



Effect of *Stevia* as a Substitute for Sugar on Physicochemical and Sensory Properties of Fruit Based Milk Shake

Mohammad Alizadeh¹, Maryam Azizi-lalabadi^{2*}, Helaleh Hojat-ansari²
and Sorayya Kheirouri³

¹Department of Nutrition and Diet Therapy, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran.

²Research committee, Tabriz University of Medical Sciences, Attar Nishabouri St., Tabriz, I R, Iran.

³Department of Community Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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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

*Corresponding author: E-mail: m_azizi766@yahoo.com;

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 [1]. 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 suiting such conditions [1,2].

Dietary risk factors are leading contributors to impede global health status. Among them, sugar consumption is particularly important due to its high calorie contents. It is generally accepted that diets with low calorie are very important in management of healthy life and consequently may help in preventing life style related diseases [2,3].

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 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 non-calorie 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]. Steviol (E960) was first commercialized as a sweetener in 1971 by the Japanese firm Morita Kagaku [13]. Despite many artificial sweeteners, Steviosides are recognized as safe supplements by JECFA, WHO and Food and Drug Administration (FDA) with relatively high upper limits. The two organizations allowed daily intake of 2 mg/kg of body weight in dietary supplements [14, 15, and 16]. However, In European Union only Steviol glycosides (E960) have been permitted to be used as food additive [17] [18]. Finally, Rebaudioside A is “Generally Recognized as Safe” as of December 2008 [19].

The most common types of these sweet herbs are: Rebaudiana, Pilosa, Eptoria, Ovatora, Plummera, Salicforian [9,10,20]. 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. 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. MATERIALS AND METHODS

2.1 Preparation of Milk Shake

Initially, 39.5 ml of kiwi juice with 70per cent total soluble solids (TSS) was purchased and one volume of such juice was added to 2 volumes of banana (TSS=20.10%) and apple (TSS= 52%) concentrates (80.25ml of each banana and apple concentrate). The characteristics of the fruit concentrates are provided in Table 1.

Table 1. Particulars of fruit juice concentrates

Constituents	Concentrate of fruit juice		
	Banana Concentrate ¹	Apple Concentrate ¹	Kiwi Concentrate ¹
Total soluble solids (%)	20.10	52.00	70.00
pH	5.00	3.80	2.90
Acidity (%) ¹	0.25	1.87	0.84
Vitamin C(mg/dl)	10.00	35.00	340.00
Reducing sugar (g/dl)	6.14	52.70	38.00
Total sugar(g/dl)	16.59	54.80	32.00

¹Expressed as % of malic acid for banana and apple juices and % of citric acid for Kiwi juice

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* white extract (Steviol Glycoside, SU 200, *Stevia* Pac) in different ratios was tried out in fruit based milk shakes. To examine the effect of substitution of sucrose with *Stevia* (Type SU200, 90% pure, Iran, *Stevia* Pac) five different treatments were adopted adding appropriate amount of sucrose and/or *Stevia* to fruit juice blend 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, percentage of 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 (Atago, Japan) 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 [21]. Further, AOAC titration method with Fehling's solution was used to measure reducing sugar 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 \pm 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 Table 2, the calorie content of beverage decreased with replacement of sucrose with *Stevia*. Caloric content of each milk shake was calculated using food analysis software (Nutritionist 4, Nutrition Marker Plus).

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 any significant change in the color or viscosity of beverage as compared to control; even 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.

