



SUBSTITUTING SUCROSE USING STEVIOSIDE AND REBAUDIOSIDE A IN COOKIES AND HIBISCUS BEVERAGE

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ABSTRACT

The effect of substituting sucrose using some of stevia sweeteners (Stevioside and Rebaudioside A) at different substitution levels (50, 75 and 100%) on physical, chemical and sensory properties of cookies and hibiscus beverage was analyzed. In cookies samples; gradual replacement of sucrose by stevia sugars, decreased energy, total sugars (reducing and non-reducing sugars), breaking force, diameter and spread ratio of cookies while caused an increase in moisture, ash and thickness. In hibiscus beverage, the increase in stevia sweeteners decreased total soluble solids, acidity, total sugars, while increased pH values, and antioxidant activity. Sensory evaluation test showed that, rebaudioside A showed more acceptability than stevioside in both cookies and hibiscus beverage samples.

Key words: Stevioside, Rebaudioside A, cookies, hibiscus beverage.

INTRODUCTION

Stevia rebaudiana (Bertoni) is a perennial shrub of the Asteraceae (Compositae) family, native to South America (Ohta *et al.*, 2010). The leaves naturally contain diterpene glycosides Stevioside and Rebaudiosides A, which are responsible for its sweet taste and have commercial value all over the world as sugar substitute in foods, beverage or medicines uses (Gupta *et al.*, 2013).

Stevia sweeteners showed to be nontoxic and possess antioxidant, antimicrobial, antifungal and anti-carcinogenic activity. Stevioside and Rebaudiosides A is likely to become a major source of high potency low calorie sweetener for growing natural cookies and beverage (Gupta *et al.*, 2013). The food industry has focused for the last several years on the production of low-calorie foods as a response to the continuing growth of public interest in these products. Cookies and other sweet baked goods contain large amounts of sugars and are usually avoided by dieters and diabetes (Drewnowski and Almiron, 2009).

Hibiscus sabdariffa L. is an herbaceous plant that belongs to the family Malvaceae (Cissé *et al.*, 2011). It is an annual herb cultivated for its leaves, stem, seed and calices (Fasoyiro *et al.*, 2005). *H. sabdariffa* calices has high antioxidant content related to the presence of anthocyanins with potent antioxidant activity (El Sherif *et al.*, 2011).

The different parts of hibiscus are the seeds, leaves and calyces and these have been used for making refreshing beverage and as food preserves. Hibiscus beverage have been found to be rich in vitamin C and other antioxidants such as flavonoids and minerals (Fasoyiro *et al.*, 2005).

The increase demand for hibiscus beverages due to its low prices, nutritional and medicinal properties is on the high side (Bamishaiye *et al.*, 2011).

Cookie is chemically leavened product, also known as biscuit. It is ideal for nutrient availability, palatability, compactness and convenience and differs from other baked products like bread and cakes because of having low moisture content, comparatively

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free from microbial spoilage and long shelf life of the product (Sharif *et al.*, 2009).

This study aimed to produce low calorie cookies and hibiscus beverage while maintaining the sweet taste of sucrose by using Stevioside or Rebaudioside A as sugar substitutions. We also aimed to analyze the effects of those two sugar alternative on the quality of the prepared cookies and hibiscus beverage.

MATERIALS AND METHODS

Wheat flour (72% extraction), sugar, shortening, salt and baking soda used to produce cookies in this study, were purchased from local market. Stevioside and Rebaudioside A obtained from El-Rebat Company for Distribution and Trade, Mansoura city, Egypt. Analytical grade chemicals and reagents were purchased from Al-Gomhuria Company, Ismailia, Egypt.

Cookies and Hibiscus Beverage Preparation

Cookies were prepared according to the method and formula of AACC (2002). Control formula of cookies was 225 g flour, shortening 64g, sugar 130 g, salt 2.1 g, baking powder 2.5g and water 49 g Hibiscus beverage was prepared according to the method and formula of AOAC (2005).

The Dried hibiscus sepals (30g) were soaked in tap water (840ml) at room temperature 25°C for 12 hours then, mixed with other contents including sweeteners (130 g sucrose or equal to the sweetness of this amount of sugar). Mixture was well mixed for two minutes and bottled in 250 ml glass bottles, heated to 90°C for another two minutes. Prepared bottles was cooled down by using tap water 25°C and stored at room temperature until analyses.

