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STUDY THE SENSORY PROPERTIES OF SOME TYPES OF JUICES THAT MADE BY USING NATURAL SWEETENER POWDER (*Stevia rebaudiana*)

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ABSTRACT

Increased diseases and obesity currently due to increased production and excessive consumption of foods manufactured from non-food sweeteners without attention to the risk of consuming those additional high calories due to consuming these refreshing products such as juices and other various drinks, especially in the summer season by most segments of Iraqi society, especially workers, children and school students the aim of this study. Therefore, the study designed to replace sucrose with 0.03, 0.04 and 0.05% of each of the white stevia crystals and milled dry stevia leaves in the laboratory manufacture of juices and its effect on the general and sensory characteristics and the extent of their acceptability among the specialized residents. In addition to the control treatment, this was manufactured according to the specifications and concentration of the ingredients of juices manufactured by one of the locally famous juices manufacturing plants (Al-Shahir Factories) as a control treatment, with an added sucrose percentage of 8%. The results showed that the addition of stevia plant at 0.03 and 0.04% was most acceptable by sensory experts. The prepared orange juice was also analyzed for pH, titration acidity, dry matter, and density. The results also showed that by increasing the concentration of sweetener with the milled stevia plant, the pH of the mixture decreased and the acidity of the juice also increased. Whereas the juices with white stevia crystals were flavored with sweetness, clearer, more firm and acceptable by panelists.

Keywords: Juices, stevia, physicochemical and sensory properties

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دراسة الخواص الحسية لبعض انواع العصائر المصنعة باستعمال المحلي الطبيعي لمسحوق نبات الستيفيا *Stevia rebaudiana*

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الخلاصة

تزداد الأمراض والسمنة حاليا بسبب زيادة الإنتاج والاستهلاك المفرط للأطعمة المصنعة من المحليات غير الغذائية دون الانتباه إلى خطر استهلاك تلك السعرات الحرارية العالية الإضافية بسبب تناول هذه المنتجات المنعشة مثل العصائر والمشروبات المتنوعة الأخرى خاصة في فصل الصيف من قبل اغلب شرائح المجتمع العراقي وخاصة العمال والاطفال

وطلاب المدارس هدف هذة الدراسة، لذا صممت الدراسة باستبدال السكروز بـ0,03 و0.04 و0.05٪ من كلا من بلورات الستيفيا البيضاء ومطحون أوراق نبات الستيفيا الجافة في تصنيع العصائر مختبريا وأثرها على الصفات العامة والحسية ومدى تقبلها لدى المقيميين المختصين، فضلا عن معاملة السيطرة والتي صنعت طبقا لمواصفات وتركيز مكونات العصائر المصنعة من قبل احدى معامل تصنيع العصائر المشهورة محليا (مصانع الشهير) كمعاملة سيطرة، وبنسبة السكروز المضافة 8٪، وأظهرت النتائج أن إضافة نبات الستيفيا وبنسبة 0,03 و0,04% هي الاكثر قبولا من قبل المقيمين المختصين حسيا، وتم تحليل عصير البرتقال المصنع لمعرفة الاس الهيدروجيني، الحموضة التسحيحية، المادة الجافة والكثافة، وكذلك بينت النتائج أنه بزيادة تركيز التحلية بمطحون نبات الستيفيا انخفض الاس الهيدروجيني للمزيج وازدادت حموضة العصير، في حين أن العصائر ذات بلورات الستيفيا البيضاء كانت منكهة بحلاوة واضحة وهادئة واكثر صلابة ومقبولة من قبل المقيمين.

الكلمات المفتاحية: العصائر، محلي الستيفيا، الخواص الفيزيوكيمائية والحسية.

