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EFFECT OF USING STEVIA LEAF POWDER IN THE MANUFACTURE OF A DAIRY PRODUCT (CREAM) AGAINST THE MICROORGANISMS THAT CAUSE FOOD SPOILAGE AND THEIR ACCEPTANCE BY THE CONSUMER

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ABSTRACT

The increase in obesity and the many accompanying diseases is attributed to the increased production and consumption of foods made of non-nutritive sweeteners without regard to the risks of consuming additional calories, and this in turn leads to hormonal imbalance and metabolic disorders and the resulting imbalance and ill health that have spread to all segments of society. During the research, 0.01, 0.02, 0.03, 0.04 and 0.05 % of stevia sweetener was added to the cream instead of the sugar used. Physical and chemical tests were performed for the stevia extract and the microbial content in the cream, as well as the sensory evaluation. It was noted that fortifying the cream with calorie-free stevia sugar led to the production of a cream with excellent texture, color and taste, with a very desirable sweetness and flavor, and the absence of any strange taste or flavor.

Keywords: Stevia sweetener, physicochemical analysis, microbial content, sensory evaluation.

تأثير استخدام مسحوق اوراق نبات Stevia في تصنيع احد منتجات الالبان (القشطة) تجاه الاحياء المجهرية المسببة لتلف الاغذية ومدى تقبلها من قبل المستهلك

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الخلاصة

تعزى زيادة السمنة والأمراض العديدة المصاحبة لها إلى زيادة إنتاج واستهلاك الأطعمة المصنوعة من المحليات غير المغذية دون اعتبار لمخاطر استهلاك سعرات إضافية، وهذا بدوره يؤدي إلى اختلال التوازن الهرموني واضطرابات التمثيل الغذائي وما نتج عن ذلك من اختلال في التوازن والاعتلال الصحي الذي انتشر وفي جميع شرائح المجتمع، وتم خلال البحث إضافة 0.01 و 0.02 و 0.03 و 0.04 و 0.05٪ من محلي الستيفيا إلى القشطة بدلاً من السكر المستخدم، وتم اجراء الفحوصات الفيزيائية والكيميائية لمستخلص الستيفيا والمحتوى الميكروبي في القشطة، فضلا عن التقييم الحسي، ولوحظ أن تدعيم القشطة بسكر الستيفيا الخالي من السعرات الحرارية ادى إلى إنتاج كريم ذو قوام ولون وطعم ممتازين مع حلاوة ونكهة مرغوبة جدا وعدم ملاحظة أي طعم او نكهة غريبة.

الكلمات المفتاحية: محلي الستيفيا، تحليل فيزيائي-كيميائي، محتوى ميكروبي، تقييم حسي