Physical Properties

Cookies spread ratio was measured according to the AACC method (2002)

After 30 minutes of removing the cookies from oven, six cookies were measured for diameter and thickness and divided by 6 to get the mean diameter and thickness of a single cookie sample. Spread ratio was calculated according to the following formula:

$$\text{Spread ratio (SR)} = (\text{Diameter D} / \text{Thickness T})$$

Moisture of cookie samples was determined according to the method described by Askar and Treptow (1993). Ash contents of cookies were determined according to the Approved Methods (AACC, 2002). Gross energy of a substance is the amount of heat measured in calories that is released when the substance is completely oxidized in a bomb calorimeter. Caloric value of the cookie samples was determined using oxygen bomb calorimeter (Werke IKA C2000) according to Krishna and Ranjhan (1981).

Breaking force of cookies was measured 30 min after removing the cookies from the oven. A three-point bend test was made on six cookies using a texture analyzer (TA-TX2, Stable Microsystem, Surrey, England) equipped with a 25-kg load cell.

The peak breaking force (g) of cookies using the force-in-compression was recorded.

Cookie samples were placed on base beams with a distance of 4 cm between the two beams. A three-point bending rig was used with an HDP/BS, knife-edge probe. The analyzer was set at a return-to-start cycle, with a pretest speed of 2 mm/sec, test speed of 2 mm/sec, posttest speed of 10 mm/sec, the trigger force was 20 g, and distance was 20 mm Abdel-Samie *et al.*, (2010).

Chemical Analyses

Total soluble solids of hibiscus beverage was determined by using refractometer following the method described by Askar and Treptow (1993). Total sugars, reducing sugars and non-reducing sugars

Table (1): Treatments of sucrose substituting.

Treatment	Sucrose (%)	Stevioside (%)	Rebaudioside A (%)
Tr ₁	100	-	-
Tr ₂	50	50	-
Tr ₃	25	75	-
Tr ₄	50	-	50
Tr ₅	25	-	75
Tr ₆	-	50	50

were determined as described in the official method of AOAC (2005). It is the method of Lane and Eyenon. pH values were measured using the pH-meter (orion, A420A) at room temperature (Askar and Treptow, 1993). The anti-oxidant activity of cookies was determined by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method according to Lee *et al.* (2003).

Acidity was determined according to the methods of AOAC (1984) it is expressed as the amount of free acid (mainly as anhydrous citric acid) in product (g/100ml beverage). Two ml of beverage was taken into 100 ml flask, 10 ml water and 1 drop of ph. ph indicator were added into the flask, then the mixture titrated against 0.1N NaOH quite rapidly unit near pH 6, then alkaline was added slowly up to pH 7. Titration was finished by adding 4 drops at time until pH 8.1. A pH-meter or titrate was used until the indicator colour went (faint pink colour). Total acidity expressed as percent of citric acid in samples.

RESULTS AND DISCUSSION

Effect of Sugar Substitution on Cookies Quality Characteristics

Moisture

Effects of sugar substitution on moisture contents of cookies were illustrated in Table (2). Significant differences were noted when control samples (Tr₁) were

compared to sugar substituted samples. It was found that maximum moisture content was that of (Tr₆) cookie samples (6.99%) which used Stevioside and Rebaudioside A (50 : 50) instead of sucrose. Minimal moisture was observed in control sample (Tr₁) which has sucrose as the only sweetener.

Moisture contents increased significantly with the decrease of sucrose from 3.96% in control sample to 4.89, 5.2, 4.79, 5.2 and 6.99, in Tr₂, Tr₃, Tr₄, Tr₅, and Tr₆ respectively. The increase in moisture content of cookies samples with the replacement of sucrose was combined with comparatively lower volume these changes may be due to the increase in flour weight to whole weight which caused higher water absorption.

These findings were in accordance with Galal (1998) who reported an increase of moisture contents of cookies with the decrease in sucrose.

Energy

Clear significant differences in energy values were observed (Table 2), control cookies (Tr₁) contained 377.1cal/100g, while substitution of 50% of sugar decreased the energy contents to 318.1 and 311.8 cal/100g in (Tr₂) and (Tr₄), respectively. When 75% Stevioside and Rebaudioside A has been used, the total energy contents were 286.4 and 285.8 in (Tr₃) and (Tr₅), respectively.