INTRODUCTION

Drinks and juices are an essential component of human food, especially for children, youth, workers and school students especially because of their inherent thirst quenching properties and fewer calories. Its stimulating and refreshing nature as well as its nutritional value **(Lemus-Mondaca *et al.*, 2012)**. Currently, most developed societies, even developing ones, have become conscious and interested in preserving public health and relying on the use of medicines originating from natural plants instead of drugs and their negative effects on the health of the body **(Pinto & Dharaiya 2014)**. Therefore, the various food industries have sought to investigate and strive to increase the nutritional value of their food products by adding functional components like, soluble or insoluble in water, antioxidants, vitamins, essential and rare mineral elements, probiotics, etc. of functional and vital components **(Rajpreet & Usha 2016)**. Also, consumers are becoming more health conscious and prefer healthy food products without affecting the natural sensory properties (**Sukhmani *et al.*, 2018)**. Therefore increased demand for foods low in sugar and calories for its importance in reducing blood sugar when consumed, which in turn reduces the incidence of obesity and diabetes type II, then decreases the incidence of heart disease and stroke (**Shivanna *et al.*, 2013)**. In the past century table sugar and industrial sweeteners were used in the manufacture of beverages, juices and refreshments necessary, especially in the summer time to resist the high temperature of the air, especially in high-temperature areas (**Ozdemir *et al.*, 2015)**. Currently, most consumers usually choose and order beverages made from calorie-free sweeteners made from natural stevia plants instead of artificial sweeteners such assaccharin, aspartame and acesulfame-K **(Chattopadhyay *et al.*, 2014**). However, this substitution of high-density sweeteners with low-density sweeteners and its effect on reducing the sensory and technical properties of beverages **(Kroyer 2010)** of beverages can be avoided, however, by using stabilizers and other fillers to enhance the taste and flavor desired by consumers **(Pinto & Dharaiya 2014)**. Moreover, calorie-free sweeteners in Stevia are provided by the desired sweetness despite the use of small quantities **(Rajpreet & Usha 2016).** It was reported that stevia has several beneficial roles on human health as antioxidant, blood glucose-lowering effect, anti-inflammatory and immunomodulatory activity **(Chatsudthipong & Muanprasat 2009; Madan *et al.*, 2010; Shivanna *et al.*, 2013)**. It also does not metabolize inside the human body and come out without providing calories harmful to health if not consumed by exercise or work to burn **(Chattopadhyay *et al.*, 2014).** So, using these stevia's compounds has increased dramatically due to the health concerns related to sucrose usage including dental caries, obesity and diabetes **(Lemus-Mondaca *et al.*, 2012).** Therefore, the study aimed to demonstrate the effect of adding different concentrations of white stevia crystals and raw stevia powder in the preparation of artificial orange juice instead of sucrose and its effect on some natural and sensory properties of set style artificial juice product.

MATERIAL AND METHODS

Stevia's leaf powder preparation

Stevia leaves were supplied from Medical & Aromatic Plants Research Unit/College of Agricultural Engineering Sciences/University of Baghdad/Baghdad/Iraq. The plant was identification and authenticated by Botanical Survey of Agric. College (No. BSI/SRC/5/23/2010-11/Tech-1585). Fresh stevia leaves were carefully isolated from bushes and exotic weeds well, and then washed thoroughly for several times with running tap water, then rinsed with distilled water, and dried from excess water by putting them in a stainless steel filter for about half an hour of time. After that it was dried in the shade under the ceiling fan for 2-3 days and finally grinded using an electric mixer. Finally, it was powdered by grinding with an electric blender, kept in a sealed polyethylene bags and stored at 4±1°C according to **(Hamdia *et al.*, 2019)**. Also, it was used natural sweetener stevia 100% stevia, Iranian product that is available in local markets (Type SU200, 90% pure, Iran, *Stevia* Pac).

Preparation of Juice mix

Initially, 39.5 mL of orange juice with 70% total soluble solids (TSS) was brought from al-Shahir plant/Iraq, that was used as normal mix of orange juice as in (Table 1). It was used fresh dried leaf stevia powder and white crystal stevia powder replacement instead of sugar as sweetener in different concentration preparation as in (Table 2).

Table (1): Component and particulars of the original orange mix that used in Iraqi al-Shahir plants.

Content (gm)	C-1(gm/6L)	C-1(gm/1L)
Sugar	815	135.83
Orange concentrate	9.5	1.58
Citric acid	7.5	1.25
Sodium benzoate	0.5	0.08
Vit. C	1	0.16
Total soluble solids (%)	70.00	70.00
pH	2.80	2.80
Acidity as citric acid	0.86	0.86

Table (2): Component of different preparation of 1L of Orange juice according to C-1(gm/L).