INTODUCTION

Milk or any of its dairy products is the main supplier of most nutritional compounds in the necessary, desirable and appropriate condition for all ages, especially for children and youth. Dairy products can provide essential proteins, necessary minerals and vitamins **(Vijayalakshmi *et. al.,* 2010)** in the desired condition and suitable for all ages. One of the dairy products is consumed, cream as a basic meal in the breakfast of most Iraqi families, in addition to its use in many pastries, sweets and various diets in Iraq **(Amer *et al.*, 2018)**. Cream is made in Iraq by the General Company for Dairy Products, and it is widely consumed in the Iraqi market, along with the surplus importer from neighboring and foreign countries **(Hamdiya *et al.*, 2019**) due to its cheap price and low income for Iraqi families despite the high percentage of this product and its known health damages **(Amer *et al.*, 2018)**. However, quality control is conducted on this product by the Central Organization for Standardization and Quality Control, as its taste, smell and texture changes. This requires conducting a lot of studies and research by fortifying this product with various healthy plant-based nutritional supplements to reduce the high fat content, which ranges from 25-30%, and replacing the added sugar with natural sweeteners without calories **(Raheleh *et al.*, 2016)** in order to reduce and avoid their health damage to the consumer **(Moussa *et al.*, 2010)**. Several centuries ago, the use of alternative sweeteners appeared in the development of most food products due to the increased consumer demand for low-calorie foods, or due to the excessive use of sugar in the diet, which led to metabolic disorders such as obesity, diabetes, cardiovascular disease **(Aureli *et al.*, 2011)** and diabetes-induced renal disorders **(Cansu *et al.*, 2011)**. Sucrose is the main sweetener that is used in dairy products, including cream and ice cream, but it contains glucose, and for diabetics, the amount of sucrose in their diet should be reduced. And other study have proven the effectiveness of stevia on pancreatic tissues and works to raise insulin and thus reduce blood sugar through Peroxisome Proliferators-Activated Receptor PPAR$^{\gamma}$, as well as the properties of stevia acting as antioxidants **(Raheleh *et al.*, 2016)**. Therefore, use stevia as an alternative to sugar because it has little effect on the level of sugar in the blood. It is a good alternative for people who follow a carbohydrate-controlled diet. Therefore, stevia is considered a natural and safe sweetener **(Puri, 2012; Balwinder *et al.*, 2014; Katarzyna *et al.*, 2015)**. Its leaves are 300 times sweeter than sucrose, as well as stable warmth and acidity **(Vishal & Dinesh, 2018)**. It also proved suitable for diabetics and hypoglycemia **(Mathur & Johri, 2016; Al-Hamdani 2019)**. Stevia has been accepted as a substitute for sugar in ice cream and whipped cream due to its sugar-like taste and often slightly aftertaste **(Alizadeh *et al.*, 2014)**. This study was conducted to study the possibilities of producing a low-calorie cream using some natural sweeteners such as stevia that replace added sugar in the manufacture of this product and the extent of its acceptance by the consumer.

MATERIALS AND METHODS

Stevia leaves preparation

Stevia leaves were supplied from Medical & Aromatic Plants Research Baghdad/Baghdad/Iraq. The plant was identification and authenticated by Research Unit/College of Agricultural Engineering Sciences/University of Botanical Survey of Agriculture College (No.BSI/ SRC/5/23/2010-11/Tech-1585). The fresh leaves were carefully cleaned to remove the dirt, washed several times with running tap water, after that rinsed with distilled water, then dried in shade and finally powdered by grinding by using an electric blender, then was kept in a sealed polyethylene bags and stored at 4 ± 1°C in a refrigerator until it used **(Abou-Arab *et al.*, 2010)**. One hundred grams of dried powdered sample was

extracted with 500 mL of distilled water for 12 h, and then filtered and phytochemical screening of the filtrate was carried out. Proximate analysis was carried out on dried powder.

Stevia's dose calculation

Natural sweetener stevia 100% (Iranian product that is available in local markets was used in this study. The amount of stevia was determined to fortify the cream product by calculating the sweetness of stevia 300 time more than sucrose, and according to the Pearson square ration formulation, 0.01 to 0.06% was added in 0.01% increments. While the sample was prepared from the cream syrup taken from the excreta for the production of regular cream, and sucrose was added as a control treatment for comparison with the stevia-supplemented sample.

Experiment

The cream mixture has been prepared so that it contains 24-28% fat. 2.5% protein was then pasteurized at 65°C with good stirring for 20 min. Then it is naturalized with a homogenizer, then it is packed and stored in the laboratory refrigerator until the required analyzes are carried out. So that 5 L of cream manufactured in the General Company for Dairy Products / Abu-Gharib / the capital, Baghdad, was taken from the homogenizer of the company. Milk is prepared for it from government milk collection centers from all sides of the Abu-Gharib / Baghdad area. The different concentrations of dry stevia's crystals were calculated: 0.01, 0.02, 0.03, 0.04, 0.5 and 0.06 g /100 g then added instead of the regular sugar used in the manufacture of cream. The control sample was made by adding sucrose without stevia for comparison. 10 sample were made for each concentration and poured into plastic containers having a capacity of 200 g. and stored at 4°C for 24 h and 10 day. Then, cream containers were sealed using a machine equipped from the German company Gasti with heat press. The chemical assessments of fresh milk and cream sample were made on the next day of manufacture according to the procedure of **(AOAC, 2005)**. Then the total number of bacteria, coliform bacteria, yeasts and molds were estimated according to American Public Health Assoc. **(APHA, 1992)** and the number of bacteria that formed spores according to the method **Bourne (2002)**. The sensory evaluation form was used according to the method **Blanc & Odet (1981**).