The lowest energy value was that of (Tr₆) with the energy value of 259.3 cal/100 g. The lower energy values in the reduced sucrose treatments was because of the removal of sucrose which is the biggest source of calories among cookies formula. These findings are in agreement with the results obtained by **Mahamoud *et al.*, (2002)** who reported that energy values decreased with the replacement of sucrose with other intense non-calorific sweeteners.

Sugars

Stevioside and Rebaudioside A are both non-carbohydrate materials, thus substitution of 50% of sugar decreased total sugars from 20% in control cookies (Tr₁) to 15% for both (Tr₂) and (Tr₄).

Further decrease in total sugars was noted with the 75% of sugars substitution at (Tr₃) and (Tr₅) with total sugar contents of 12% for each. Minimum total sugar contents (4.0%) was noted in (Tr₆) where no sucrose was added to the formula.

These results were in agreement with the results of **(Askar and Treptow, 1985)**, who reported that total sugars were decreased when sucrose has proportionally removed from cookies formula. The use of Stevioside and Rebaudioside A instead of sucrose in cookies led to a decrease in reducing sugars. When Stevioside or Rebaudioside A was used as a substitution instead of 50% of sugars, reducing sugars were decreased from 8 in control cookies (Tr₁) to 4% for both (Tr₂) and (Tr₄). Further decrease in reducing sugars was noted in the 75% of sugars substitution at (Tr₃) and (Tr₅) with a reducing sugars contents of 3% in both treatments. Minimum reducing sugars contents was noted in (Tr₆) (with no sucrose added).

Removing sucrose from formula led to the decrease of non-reducing sugars. In 50% of sugar substitution, non-reducing sugars decreased from 12% in control cookies (Tr₁) to 11% in both (Tr₂) and

(Tr₄). Further decrease in non-reducing sugars was noted with the 75% of sugars substitution at (Tr₃) and (Tr₅) with a contents of 9% for each.

The minimum non-reducing sugar content (3%) was noted in (Tr₆). This trend was in agreement with the results obtained by **Askar and Treptow (1985)** whose results showed that substituting sucrose for alternative sweeteners reduced non-reducing sugars in cookies.

Ash Contents

Control treatment (Tr₁) contained 0.56% of ash contents, while in 50% sugar substitution showed an increase in ash contents (0.75% and 0.85%) was noticed in (Tr₂) and (Tr₄) in Stevioside and Rebaudiosid A added cookies respectively. The 75% sugar substitution showed further increase in ash content (1.02 and 1.03) for (Tr₃) and (Tr₅), respectively. Maximum ash contents (1.56%) was that of the 100% sugar substitution using 50% Stevioside and 50% Rebaudioside A. These findings are in line with the results obtained by **El-Azab and Bothayna (1997)** who reported that alternative sweeteners led to an increase in ash contents due to the removal of sucrose which is very poor in minerals and increase of flour ratio which is higher in ash content.

Breaking Force

One of the important roles of sugar in cookies is the adsorption of water in a competition with the flour, so as to build the gluten network. Thus, removing of sucrose, as the hygroscopic material which carry water on its surfaces disturb the structure and will surely reduce breaking forces, as could be seen in Table (2).

Control treatment (Tr₁) had the maximum breaking force with an average of 5.51 kg, while the application of Stevioside and Rebaudioside A in the 50% substituted cookies decreased the breaking force to 4.31 kg, and 4.30 kg in Tr₂ and Tr₄, respectively. The minimum breaking

Table (2): Effect of sugar substitution on cookies quality characteristics.