Contents (gm)	Orange juice (Control) 1=T	White stevia 1L 2	White stevia (%)			Leaf stevia powder 1L 6	Leaf stevia powder (%)		
			3	4	5		7	8	9
Stevia	-	25	0.3	0.4	0.5	25	0.3	0.4	0.5
Sugar	135.83	-							
Orange concentrate	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Citric acid	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Sodium benzoate	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Vit. C	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

The processed juices were homogenized well by using the electric stirrer according (Heidolph RZR 2012 control, Japan at 800 rpm for 1 minute). The amount of sugar and/or Stevia white extract (Type SU200, 90% pure, Iran, *Stevia* Pac) or stevia's leaf powder in different ratios were tried out in fruit based orange juices as in (Figure 1). To examine the effect of substitution of sugar as sucrose with white Stevia nine different treatments were conducted by adding appropriate amount of sucrose and/or *Stevia* to fruit juice blend as follows: Treatment (1) using solely 135.83 gm sucrose (TA) as control treatment; (2) using 25 g white stevia, (3-5) using 0.3, 0.4 and 0.5% white *Stevia* respectively; (3) using 25 g stevia's leaf powder; (7-9) treatments were used 0.3, 0.4 and 0.5 mg stevia's leaf powderrespectively. Finally, all the beverages were pasteurized (in a glass bottle) in water bath at 80ºC for 7 minutes, cooled to 4ºC and subjected to further analysis.

HPLC analysisfor vitamin C determination

Samples were analyzed by HPLC system which consist of column oven and an ultraviolet (UV) detector at 230 nm. The HPLC technique is composed of a Shimadzu LC solution 20A technique with UV detector at 230 nm. Mobile phase combination was examined with Acetonitrile 40% and KH_2 PO_4 60% at pH=3. The Superspher C18 column was used (250×4.6 mm) and the mobile phase (pH=3.0) by dissolving 1.5 g sucrose in 500 mL of acetic acid (99.8%) with a good mixing. Then the routine evacuation of the mobile phase was achieved by passing it through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) and the flow rate was 1.0 ml/ min at 25^{o}C. The low temperature 25^{o}C was used in this study to achieve the stability of ascorbic acid and get ride from decomposes by increasing temperature. The injection volume was 20 µl **(Aliaa *et al.*, 2019)**.

Physico-chemical analysis

The physico-chemical analysis of processed orange juice such as pH, acidity, percentage of total soluble solids (TSS), total sugar, and Vitamin C were studied. pH was measured by using a pH meter (Net ohm 827, pH lab model, Swiss). The TSS of juice and beverage was measured with a digital refractometer (Atago, Japan). Acidity was determined using conventional titration method **(AOAC, 2000)**. Total sugar content of the juice was estimated using the method of **Madan *et al.* (2010)**.

Sensory evaluation

Sensory evaluation of the juice 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 group of encoded cups containing 50 mL of juice, with different proportions of both white stevia crystals and dried raw powder of stevia plant were evaluated to team panelists, then the intensity of each sensory feature was rated between 0 (unmarked intensity) and 5 (Very strong intensity). Results were analyzed statistically, all trials were conducted in triplicate and the results were reported as mean values **(Agarwal *et al.*, 2010)**.

Statistical analysis

The Statistical Analysis System **(SAS 2012)** program was used to demonstrate the effect of difference factors on all sensory properties in this study. A less significant difference -LSD test enabled a significant comparison between means of the various factors in this study.

