.9 ml of the media is pored over it and mixed gently. There Should be 2 set of Microbial analysis for each dilution and a control which must contain media Except the sample.

Plates are left to solidify with the agar and later Incubated inverted for 2-3 days at 25°C. Analysis is performed in sterilized conditions [12].

Nutrient Agar Analysis

Components required for Nutrient Agar

Beef Extract	1.5 gm
Peptone	2.5 gm
Sodium Chloride	2.5 gm
Agar	7.5 gm
Distilled Water	500 ml
pH -7.2	

Preparation of the Nutrient Agar

Take 500 ml clean and dry conical flask. Weigh 1.5 gm of Beef Extract, 2.5 gm of Peptone, 2.5 gm of Sodium chloride maintain the pH and add 7.5 gm of Agar powder pour them into clean and dry conical flask. Measure 500 ml of Distilled water in a measuring cylinder and pour down distilled water to make up the volume 500 ml shake it well to dissolve all the components. Plugged with cotton plugs and autoclaved at 121 psi for 45 min.

Nutrient Agar Analysis

Take 31 clean and dry petri plates pre autoclaved at 121 psi for 15 min. Pour 1 ml of the sample from the dilution test tubes which are tagged with the sample code and the dilution number on to the plates.9 ml of the media is pored over it and mixed gently. There Should be 2 set of Microcial analysis for each dilution and a control which must contains media Except the sample. Plates are left to solidify with the agar and later Incubated inverted for 48 hr at 30°C. Analysis is performed in sterilized conditions [13].

Results and Discussion

In the present study the nutritive value of flavored milk and custard pudding was analyzed

Nutritive value of the products

Energy-Energy was observed higher in smoothie, in all the three treatments T_1,T_2&T_3 324 kcal followed by flavoured milk with172.59 kcal/100ml,custard pudding with 169.07 kcal/100gm.

Fat –Highest fat value was found 92.4gm/100ml in custard pudding and followed by flavoured milk with 12.4gm/100ml.

Carbohydrates Among the products custard pudding, values 24.66gm/100ml,where as flavoured milk was lowest with the value 9.61gm/100ml.

Moisture- Moisture percentage was found to be for flavoured milk (114%) and lowest percentage was observed in custard pudding (99.0%).

Calcium- Calcium content in flavoured milk was observed and it was found that among all the products smoothie had higher calcium content 430.5mg/100ml,(T_1),430.60mg/100ml(T_2) 430.61mg/100ml.(T_3) and (T_0)Control group was 289.5gm/100ml followed by strawberry shrikhand 421.0 mg/100ml,(T_1),421.1mg/100ml (T_2) and 421.11mg/100ml.(T_3) and (T_0)Control group was 234.0gm/100ml, flavoured milk 189.9 mg/100ml,(T_1),190.0mg/100ml (T_2) and 190.3 mg/100ml.(T_3), lowest calcium was observed in custard pudding ,gajer halwa and Phirini respectively.

Vitamin A- custard pudding1934.6 flavoured milk 203.1 I.U.

Vitamin C-Lowest Vitamin was found in flavoured milk and custard pudding 1.5 /100ml T_1,T_2 &T_3,2.5 /100ml in T_0. **(**T_1,T_2 &T_3 < T_0)

Iron- Flavored milk (4.9 -5.0 gm/100 ml) lowest value was observed in custard pudding (0.58 -0.68gm/100ml).

b. shelf life of the products prepared from stevia powder – A)Test Result for the **Mac'Conkey Analysis** (coliform test)

Table 1

	DILUTION 10^1			DILUTION 10^2			DILUTION10^3		
Sample	Colour change to yellow		Growth	Colour change to yellow		Growth	Colour change to yellow		Growth
	A	B		A	B		A	B	
Flavored Milk	+	+	Excellent	+	+	Excellent	+	+	Excellent
Custard pudding	-	-	Fair	-	-	Fair	-	-	Fair

Table 2.