*Treatment	Moisture (%)	Energy cal/100g	Sugars (%)			Ash (%)	Breaking force (kg)	Spread		
			Total	reducing	Non reducing			Diameters (cm)	Thickness (cm)	Spread ratio
Tr ₁	*3.96 ^d	377.1 ^a	20 ^a	8.0 ^a	12.0 ^a	0.56 ^d	5.51 ^a	6.45 ^a	1.00 ^d	6.45 ^a
Tr ₂	4.89 ^c	318.1 ^b	15 ^b	4.0 ^b	11.0 ^b	0.75 ^c	4.31 ^b	6.27 ^b	1.10 ^c	5.70 ^b
Tr ₃	5.20 ^b	286.4 ^c	12 ^c	3.0 ^b	9.00 ^c	1.02 ^b	3.17 ^c	5.77 ^c	1.20 ^b	4.81 ^c
Tr ₄	4.79 ^c	311.8 ^b	15 ^b	4.0 ^b	11.0 ^b	0.85 ^c	4.30 ^b	6.05 ^b	1.10 ^c	5.50 ^b
Tr ₅	5.20 ^b	285.8 ^c	12 ^c	3.0 ^b	9.00 ^c	1.03 ^b	3.14 ^c	5.74 ^c	1.20 ^b	4.79 ^c
Tr ₆	6.99 ^a	259.3 ^d	4.0 ^d	1.0 ^c	3.00 ^d	1.56 ^a	2.88 ^d	5.08 ^d	1.40 ^a	3.88 ^d

force was noted in the non - added sucrose treatment (tr₆) with a score of 2.88 kg. Those findings were in line with those mentioned by **Mahamoud *et al.* (2002)** who reported a decrease in breaking forces of cookies with application of alternative sweeteners in cookies formulation.

Spread of Cookies

Cookies spread are a ratio between diameter and thickness. As a result of a decreased diameter and relatively increased thickness, cookies spread ratios decreased with the sugar substitution. Spread ratio of the control samples Tr₁ was 6.45 cm and it was the maximum spread value.

Application of Stevioside and Rebaudioside A decreased the spread ratio to 5.70 and 5.50 in the 50% replacement of sugar in Tr₂ and Tr₄, respectively. Cookies spread decreased to 4.81 and 4.79 in the 75% substituted sugar cookies. Same trends were noted with Tr₆ with a minimum spread ration compare to all other treatments, with a spread score of 3.88. The decreased spread ratio when sugar was substituted using alternative sweeteners including Stevioside and Rebaudioside A might be due to the harder gluten network formed with the absence of sucrose which play a role in a loose texture by adsorbing water on its surface and to reduce water available used for the gluten network to develop. That comes in line with results of **Peck (1994)**.

Effect of Sugar Substitution on Hibiscus Beverage Quality Characteristics

Total Soluble Solids (TSS)

Table (3) shows the changes in T.S.S percentage in hibiscus beverage due to the effect of sugar substitution. Significant decrease was noted when control samples were compared to sugar substituted hibiscus beverage samples. The maximum T.S.S content (14.8) was recorded in (Tr₁) which contain 100% sucrose. While the minimum content was recorded in (Tr₆) where sugar was substituted with Stevioside and Rebaudioside A (50:50). Tr₂, Tr₃, Tr₄ and Tr₅ contained T.S.S of 10.3, 8.1, 9.0 and 8.4, respectively which were all lower than T.S.S in control sample Tr₁. This observation was in agreement with the results obtained by **El-Zoghbi and Siliha, (1992)** who reported that T.S.S decreased when sucrose was removed from apricot nectar.

Total Acidity

It was clear that added sweeteners decreased the acidity values of hibiscus beverage significantly. The highest acidity value was noted in control treatment Tr₁ (0.96), while lowest value was recorded in Tr₆ when Stevioside and Rebaudioside A were used instead of sucrose at ration 50:50. This decrease in acidity may be due to the absence of sucrose. These results are in agreement with the results of **Abdel-Samie (2007)** who reported an increase in

Table (3): Effect of sugar substitution on hibiscus beverage quality characteristics.

Treatment	T.S.S (%)	Acidity (%)	pH	Anti-oxidant (%)	Sugars (%)		
					Total	Reducing	Non Reducing
Tr ₁	14.8 ^a	0.96 ^a	3.10 ^d	61.05 ^d	12.5 ^a	5.50 ^a	7.00 ^a
Tr ₂	10.3 ^b	0.85 ^b	3.25 ^c	72.47 ^c	7.50 ^b	2.00 ^b	5.50 ^b
Tr ₃	8.10 ^c	0.81 ^c	3.42 ^b	76.19 ^b	6.00 ^c	1.50 ^c	4.50 ^c
Tr ₄	9.00 ^b	0.87 ^b	3.28 ^a	74.83 ^c	7.50 ^b	2.00 ^b	5.50 ^b
Tr ₅	8.40 ^c	0.82 ^c	3.41 ^c	77.61 ^b	6.00 ^c	1.50 ^c	4.50 ^c
Tr ₆	6.30 ^d	0.65 ^d	3.81 ^a	78.12 ^a	4.00 ^d	1.00 ^d	3.00 ^d

acidity values with storage of guava and mango sugar substituted beverages.

