Effect of Stevia as a Substitute for Sugar on Physicochemical and Sensory Properties of fruit based milk shake

Mohammad Alizadeh 1, Maryam Azizi-lalabadi†2, Helaleh Hojat-ansari 3, Sorayya Kheirouri

1 M.D., Ph.D., Assistant Professor, Department of Nutrition and Diet therapy, Faculty of Nutrition, Tabriz University of Medical Sciences. Tabriz, Iran
2,3 MSc student in Food Sciences, Students’ research committee, Tabriz University of Medical Sciences, Attar Nishabouri St., Tabriz, I. R. Iran
4 Ph.D., Assistant Professor, Department of Community Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences. Iran

ABSTRACT

A food with low calorie density has high importance in managing health concerns attributed to food and nutrition. Replacing sucrose with natural intense sweeteners such as Stevia has been suggested as a way to produce foods with low caloric value. The present study has been conducted to formulate novel fruit based milk shake using a mixture of Kiwi, apple and banana concentrates by replacing sucrose partly with Stevia. Five different treatments of fruit milk shakes were prepared with sucrose/Stevia ratios of 100:0 (TA), 75:25 (TB), 50:50 (TC), 25:75 (TD) and 0:100 (TE). The physico-chemical properties of the beverage were examined using conventional methods. All the products were evaluated by sensory expert evaluators. Substitution of sucrose with Stevia had no remarkable effect on pH, acidity and vitamin C content of beverage. Total soluble solids of the beverage decreased in proportion to the reduction in sucrose content (P<0.05). Control treatment (TA) had the lowest total sugar content which was significantly different from TB, TC and TD (p<0.01). Among Stevia containing beverages, the best mean liking score belonged to sample TD followed by TB, TC and TE. The recommended ratio of sucrose/Stevia in beverage was 25:75. Stevia free beverage had the best sensorial acceptance. It is concluded that Stevia is a good choice to develop low sucrose beverages including fruit based milk shakes. Although Stevia had no adverse impact on physico-chemical properties, its sensorial acceptance is affected by its rate of addition.

Keywords: Total soluble solids, Stevia, total sugar, fruit based milk shake
1. INTRODUCTION

In the recent decades, prominent changes have been performed to comprehend food role in promoting human health. Consumers believe that food plays a key role in improving quality of their life. Therefore, food science and technology has been directed to produce foods which possess high nutritional value with good sensory acceptance. Further, recent changes in global burden of diseases and increased incidence of non-communicable diseases has resulted in an increasing attention by food technologists to produce diets and drinks with specialized formulae suitting such conditions.

Dietary risk factors are leading contributors to impede global health status. Among them, high sugar consumption is particularly important due to its high calorie (fat gives high calorie, sugar and protein rather contributes less calorie! – remove word high) contents. It is generally accepted that diets with low calorie are very important in management of healthy life and consequently may help in preventing lifestyle related diseases.

Although products containing artificial sweeteners have fewer calories, such additives may exert adverse health effects such as increased risk of weight imbalance, toxicity, allergy, fetal malformations and physiological effect on body metabolism. Consumption of high concentrations of such compounds may induce cell proliferative and genetic disorders. For these reasons regulations has been set to minimize their usage by children, pregnant or lactating women and other vulnerable groups [4, 5]. Taking into account such considerations, application of natural intense sweeteners in the food industry receives increasing interest by many researchers. Excluding glucose, other natural sweeteners have no remarkable side effects on human health and hence they can be considered as suitable alternatives to artificial sweeteners. Although sucrose has many advantage as a natural sweetener [5], its calorie contribution results in restricted consumption for several individuals in particular overweight and obese ones. High intake of sucrose may result in hypertension, ischemic heart diseases, dental decay, overweight, and insulin resistance [5, 6].