	DILUTION 10^1		DILUTION 10^2		DILUTION10^3	
Sample	Gas formation		Gas formation		Gas formation	
	A	B	A	B	A	B
Flavored Milk	+	+	+	+	-	-
Custard pudding	+	+	+	+	-	-

As it was observed in table 1 (colour changed to yellow) all the three dilution respectively 10^1, 10^2 &10^3 (coliform test) was observed and it was found that in dilution 10^1 excellent growth of coliform was in flavored milk.

Table 2 showed positive gas formation In dilution 10^2 flavoured milk and custard pudding had gas formation and dilution10^3 products were having no gas formation.

B) (Total plate count) (a) Potato Dextrose Agar Analysis at 10^1 Dilution, 10^2Dilution & at 10^3Dilution

	10^1Dilution		**10^2Dilution**		**10^3Dilution**	
Sample	Colony Count in 1 quarter of plate	Total Colony on Petri Plate	Colony Count in 1 quarter of plate	Total Colony on Petri Plate	Colony Count in 1 quarter of plate	Total Colony on Petri Plate
Flavored Milk	31	126 x 10^1	16	62 x 10^2	10	40 x 10^3
Custard pudding	33	135 x 10^1	16	63 x 10^2	13	50 x 10^3

(b) Nutrient Agar Analysis at 10^1Dilution,10^2Dilution & at 10^3Dilution

	10^1Dilution		**10^2Dilution**		**10^3Dilution**	
Sample	Colony Count in 1 quarter of plate	Total Colony on Petri Plate	Colony Count in 1 quarter of plate	Total Colony on Petri Plate	Colony Count in 1 quarter of plate	Total Colony on Petri Plate
Flavored Milk	46	183 x 10^1	38	153 x 10^2	31	124 x 10^3
Custard pudding	45	179 x 10^1	36	144 x 10^2	27	108 x 10^3

Potato dextrose agar analysis for total plate count was carried out and was observed (Table a) that in 10^1 Dilution maximum Total Colony on Petri Plate in custard pudding(135 x 10^1/ml), flavoured milk(126 x 10^1 /ml) least growth was in flavoured milk(62 x 10^2/ml) in 10^3Dilution custard pudding had highest growth was 50x10^3/ml (Table b) The Nutrient Agar Analysis at 10^1Dilution was calculated (Total Colony on Petri Plate) and custard (108 x 10^3/ml)and Strawberry shrikhand(114 x 10^3/ml). The shelf life of the products were close to the values of Savita [14-15].

Conclusion

Based on this study it can be concluded that Stevia products has High Stability & Unique Properties. s a versatile new food ingredient it functions effectively as a sweetener and flavor enhancer. Stevia is Non-fermenting so improves shelf life of dairy products. Perfect for storage as it has high shelf-life properties and can adapt to normal warehouse conditions.

Acknowledgement

The authors are grateful to the SHIATS, Allahabad for providing the facilities and support to complete the present research work.

References

1. **Akashi, H. & Yokoyama, Y**. "Dried-leaf extracts of stevia. Toxicological test." Shokihin Kokyo, Tokyo, 18(20), 34-43, 1975.

2. Alvares, M., et.al, Abstract Pap., Semin. Bras. Stevia Rebaudiana Bertoni 1st, 1981, p. XIII.I.

3. **Aritajat S., Kaweewat K., Manosroi J., Manosroi A.,** "Dominant lethal test in rats treated with some plant extracts", Southeast Asian J Trop Med Public Health 2000;31 Suppl 1:171(http://www.ncbi.nlm.nih.gov:80/entrez/query/fcgi?cmd=Retrieve&db=PubMed&list_uids=11414451&dopt=Abstract)

4. **Boeckh, E.M.A.,** "Stevia rebaudiana (Bert.) Bertoni: clinical evaluation of its acute action on cardio-circulatory, metabolic and electrolytic parameters in 60 healthy individuals." Third Brazilian Seminar on Stevia Rebaudiana (Bert.) Bertoni, (Summaries), Angelucci, E. (Coordinator), July, 1986, pp. 22-23.