pH Value

pH values of hibiscus beverage are shown in Table 3. It is clear that addition of the sweeteners increased the pH values of hibiscus beverage. Control treatment (Tr₁) had the lower pH value (3.10), while the higher value (3.81) was recorded in Tr₆ when Stevioside and Rebaudioside A in ratio 50:50 was used instead of sucrose.

Abdel-Samie (2007) reported that pH values increased when sucrose has been removed from guava beverage.

Anti-oxidant Activity

The use of Stevioside and Rebaudioside A instead of sucrose in formula led to the increase of antioxidant activity in all treatments compared to the control treatments with score of 61.05%. The substitute of 50 % of sugar increased the antioxidant activity to 72.47 and 74.83% in Tr₂ and Tr₄, respectively. The higher values of antioxidant (78.12) were obtained when sucrose removed completely in Tr₆. These results are in agreement with the results of **Delva *et al.* (2005)** who reported that antioxidant value increased when sucrose was substituted using stevia sweeteners.

Total Sugars

Table 3 presents the sugars concentrations (total, reducing and non-reducing sugars) in beverage, respectively. Stevioside and Rebaudioside A are both non-carbohydrate materials thus substitution of 50% of sugar decreased total sugars from 12.5% in control cookies (Tr₁) to 7.5% and 7.5% in (Tr₂) and (Tr₄), respectively. Further decrease in total sugars was noted with the 75% of sugars substitution in (Tr₃) and (Tr₅) with a contents of 6.0 and 6.0, respectively. The minimum total sugar content was noted in (Tr₆) without sucrose. This result was in agreement with the results obtained by **Abdel-Samie (2007)**.

On the other hand, minimum reducing sugars was recorded in Tr₆ when no sucrose was added to hibiscus beverage. While maximum reducing sugars (Tr₁) (5.5%) was that of control hibiscus beverage.

On contrary, control sample Tr₁ had that maximum content (7%) of non-reducing sugars, while sugar substituted hibiscus beverage had less non-reducing sugar content of 5.5, 4.5% in Stevioside containing hibiscus beverage and 5.5 and 4.5% in Rebaudioside A containing hibiscus beverage.

Sensory Evaluation Test

Sensory Evaluation of Cookies

Fig. 1 shows appearance, texture, flavor-taste and overall acceptability of cookies.

Appearance

There were significant differences between treatments. The best appearance was scored in Tr₁, Tr₂ and Tr₅. Samples with Stevioside at of 50% sucrose substitution and Rebaudioside A instead of 75% sucrose recorded the best appearance. But when Stevioside used at substitution level 75% (Tr₃) recorded lower appearance score. Rebaudioside A as 50% sugar substitution (Tr₄) and Stevioside with Rebaudioside A 50:50 recorded the lowest score of appearance. Less colour score for Tr₂, Tr₃, Tr₄, Tr₅ and Tr₆ was due to the absence of brown colour of Millared reaction with the absence of sucrose.

Texture

The best texture was recorded with Tr₅ when Rebaudioside A used as 75% sugar substitution. The lowest texture score was recorded with Tr₆ when Stevioside with Rebaudioside A used in ratio 50:50 without sucrose addition. Less scores of texture of sugar substituted cookies might be related to the higher moisture contents.

Flavor and Taste

There were significant differences between treatments. The best flavor and taste was scored in Tr₅ that used Rebaudioside A instead of 75% sucrose, while the worst flavor and taste scored in Tr₆ with no sucrose added. It is clear that Tr₁, Tr₃, Tr₄ recorded the same lower score. Decreased flavor in Stevioside added cookies may be because the after taste effect of Stevioside.

Overall Acceptability

There were significant differences between treatments. The highest score was

achieved with Tr₁, Tr₂ and Tr₅ with excellent acceptability.

It is clear that treatments which use stevioside instead of 75% sucrose and Rebaudioside A instead of 50% sucrose recorded lower score. But when Stevioside with Rebaudioside A 50:50 recorded lowest score of overall acceptability.