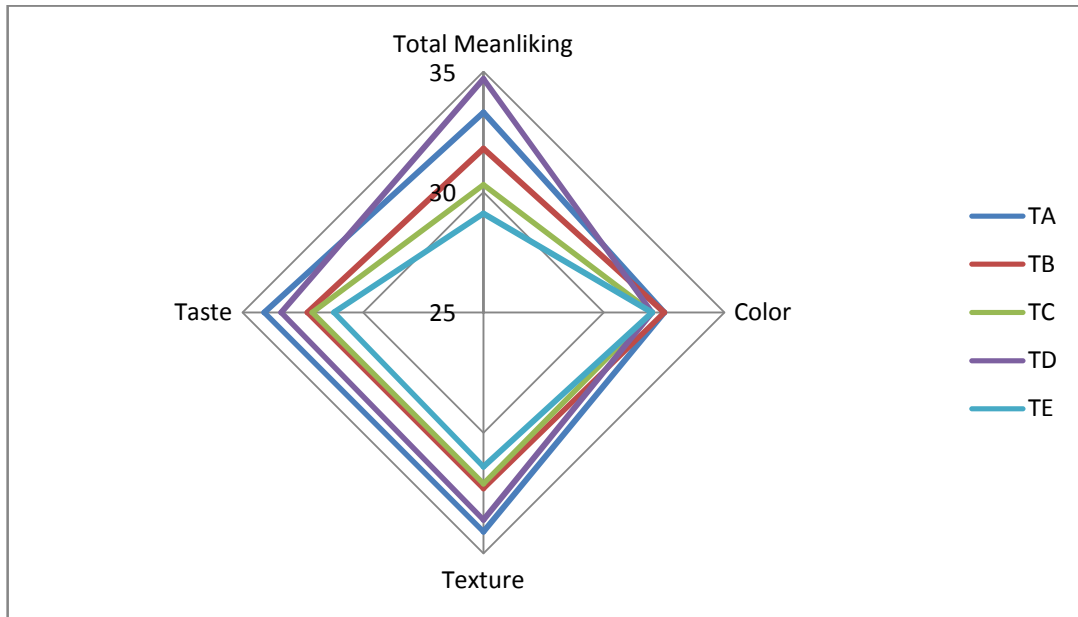


Fig. 1. Sensory evaluation of milk shakes with sucrose: *Stevia* ratio: TA: 100:0, TB=75:25, TC=50:50, TD=25:75 and TE=0:100

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

Treatments	TA	TB	TC	TD	TE
Sucrose/ <i>Stevia</i> ratio	100:0	75:25	50:50	25:75	0:100
Sucrose (g) ^a	36	27	18	9	0
<i>Stevia</i> (mg) ^b	0	40	90	100	180
Total soluble solids (%)	16.77±0.21 ^a	15.40±0.10 ^b	14.37±0.12 ^b	14.37±0.12 ^b	14.03±0.06 ^b
pH	4.37±0.02	4.35±0.02	4.34±0.02	4.33±0.04	4.33±0.03
Acidity (% Malic acid)	0.52±0.01	0.51±0.01	0.52±0.01	0.52±0.02	0.53±0.01
vitamin C (mg/dl)	11.28±0.02	11.28±0.02	11.28±0.02	11.28±0.02	11.28±0.02
Reducing sugar (g/dl)	3.62±0.02 ^a	6.83±0.01 ^b	8.30±0.1 ^c	6.9±0.17 ^b	7.64±0.02 ^d
Total sugar (g/dl)	3.96±0.02 ^a	8.67±0.15 ^b	10.43±0.21 ^c	7.77±0.06 ^d	7.81±0.0 ^d
Total calorie (Kcal per 100 g of beverage)	338.9	302.9	266.9	230.9	194.9

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 ($P < 0.01$))

4. DISCUSSION

Many strategies have been developed to manage increasing burden of life style related diseases. Such strategies involve reducing level of fat in foods, intake of fiber through consumption of appropriate portions of fruits and vegetables and reducing the total calorie intake [22]. However, there are little adherence to the recommendations mainly among children and adolescences 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 [22,23]. 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 non-caloric alternative sweetener. The most common types of these herbs are; Rebaudiana, Pilosa, Eptoria, 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* herb [24]. *Stevia* is known to possess antioxidant and anticancer properties [13,23] and being non-caloric makes it a suitable component to be used by individuals who are health conscious [25].

Physico-chemical parameters are critical determinants of quality of food stuffs [13], especially in fruit based drinks [26]. 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 to the standards of Codex Alimentarius for fruit juice, for the mentioned character [27]. There was a mild reduction in the vitamin C content of milk shakes upon thermal processing. This was inconsistent with findings by Ranuet et al[28] who reported significant loss of vitamin C content of pomegranate 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 overall other *Stevia* containing samples in terms of overall quality. In line with the other reports [29], 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 [11]. Lisak et al. suggested that a combination of sucrose and *Stevia* used at 4.5% concentration in strawberry flavored fresh yoghurt was most accepted by expert panelists [29]. 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* sweeteners is frequently associated with a bitter aftertaste [30, 31]. The sweet sensation of a product, sweetened in absence of sucrose, is perceived slowly. It is suggested that the bitter aftertaste is predominant when high concentrations of *Stevia* is used. It has been reported that this bitterness starts at concentrations equi-sweet to 20% sucrose or higher [32]. We 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 of co-sweeteners, temperature of medium, and viscosity of food and characteristics of the dispersion media [33]. Therefore, further research is needed to examine sensory profile of *Stevia* sweetened beverages in reference to above mentioned parameters.

5. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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