RESULT AND DISCUSSION

The evaluation of Stevioside in Stevia leaves

Stevia extract has been used as a dietary supplement and is one of the sweetest and lowest calorie sweeteners, due to the main sweetener ingredients for stevia are Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A, which are 200-300 Sweetest times of sucrose **(Asrul *et al.*, 2013)**. Stevia extract (Steviol glycosides) steviol glycosides are extracted from leaves of the plant Stevia RebaudianaBertoni as shown in (Figure 1, 2 and 3). It was founded that Stevioside,Rebaudioside and total steviol were 5.26, 2.587 and 7.86% respectively as in (Figure 4). Previouse study was founded the main constituents in stevia plant extracts as expressing high sweetness intensity as stevioside and Rebaudioside A according to **(Madan *et al.*, 2010)** and are also known as bio-sweeteners **(Goyal *et al.*, 2010)**. These are shelf stable in solid form and have better stability than aspartame and acesulfame-K in liquid form. In the beverages processing, were showed an excellent stability under normal conditions, whereas chemical degradation occurs under extreme conditions of high temperature and pH by **(Kroyer 2010)**. There is no evidence of steviol accumulation in the body from successive ingestions **(Wheeler *et al.*, 2008)**. Nutritional and toxicological studies indicated that Stevioside or Rebaudioside A for Stevia have no health effect and do not pose a serious health threat to different animals by **(Toskulkao *et al.*, 2013).**

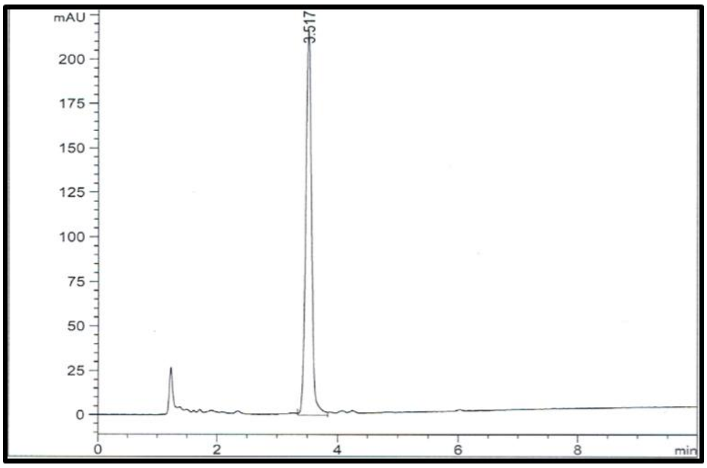

Figure (1): HPLC chromatogram for Stevia (Stevioside standard) **(Hamdia *et al.*, 2019**).

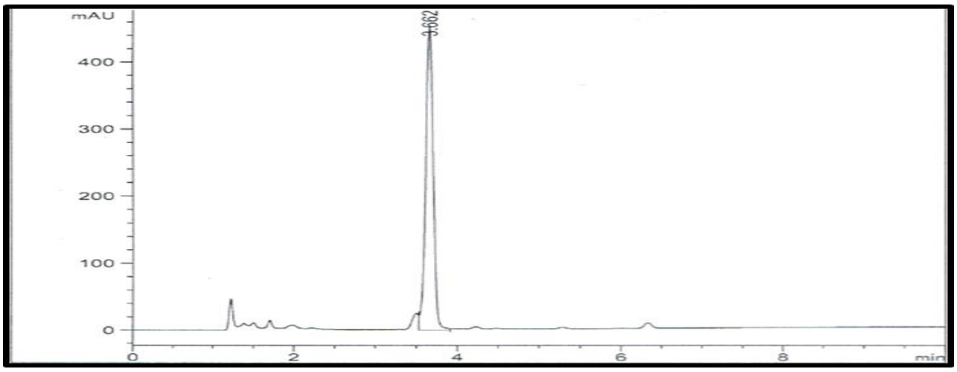

Figure (2): HPLC chromatogram for Stevia (Reb A standard) **(Hamdia *et al.*, 2019).**