Phytochemical screening

Phytochemical screening procedures were carried out by the method of **(Sato *et. al,* 2000)**. The crude stevia rebaudiana extract (SME) were screened for the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, triterpenes, glycosides, catechin, coumarin, quinone and xanthoprotein.

Aqueous extraction of stevia leaves powder

Three grams of stevia rebaudiana powder were transferred to a 250 mL beaker; 100 mL of distilled water was added and boiled for five minutes. Then, the material was decanted and filtered under vacuum. The procedure was repeated twice with 100 mL and 50 mL of water, respectively. After the last filtration, the extract was transferred to a 250 mL volumetric balloon and filled up with distilled water. It was used this extract to analyze glycosides, phenolic compounds and flavonoids.

Alcoholic extraction of stevia leaves powder

Stevia rebaudiana powder 3 g were extracted once with 45 mL of 80% ethanol for 20 min by ultrasound. The ethanol extract was then filtered and ethanol was added to a final volume of 100 mL.

Microbiological study

Three bacterial strains, *Staphylococcus aureus, Pseudomonas* and *Escherichia coli* were selected for the experiments (Merck, Germany) at 37°C for 24 h. Final cell concentrations

were 108 cfu/mL according to the McFarland turbidometry McFarland turbidometry. 100 µL of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). (Merck, Germany). Three different concentrations of the *Stevia rebaudiana* with each aqueous and alcoholic extract (100, 200, and 300 mg/mL, respectively) were prepared. The sterile filter paper disks (6mm in diameter) were saturated with 50 µL of each concentration of the extract. The plates were incubated at 37°C for 24 h and the diameters of inhibitory zones were measured. The assay was carried out three times for each extract. Disks containing different concentrations of antibiotics were used as reference to compare the sensitivity of each tested bacterial species **(Hsieh *et al.*, 2001)**. Yeast and mold were determined according to the Standard Methods for Examination of Dairy Products by Iraqi Quality Standard (*The Central Agency for Standardization and Quality Control, Iraqi Standard No.* 609, 1988). For microbial analysis, all creams samples were homogenized and serial dilution were prepared. Creams samples were analyzed at 1st and 10th day. All relative data were transferred using a based-10 logarithm.

Microbiological analysis

The resultant Cream products were microbiologically examined for total bacterial count, mold and yeasts count and coliform group according to American Public Health Association **(Vinderola & Reinheimer, 2000)**.

Sensory evaluation

Cream with fresh stevia powder was evaluated for color, flavor and taste by 10 panelists of the staff members of dairy and processing of Food product Abu-Ghraip/ Ministry of Element & Industry/ Baghdad/Iraq. The sensory evaluations were determined according to the method described by **(Blanc & Odet, 1981)**.

Proximate composition

Proximate composition was carried out according to the procedure of Association of the Official Analytical Chemist **(AOAC, 2005)**.

Statistical analysis

The Statistical Analysis System- program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study **(SAS, 2012)**.

RESULTS AND DISSCUSION

Physio-Chemical composition of used milk

It was found there were no significant differences in measuring chemical contents of milk that used in flavored milk making with different methods as shown in (Table 1).

Table (1): Chemical composition of used milk in cream processing in this study.

Parameters (%)	Methods of analysis			LSD value
	Funke Gerber	Ultrasonic Milk analyzer	Traditional methods	
Protein	2.35±0.06 b	2.45±0.08 b	2.76±0.08 a	0.287*
Fat	2.10±0.15	2.09±0.05	2.08±0.06	0.261 NS
NFSM	6.37±0.28 ab	6.85±0.19 a	6.32±0.22 b	0.409*
Lactose	3.89±0.05 b	4.46±0.12 ab	5.07±0.14 a	0.773*
Density	23.00±1.53	25.30±1.48	24.22±1.08	3.694 NS
Freezing point	-0.380±0.07	-0.411±0.09	-0.399±0.06	0.188 NS
pH	-	-	6.4±0.17	---
Acidity	-	-	11.5±0.09	---
Means having with the different letters in same row differed significantly. * (P≤0.05).				