Hence, researches are in progress to replace sucrose with natural intense sweeteners. Taking into account both health and technological aspects sucrose is used with caution due to its calorie contribution (repeating again!) which is correlated with adverse metabolic consequences, and cariogenic effects [7, 8]. Stevia, as a non-caloric intense sweetener is useful as a healthy substance [6]. Substitution of sucrose with low-calorie compounds is thought to be helpful in the management of obesity and hypertension [9]. Accordingly, a safe natural sweetener such as Stevia may be useful in preparation of fruit based milk shake.

**Stevia rebaudiana** Bertoni is a natural sweetener with relative sweetness 250-300 as compared to sucrose. It is a very low calorie (low calorie or non-caloric as written against line 50 above!) compound which makes it a good alternative of sugar for patients suffering from serious disease and other sedentary life related diseases [10]. This plant has been used in formulation of many sugar-free foods including Custard, Kulfi, Sandesh [11] and biscuit [12]. Despite many artificial sweeteners, Stevioside are recognized as safe supplements by JECFA, WHO and Food and Drug Administration (FDA) with relatively high (mention limits!) upper limits [13, 14, 15].

The most common types of these sweet herbs are: Rebaudiana, Pilosa, Epatoria, Ovatora, Plummera, Salicforian and Salicforian [9, 10, 16]. Nowadays, people prefer to consume juices that are mixtures of several fruits over single fruit. A combination of many fruits is richer in vitamin, isoflavones and antioxidant contents than an individual fruit juice (delete rest). Milk is a rich source of protein, calcium and other minerals that are useful during childhood and even to the elderly. Therefore, combination of milk and fruit juice makes an excellent nutritive drink. The aim of the current research was to look for a possible formulation of novel fruit based milk shake drink using Stevia, as an alternative sweetener, with or without added sucrose, comprising of mixture of kiwi, apple and banana juice concentrates and to examine the products physico-chemical and sensory properties.
2. MATERIAL AND METHODS

2.1 Preparation of milk shake

Initially, kiwi juice (70 per cent total soluble solids (TSS)) was purchased and one volume of such juice was added to 2 volumes of banana (TSS of 23%) and apple (TSS of 38%) juice concentrates. The characteristics of the fruit concentrates are provided in Table 1.

What was the quantity of one batch of fruit based milk shake (i.e. individually for samples TA, TB, TC, TD and TE) Values of TSS depicted in Table 1 and above para for juice concentrates do not match (e.g. Kiwi – 70% vs. 52.0% in Table 1)

Table 1: Particulars of fruit juice concentrates

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Concentrate of fruit juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Banana Concentrate†</td>
</tr>
<tr>
<td>Total soluble solids (%)</td>
<td>20.10</td>
</tr>
<tr>
<td>pH</td>
<td>5.00</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin C(mg/dl)</td>
<td>10.00</td>
</tr>
<tr>
<td>Reducing sugar (g/dl)</td>
<td>6.14</td>
</tr>
<tr>
<td>Sucrose (g/dl)</td>
<td>9.89</td>
</tr>
<tr>
<td>Total sugar (g/dl)</td>
<td>16.59</td>
</tr>
</tbody>
</table>

†Expressed as % of malic acid for banana and apple juices and % of citric acid for Kiwi juice.

Keep left alignment for parameters of Constituents

What is the need to mention sucrose in Table 1 (reducing sugar and total sugar is enough)

The resultant beverage was homogenized with a stirrer (Heidolph RZR 2012 control, Japan) at 800 rpm for 1 minute. To prepare milk shake, 100 ml of pasteurized and cooled milk (1.5% fat) was added to 200 ml of fruit juice concentrate blend and again homogenized with the stirrer (again 1 min at 800 rpm). The amount of sugar and/or Stevia (200SUD, 90% pure, Iran, Stevia Pac) in different ratios was tried out in fruit based milk shakes. To examine the effect of substitution of sucrose with Stevia, five different treatments were adopted adding appropriate amount of sucrose and/or Stevia to 120 mg† of fruit juice blend (fruit juice cannot be 120 mg – this may be stevia amount!) as follows: treatment (1) using solely 36 g sucrose (TA), (2) using 27 g sucrose and 40 mg Stevia (TB), (3) using 18 g sucrose and 90 mg Stevia (TC), (4) using 9 g sucrose and 100 mg
Stevia (TD) and using solely 180 mg Stevia (TE). Finally, all the beverages were pasteurized (in a glass bottle) in water bath at 80°C for 7 minutes, cooled to 4°C and subjected to further analysis.