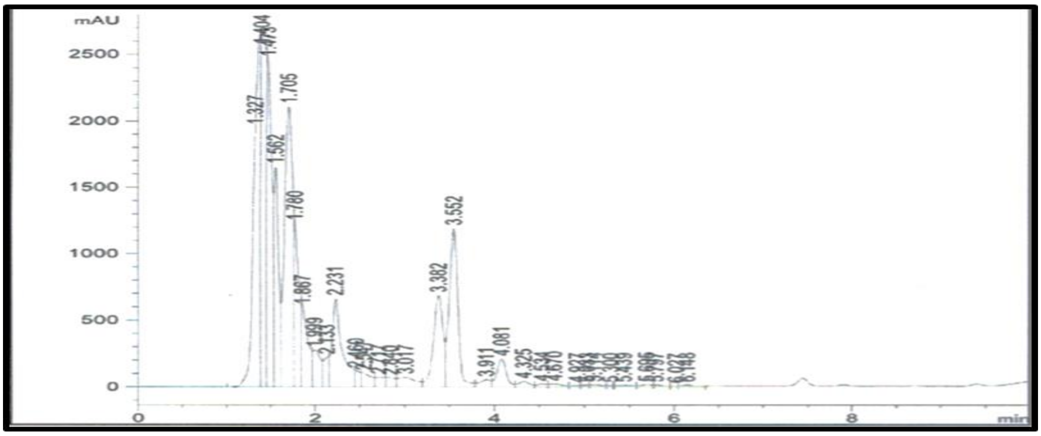

Figure (3): HPLC chromatogram for stevia leaves **(Hamdia *et al.*, 2019)**.

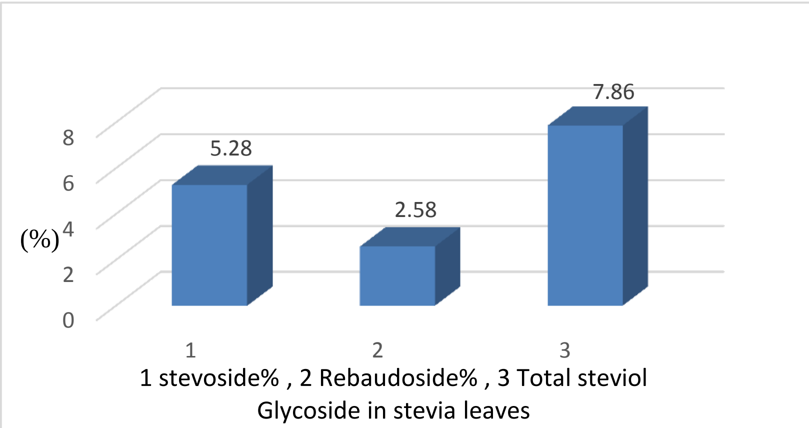

Figure (4): shows the percentage of Stevoside, Reb A and the total steviol (St and Reb A) in stevia leaves.

Globally, scientists have proved through long-term research that it is safe to eat natural stevia instead of artificial sweeteners for all age groups for humans. It was Proved that eating stevia leaves or any other form of plant extract for stevia such as stevioside, rebaudioside A and steviol glycosides is safe use by the U.S. Food and Drug Administration and approved as a dietary supplement considered (generally recognized as safe) in the United States (GRAS Notification 287) for Steviol Glycosides with Rebaudioside A and Stevioside as the main components and the appropriate allowances daily intake (ADI) is 25 mg/kg body weight per day **(Goyl *et al.*, 2010)**, following 100-fold safety factor, commonly seen in ADI values in rats which is around 7.9 mg/kg in humans. Also, it was shown that daily human consumption of 5 to 6 mg of Stevia leaf extract as a dietary sweetener per kg of body weight is safe **(Chattopadhyay *et al.*, 2014)**. In addition, currently the Joint Committee of Experts between the Food and Agriculture Organization and the World Health Organization on food additives (JECFA) conducted extensive scientific studies of all available scientific data and concluded that stevia is safe to eat in different foods and drinks instead of other sweeteners, which have side effects on public health. Also, **Ena *et al.* (2013)** pointed that a daily intake of steviol glycoside of up to 4 mg/kg of body weight was safe. Other study proved that stevia used as a natural, sweetener that does not lead to obesity and is considered a safe alternative to sugar, and also has no side effects on human health after consumption **(Assaei *et al.*, 2016)**.