As a matter of fact overall acceptability a measure of liking to panelists which may be an average of all previously mentioned sensory parameters, so overall acceptability of sugar substituted prepared products were lower than control.

Sensory Evaluation of Hibiscus Beverage

Fig. (2) sensory evaluation of hibiscus beverage showed that control sample Tr₁ was better than sugar substituted samples. Color score of control hibiscus beverage was 8 while other treatments Tr₂, Tr₃, Tr₄, Tr₅ and Tr₆ ranged from 7–8. Odor had no differences between treatments.

Flavor scores decreased gradually with the application of Tr₂, Tr₃, Tr₄, Tr₅ and Tr₆. Overall acceptability, control samples got a score of 8 without differences with Tr₂, Tr₃ and with differences with Tr₄, Tr₅ and Tr₆ which ranged in 6-7 scores. Less sensory scores might be due to the desired taste profile of sucrose loved by consumers and also because of the after taste of the stevioside.

Colour

The best colour was scored with Tr₂, Tr₃, Tr₄ and Tr₅. It was found that treatments which use Stevioside with Rebaudioside A 50:50 recorded lower colour score.

Odor

There were significant difference in Tr₆ when Stevioside with Rebaudioside A were a sugar substitution 50:50 scored a lowest odor score compared with all other treatments Tr₁, Tr₂, Tr₃, Tr₄ and Tr₅ were get the same score.

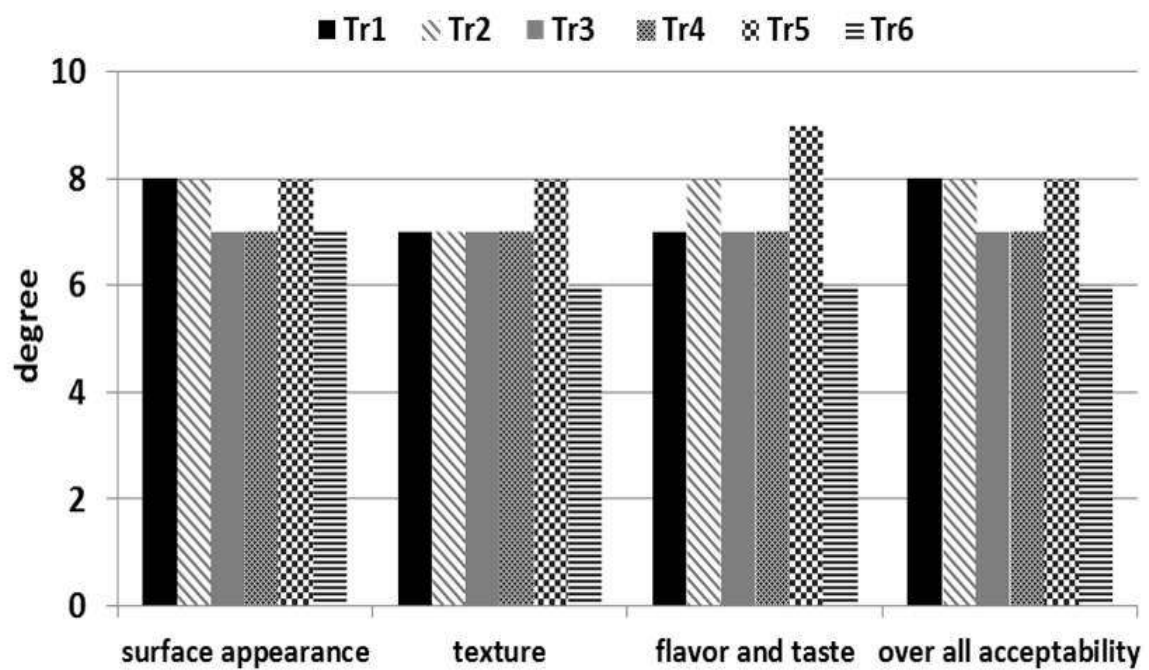


Fig. (1): Effects of sugar replacement on sensory evaluation of cookies.