2.2 Physico-chemical properties of beverage

The physico-chemical properties of milk shake such as pH, titratable acidity, total soluble solids (TSS), total sugar, and Vitamin C were studied. pH was measured using a pH meter (Net ohm 827, pH lab model, Swiss), calibrating with buffers of 4 and 7 pH. The TSS of beverage was measured with a digital refractometer (Pal-3maleatago, Japan – range 0.0 to 93.0) and expressed as per cent TSS. Acidity was determined using conventional titration method and Titrisol (Germany brand, class 50ml) tool. Total sugar content of the milk shake was estimated using the method of Lane and Eynon [17]. Further, AOAC titration method with Fehling’s solution was used to measure sucrose content of the samples.

2.3 Sensory properties of beverage

Sensory evaluation of the beverage was conducted by eight member group of well trained expert panelists. Their ability to perform sensory evaluation was judged by subjecting them to recognition of basic tastes (sweet, bitter) at low concentrations; determination of minor concentration differences of basic tastes; determination of taste threshold; after taste recognition. A set of coded cups containing 50 ml of milk shake was presented to the panelists and then the intensity of each sensory attribute was ranked between ‘0’ (uncharacterized intensity) and ‘5’ (very strong intensity). The average score for each descriptive attribute was plotted in a web diagram. All experiments were carried out in triplicate and the results were reported as the mean values.

2.4 Statistical analysis

All the treatments were replicated thrice. Data were first examined by Kolmogorov– Smirnov test to ensure normality. They were then expressed as mean ± standard deviation. Comparison within each group was done by one way analysis of variance followed by Tukey test. To determine effect of different proportion of Stevia with sucrose, Pearson correlation analysis was performed. All the analysis was performed using SPSS software, version 17.1.

3. RESULTS AND DISCUSSION

3.1 Effect of replacement of sucrose with Stevia on the physico-chemical properties of milk shake

As shown in Table 2, substitution of sucrose with Stevia did not result in significant (P < 0.01) change in vitamin C, pH, acidity, sucrose and TSS content of beverage. Vitamin C, pH and acidity were not different for the five juice blends (A-E) irrespective of the treatment used. The maximum acidity and pH were found in fruit based milk shake containing 100% Stevia and 100% sucrose respectively. As expected, TA had the lowest total sugar content which was significantly different (p< 0.01) from that of samples TB, TC and TD. Significant difference (p< 0.01) with regard to total sugar was not noted between samples TD and TE. As shown in the Table 2, the highest (16.77±0.21) and lowest (14.03 ± 0.06) TSS was associated with sample TA and TE (i.e. with Stevia alone). There was significant difference (P<0.05) in the TSS of all the fruit based milk shakes as compared to control. The TSS content of beverage tended to decrease as the sucrose content decreased. The mean calorie value of fruit based milk shake is shown in
The calorie content of beverage decreased with replacement of sucrose with Stevia. Caloric content of each milk shake (not treatment) was calculated using food analysis software (Nutritionist 4, Nutrition Marker Plus).