Estimation of vitamin C in packed juices with HPLC technique

Supplementation by ascorbic acid to prevent oxidation by decreasing the available oxygen in the medium. Ascorbic acid is preferably used as antioxidant that oxidized to the dehydroascorbic acid (DHA) form, thus prohibition the oxidation of the matrix **(Asrul *et al.*, 2016)**. Preferable technique HPLC in this research does not need any reagent or sample preparation, simple and having less time. In addition, it is possible to analyze more samples concurrently than other old methods. The particularity of use HPLC instrument makes them an ideal quality control tool for many food, raw material, fruit, fruit juice and pharmaceutical industries **(Aliaa *et al.*, 2019)**. Furthermore, other methods do not determine all amount of ascorbic acid due to the reduction of dehydro-ascorbic acid, so ascorbic acid by high temperature of processing so it cannot be measured well **(Aliaa *et al.*, 2019)**. Vitamin C determination of this study was presented in (Table 3).

Effect of adding Stevia instead of sugar onphysico-chemical Properties in processed orange juice

Physico-chemical parameters are the determinants of the quality of the foodstuff produced, in particular in fruit-based juices and drinks **(Aliaa *et al.*, 2019)**. Among them, TSS is especially important in both fruit juice and milk fruit mix due to its effect on both sensory and immutability aspects. Results were presented the effect of sucrose substitution with *Stevia* on physio-chemical properties of produced orange juice as in (Table3). The results of the study showed no significant differences in the vitamin C content in the juices produced due to heat treatment. This was inconsistent with the findings of **(Ranu & Uma 2012)** who showed significant loss of vitamin C content from pomegranate juice has been reported when heat treatment. It was founded no significant difference (P<0.01) in moisture, pH and acidity with the different samples. With respect to the total sugar values of all orange juices treatments,there was a significant difference (P<0.05) between the different treatments. It was notice that the powder of leaf stevia in orange juice making is not good as stevia's white crystals powder, due to the change the color of the orange juice to unacceptable green-brownish and to the bad flavor and taste. This darkened color and bad taste could be associated with nonenzymatic processes with the formation of caramel colored pigments **(Cadena *et al.*, 2013)**.

Table (3): Physico-chemical properties of different stevia replacement instead of sucrose in orange juice making.

Parameters	Orange juice with sugar gm/100mL (Control)	White stevia gm/100mL	White stevia (%)			Leaf stevia powder 100mL	Leaf stevia powder (%)			LSD value
	Percent of different addition of stevia									
	13.583	2.5000	1.3	1.8	2.3	2.5	1.3	1.8	2.3	2.48 *
Vit. C (mg/mL)	2.01	1.95	2.05	2.04	1.95	1.95	1.93	1.92	1.90	0.276 NS
TSS	17.02	14.05	13.90	13.85	14.0	15.56	14.54	14.86	15.50	2.09 *
Moisture	94.50	93.56	93.86	93.56	92.80	90.55	90.65	91.06	90.26	5.27 NS
pH	2.80	2.73	2.75	2.74	2.74.	2.85	2.86	2.85	2.90	0.286 NS
Acidity	0.56	0.52	0.51	0.53	0.52	0.58	0.51	0.52	0.52	0.188 NS
Total sugar	3.88	7.78	7.55	7.65	7.72	7.56	7.45	7.59	7.64	2.16 *

* (P≤0.05)=significant, NS: Non-Significant.

Effect of adding Stevia instead of sugar on the sensory evaluation in processed orange juice

Orange juices with fresh stevia powder and crystal stevia powder (white powder) was evaluated for color, flavor, taste, sweetness and bitterness by 10 panelists of the staff members of Food processing product/ Abu-Ghraib/ Ministry of Element & Industry/ Baghdad/Iraq. The sensory evaluations were determined according to the method described by **(Agarwal *et al.*, 2010)**.

Color

The analysis of the color of the produced juice is an important factor as it affects the acceptability of the product and is directly affected by the raw materials used in the composition **(Ozdemir *et al.*, 2015)**. Results of this study was presented in (Table 4), it was found the color of the low concentration of crystal stevia powder significantly acceptable compared with control. While the powder of stevia leaf were given lowest score by all the 10th panelists even with low concentration of stevia powder.

Flavor

Flavor Analysis of the juice is an important factor which affects the acceptability of the product and is directly influenced by the raw materials used in the formulation. Results of this study was presented in (Table 4), it was found the flavor of the white crystal stevia powder significantly acceptable compared with control. While fresh powder of stevia leaf were given lowest score flavor by all the 10th panelists even with low concentration of stevia powder.