5. **Bonvie L., Bonvie B. and Gates D.**, "Stevia: The Natural Sweetener That Frightens NutraSweet", Earth Island Journal, [Northern] Winter 1997-98, [Southern] Summer 1997-98

6. **Brandle, Dr J.**, "Frequently Asked Questions about Stevia, Nature's Low Calorie Sweetener", Agriculture and Agri-Food Canada, at: (http://res2.agr.ca/london/pmrc/english/faq/stevia.html,) last modified 21 November 2002

7. **Brandle, J.E., Starratt A.N. and Gijzen M.,** "Stevia rebaudiana: Its biological, chemical and agricultural properties", Agriculture and Agri-Food Canada, at: http://res2.agr.ca/london/pmrc/english/faq/stevia-rev.html, last modified 12 November 2002

8. **Cbc.ca,** "Stevia: A brief history", at: http://cbc.ca/consumers/market/files/food/stevia/history.html, downloaded 28 November 2002

9. **Chan P., Tomlinson B., Chen Y.J., Liu J.C., Hsieh M.H., Cheng J.T.,** "A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension", Br J Clin Pharmacol 2000 Sep;50(3):215-20, Abstract at: http://www.ncbi.nlm.nih.

10. **Chen J, Jeppesen PB, Nordentoft I, Hermansen K.** Metabolism. 2006 Dec;55(12):1674-80 Stevioside counteracts the glyburide-induced desensitization of the pancreatic beta-cell function in mice: studies in vitro

11. **Chubb L.,** "FDA stonewalling on sweetener, activists say", Environmental News Network, at: http://www.enn.com/news/enn-stories/1999/10/101999/headache_6491.asp, 19 October 1999

12. Comparative effects of Stevia rebaudiana leaves and stevioside on glycaemia and hepatic gluconeogenesis

13. **Daniel Stephen** Stevia tops preferences for low-cal chocolate Source: *JAMA* 2010, Volume 303, Issue 15, Pages 1490-1497 *"Caloric Sweetener Consumption and Dyslipidemia Among US Adults"*

Jeppesen P.B., Gregersen S., Alstrup K.K., Hermansen K., "Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats", Phytomedicine 2002 Jan;9(1):9-14, Abstract at: http://www.ncbi.nlm.nih.gov:80/entrez/query/fcgi?cmd=Retrieve&db=PubMed&list_uids=11924770&dopt=Abstract

14. **Jeppesen P.B., Gregersen S., Poulsen C.R., Hermansen K.**, "Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity", Metabolism 2000 Feb;49(2):208-14, Abstract at: http://www.ncbi.nlm.nih.gov:80/entrez/query/fcgi?cmd=Retrieve&db=PubMed&list_uids=10690946&dopt=Abstract

15. **Joint FAO/WHO Expert Committee** on Food Additives (JECFA)'s Evaluation Report on Stevioside (Extracted from WHO Technical Report Series 891, June 2008, Sweetening agentstevioside,(http://www.info.gov.hk/fehd/textmode/safefood/report/stevioside/who.html)

Table 1. Nutritive value of products prepared by incorporation of stevia powder

Treatments	Energy (Kcal.)	Protein (g)	Fat (g)	Carbo hydrates (g)	Moisture (%)	Ash (g)	Fibre (g)	Calcium (mg)	Vit. A (µg)	Vit. C (mg)	Iron (mg)
a). Flavoured Milk											
T_0	274.38	5.4	18.5	17.77	104.5	0.53	2.8	206.7	125	2.5	2.0
T_1	172.59	4.9	12.4	9.61	114.5	0.60	2.8	189.9	203.1	1.5	1.98
T_2	172.59	4.9	12.4	9.61	114.5	0.60	2.8	190.0	203.1	1.5	2.04
T_3	172.59	4.9	12.4	9.61	114.5	0.60	2.8	190.3	203.1	1.5	2.09
(b) Custard Pudding											
T_0	192.37	4.5	98.5	32.82	89.0	0.53	2.8	166.7	1856.5	2.5	0.6
T_1	169.07	4.0	92.4	24.66	99.0	0.608	0	149.9	1934.6	1.5	0.58
T_2	169.07	4.0	92.41	24.66	99.0	0.609	0	150.0	1934.6	1.5	0.63
T_3	169. 07	4.0	92.41	24.66	99.0	0.610	0	150.1	1934.6	1.5	0.68