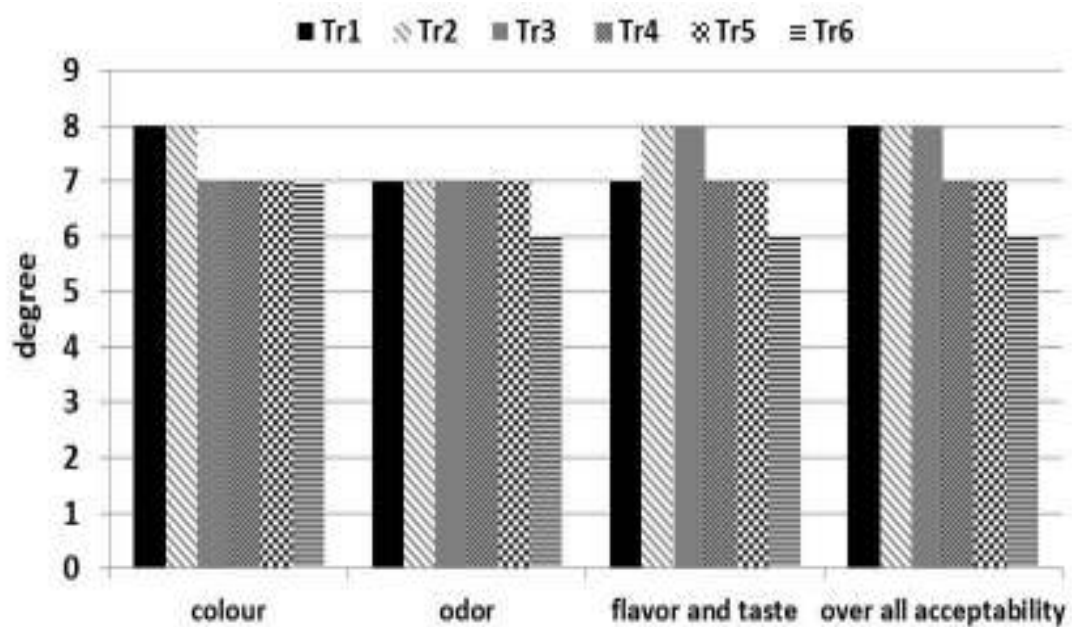


Fig. (2): effects of sugar replacement on sensory evaluation of hibiscus beverage.

Flavor and taste

The best flavor and taste was recorded with Tr₂ and Tr₃ that used Stevioside and rebaudioside A instead of 50% sucrose, while worst flavor and taste scored in Tr₆ with no sucrose added. it is clear that Tr₁,Tr₄,Tr₅ recorded same lower score.

Overall acceptability

The best score in overall acceptability was scored in control treatment Tr₂ and Tr₃ that used Stevioside and Rebaudioside A instead of 50% sucrose, while the lowest overall acceptability was observed in Tr₆ with any sucrose added.

CONCLUSION

It can be concluded that both Stevioside and Rebaudioside A can be used instead of sucrose to produce low-calorie cookies and beverage; it is safer, economical and has no bad effects on the chemical and physical properties of the prepared cookies and hibiscus beverage.

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المخلص العربي

استبدال السكرز بالاستيفوسيد والريبيدوسيد A في المخبوزات ومشروب الكركديه

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تم دراسة تأثير استبدال السكرز بمحليات بديلة مستخلصة من نبات الإستيفيا (سيفوسيد، ريبودوسيد A) على الخصائص الكيميائية والفيزيائية والحسية للمخبوزات ومشروب الكركديه، حيث تم استبدال السكرز بنسبة ٠,٥٠، ٠,٧٥، و ١,٠٠%، وقد أوضحت النتائج زيادة نسبة الرطوبة والرماد مع تطبيق المعاملات، في حين انخفض محتوى كلاً من السكريات و السرعات الحرارية وانخفاض قوة الكسر ومعامل التمدد للمخبوزات، وبالنسبة لمشروب الكركديه فقد انخفض كلاً من الحموضة والمواد الصلبة الذائبة والسكريات مع تطبيق المعاملات، وارتفع كلاً من الرقم الهيدروجيني ونسبة مضادات الأكسدة، وقد أوضحت نتائج التقييم الحسي أن نتائج المخبوزات والمشروبات التي تم استبدال السكرز بها قد لاقت قبول من حيث الطعم واللون والرائحة ومستوي القبول العام.

الكلمات الإسترشادية: السكرز، الإستيفوسيد، الريبيدوسيد A، مشروب الكركديه، المخبوزات.

المحكمون:

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