Table 2. Physico-chemical and sensory properties of milk shake

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TA</th>
<th>TB</th>
<th>TC</th>
<th>TD</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose/Stevia ratio</td>
<td>100:0</td>
<td>75:25</td>
<td>50:50</td>
<td>25:75</td>
<td>0:100</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>36</td>
<td>27</td>
<td>18</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Stevia (mg)</td>
<td>0</td>
<td>40</td>
<td>90</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>Total soluble solids (%)</td>
<td>16.77±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.40±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.37±0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.37±0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.03±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>4.37±0.02</td>
<td>4.35±0.02</td>
<td>4.34±0.02</td>
<td>4.33±0.04</td>
<td>4.33±0.03</td>
</tr>
<tr>
<td>Acidity (% Malic acid)</td>
<td>0.52±0.01</td>
<td>0.51±0.01</td>
<td>0.52±0.01</td>
<td>0.52±0.02</td>
<td>0.53±0.01</td>
</tr>
<tr>
<td>vitamin C (mg/dl)</td>
<td>11.28±0.02</td>
<td>11.28±0.02</td>
<td>11.28±0.02</td>
<td>11.28±0.02</td>
<td>11.28±0.02</td>
</tr>
<tr>
<td>Reducing sugar (g/dl)</td>
<td>3.62±0.02</td>
<td>6.83±0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.30±0.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.9±0.17&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.64±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total sugar (g/dl)</td>
<td>3.96±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.67±0.15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10.43±0.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.77±0.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.81±0.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrose (g/dl)</td>
<td>0.33±0.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.74±0.15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.03±0.24&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.82±0.22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.16±0.01&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total calorie per</td>
<td>338.9</td>
<td>302.9</td>
<td>266.9</td>
<td>230.9</td>
<td>194.9</td>
</tr>
</tbody>
</table>
When 36 g sucrose is used (can't understand in how much total beverage quantity), how the sucrose can be analyzed to have 0.33% only! Added sucrose is decreasing thereafter i.e. in samples TB, TC, TD and TE, but sucrose content (g/dl) is increasing in Table i.e. 1.74 to 2.03, then decreasing again 0.82 and 0.16. Cannot understand!

a, b: in 120 ml of fruit juice blend (calculate to 100 mL or to 1 L).

Different alphabets in each row represent statistically different (P<0.01).

3.2 Effect of sucrose replacement with Stevia on the sensory properties and the calorie of fruit based milk shake

As shown in Fig.1 different proportions of Stevia and sucrose did not result in significant change in the color or viscosity of beverage as compared to control; the experimental beverages did not differ from each other in these respects. However, substitution of sucrose with Stevia had some adverse effects on the taste of the beverage, regardless of sucrose/Stevia ratio. The taste was especially acceptable in case of beverages TA and TD as compared to TB, TC and TE. The maximum score for ‘mean liking’ was associated with products TA and TD, TA being best followed by TC and TE (delete respectively).
**Fig. 1.** Sensory evaluation of milk shakes with sucrose:stevia ratio: TA: 100:0, TB=75:25, TC=50:50, TD=25:75 and TE=0:100. Different alphabets represent statistically different (P<0.05) (I cannot see any alphabets as superscript, etc.).

4. DISCUSSION

Many strategies have been developed to manage increasing burden of lifestyle related diseases. Such strategies involve reducing level of fat in foods, intake of fiber through consumption of appropriate portions of fruits & vegetables and reducing the total calorie intake [18]. However, there are little adherence to the recommendations mainly among children and adolescence due to healthy, mental and subjective pressures. Healthy food choice, for individuals suffering from impaired health status are receiving much attention by both nutritionists and food technologists [18, 19]. Since carbohydrates have been recognized as the major calorie source in many food baskets, most attention has been focused on substitution of sucrose with non-caloric sweeteners. Although both natural and synthetic sweeteners have been used in the formulation of food stuffs, deep concern is raised regarding safety aspects of synthetic ones. Application of natural non-caloric sweeteners has been suggested as an alternative choice.

*Stevia rebaudiana* is receiving an increasing attention to be used as a (a two times!) non-caloric alternative sweetener. The most common types of these herbs are; Rebaudiana, Pilosa, Epatoria, Ovatora, Plummera, Salicforian and Salicforian [10]. This herb consists of glycoside compounds such as Stevioside, A-rebaudioside, and Brebaudioside. So far nine diterpenoid glycoside compounds have been extracted from *Stevia rebaudiana* [20]. Stevia is known to possess antioxidant and anticancer properties [13,19] and being non-caloric makes it a suitable component to be used by individuals who are health conscious [21].