Taste

Among the arguments in favor of allowing the use of steviol glycosides most important are their natural origin, non-toxic, high solubility, stable in aqueous solution over a wide range of pH values and temperatures, non-fermentative, safety for diabetics and people with phenylketonuria **(Tondare & Hembade 2019)**. It was found with the taste of the 2.5% of crystal stevia's powder gives lower score than 1.3% compared with control as shown in (Table 4). In contrast, stevia's leaf powder juices gives worse taste and it is rejecting able by most of the panelists as shown in (Table 4).

Sweetness and Bitterness

The concentration of stevia most widely accepted by consumers varies with different food products **(Ena *et al.*, 2013)**. It has been proven that Stevia's 0.25% concentration is the best receptive to fermented dairy products **(Agarwal *et al.*, 2010)**. Two high stevia concentrations were previously found to correlate with the bitter taste of the resulting juice **(Rajpreet *et al.*, 2016; Sukhmani *et al.*, 2018)**. Result was founded a significant difference ($P \leq 0.05$) in panelist ratings for sweetness sugar-replacement crystal stevia's juice compared with control. Previous study appeared that the amount of crystal and powder stevia juice added to the formulations is theoretically equal to the amount of sucrose in the basic formulation of the normal basic forma of orange juice, since stevia product is 250 time sweeter than sucrose **(Chatsudthipong *et al.*, 2014)**. Also it was founded processed juices with fresh powder of stevia leaf gives lowest score by all the 10th panelists even with low concentration of stevia powder. The reason for the low evaluation in the sweetness and bitterness properties of the juice to which the dry powder of stevia is added is due to the pungent taste and the strong flavor of stevia, which negatively affected the acceptance by the panelists as shown in (Table

4). While, results of juices sweetened with white stevia extract powder gave a high affinity for glucose with regard to taste properties, smells, flavor, texture and general impression (P<0.05). Therefore, white stevia crystals with low concentrations are desirable for drinks because of the sweet and desirable taste and free from any unpleasant flavor, especially for those who are concerned with public health in terms of free staining of the, bitter taste, and mineral flavor. Result of this study is accordance with **(Agarwal *et al.*, 2010)**. Also, used high stevia concentrations were correlated with the bitter taste and aftertaste of the resulting juice as mentioned by **(Madan *et al.*, 2010; Sukhmani *et al.*, 2018)**.

Table (4): Effect of replacement of Sucrose with Stevia on the sensory evaluation of orange juice**.**

Sensory Evaluation	**Orange juice with sugar gm/100mL (Control)**	**White stevia gm/100mL**	**White stevia (%)**			**Leaf stevia powder 100mL**	**Leaf stevia powder (%)**			**LSD value**
	Percent of different addition of stevia									
	13.583	2.5000	1.3	1.8	2.3	2.5	1.3	1.8	*2.3*	2.48 *
Color	5	5	5	5	5	0	2	0	0	1.75 *
Flavor	5	3	3	2	2	0	2	0	0	1.44 *
Taste	5	1	4	3	1	0	3	0	0	1.51 *
Sweetness	4	1	4	3	2	0	2	0	0	1.38 *
Bitterness	1	0	4	3	1	0	1	0	0	1.72 *
* (P≤0.05), NS: Non-Significant.										

CONCLUSION

Large quantities of table sugar and industrial sweeteners are used in many processed food products. In parallel, many diseases have increased for all segments of society, including young people and children, which raise the concern and interest of consumers and manufacturers to rely on high - density natural sweeteners in the world of beverages and juices of various kinds. Results showed the white crystal stevia's powder with small amount is a good choice, for replacing sucrose, for the development of different artificial fruit juices. So, it recommended that stevia is an excellent sweetener and can be used instead of sucrose which used in beverage processing. This study concluded that overall sensory properties affected by possible interactions between natural sweetening components of the processed fruit juice. Therefore, more research and investigation is required to produce drinks using natural, calorie-free sweeteners without affecting the physic-chemicals and sensory properties using other ingredients that overwhelm the sharp taste of stevia. .

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