Physico-chemical parameters are critical determinants of quality of food stuffs [13], especially in fruit based drinks [22]. Among them, TSS is particularly important in both fruit juice and fruit based milk shake due to its influence on both stability and sensory aspects. Interestingly, all the beverages conformed with the standards of Codex Alimentarius for fruit juice, for the mentioned character [23]. There was a mild reduction in the vitamin C content of milk shakes upon thermal processing. This was inconsistent with findings by Sánchez- Monreno et al [24] who reported significant loss of vitamin C content of (Rather quote reference of some citrus fruit juice where vitamin C stability has been studied due to processing) tomato juice upon thermal processing.

The effect of Stevia and Stevia/sucrose combination on the sensory properties of fruit based milk shake is discussed herein. The sample sweetened with sucrose /Stevia of 25:75 (TD) was more preferred over all other Stevia containing samples in terms of overall quality. In line with the other reports (which Ref. no.), the beverages devoid of Stevia were preferred over the Stevia sweetened ones. The most acceptable level of Stevia varies according to the food products. It has been reported that a concentration of 0.25% Stevia in fermented dairy products was most acceptable (delete level – concentration means same) [11]. Lisak et al, suggested that a combination of sucrose and Stevia used at 4.5% concentration (in which product?) was most accepted by expert panelists [25]. The mean liking score of the tested samples may have been affected by flavouring constituents of fruit juice concentrates. Interestingly, TE samples, having 100% Stevia, had similar
‘mean liking score’ to that of control (i.e. TA). This might be attributed to the sour taste of kiwi fruit and its masking effect on bitter aftertaste induced by Stevia. High concentration of Stevia sweetener is frequently associated with a bitter aftertaste [26, 27]. The sweet sensation of a product, sweetened in absence of sucrose, is perceived slowly. It is reported that the bitterness of beverage increases when using high concentration (sucrose with 10% concentrations and Stevia with 20% or higher concentrations – how can Stevia be used at concentration greater than sucrose (i.e. 20 vs. 10% for sucrose?)) [28]. It was hypothesized that overall sensory characteristics of the beverage are related to possible interactions between natural sweetening components of fruit juice and the added sweeteners. Also, it appears that sweetening potency and the lingering of sweet aftertaste of Stevioside are affected by factors such as concentration of stevia, synergistic or inhibitory effects (by what?), temperature of medium, and viscosity of food and characteristics of the dispersion media [29]. Therefore, further research is needed to examine sensory profile of Stevia sweetened beverages in reference to above mentioned parameters.

In conclusion, Stevia is a good choice, for replacing sucrose, for the development of fruit based milk shakes. Inclusion of Stevia in combination with sucrose does not have any adverse effect on the physico-chemical characteristics of the beverage. Fruit based milk shake made using sucrose/Stevia (25:75) has the highest consumer acceptance among Stevia containing formulations. Further research in this aspect is needed to elucidate the sensory profile of the beverage involving different fruits and with varying concentrations of the sweetener blend.

ETHICAL APPROVAL
All authors hereby declare that specific national laws were followed where applicable. All experiments have been examined following a study proposal approved by the ethics committee of the Tabriz University of Medical Sciences, Tabriz, Iran.

REFERENCES
5. Leung A, Foster S. Encyclopedia of common natural ingredients used in food, drugs and cosmetics, Food Chemistry. 50(4);1996:22.


28. Cardello HM, Da Silva MA, Damasio MH. Measurement of the relative sweetness of *Stevia* extract, aspartame and cyclamate/saccharin blends as compared to sucrose at different concentrations, Plant Foods Hum Nutr. 54;1999:119-30.

29. Weber A, Hekmat S. The effect of *Stevia rebaudiana* on the growth and survival of *Lactobacillus rhamnosus* GR-1 and sensory properties of probiotic yogurt. Journal of Food Research, 2(2);2013; (Name of bacteria in Italics; small letters for first alphabet after ‘The’)

293

294