Each unit were triplicates; NFSM=Non- fat solid material.

The results of this study showed the chemical analysis of cream samples immediately after filling and packing tightly by using the machine (heat closing) as shown in (Table 2), and

it is noted that its percentages are identical to the percentages of the Iraqi standard quality **(ISQ, 1988).**

Table (2): Chemical components of the cream manufactured immediately after filling.

Chemical Tests	(%)	Iraqi standard specifications
Moisture	64.0 ±2.59	-
Fatty substances	28.5 ±1.08	Not less than (30%)
Protein	2.1 ±0.05	-
Lactose	2.48 ±0.09	-
Non-Fatty solids substance	3.85 ±0.13	Not more than (8%)
Ash	0.30 ±0.02	-
pH	6.75 ±0.22	-
Acidity	11.6 ±0.16	-

Each unit were triplicates; NFSM=Non- fat solid material.

Results of the chemical analysis of stevia leaf powder are shown in (Table 3). The results of the study proved that stevia is local, nutritious, and very healthy for diabetics because it has very low calories **(Mathur & Johri 2016)**. A study **(Al-Hamdani, 2019)**, also demonstrated that stevia contains two compounds of stevioside and ribodioside that are high in sweetness. As well as it contains many active and vital substances, which are mentioned later in the following (Table, 3).

Table (3): Chemical composition of stevia leaf powder

Chemical composition	
Chemical content	Amount (g./kg^{-1})
Moisture	0.66±0.04
Protein	0.88±0.07
Fat	0.49±0.02
Crude fat	1.32±0.08
Total carbohydrate	6.26±0.28
Ash	1.04±0.06
Iron	0.28±0.02

Each unit was triplicates.

Phytochemical screening

Result of this study showed the phytochemical analysis of the stevia's leaf extract revealed the presence of alkaloids, tannins, flavonoids, glycosides, saponins, quinone and triterpenes as in (Table, 4). The most abundant compounds in the alcoholic leaf extract were the flavonoids and glycosides. Tannins are seen in least amount. The test for catechins, coumarins, quinones and xanthoproteins showed negative result. The results of this study came close to what was found **(Kujur *et al.*, 2010)**.The presence of these bioactive compounds suggests that the plant might be of industrial and medicinal importance (**Sheeja & Beena, 2015**).

Table (4): Phytochemical scanning of Stevia rebaudiana leaf with ethanol extract.

Phytochemical constituents	Result
Alkaloids	++
Flavonoids	+++
Tannins	++
Glycosides	+++
Catechin	-
Coumarins	-
Saponins	+
Quinone	-
Triterpenes	++
Xanthoproteins	-
LSD value	1.066 *

+++ Strongly present; ++ Present; + Weakly present; - Absent (**Hamdia *et al.*, 2019**).
* (P≤0.05).

Microbial study

Microbial count was studied for the cream fortified with different concentrations with stevia and stored for 1 and 10 d as shown in (Table 5). The results showed that the total count and coliform bacteria and spore bacteria were within the limits permitted by the Iraqi standard. Molds and yeasts were also not detected in most of the samples, due to good sanitary conditions during manufacture and storage. However, a number of denominations were found in the cream samples fortified with high concentrations of stevia, and after storage 10 day, the reason may be attributed to the increased sweetness due to the increased concentration, which led to the growth of molds known to favor sugars. These results were similar to what they found **Meyer & Riha (2002); Mona *et al.* (2005)**, as stevia inhibits the growth and reproduction of some bacteria that cause tooth decay and gum disease.

Table (5): Microbial content in Stevia's cream product after 1 and 10 day on refrigerator temperature.

Treatments Mg/g milk	Total bacterial count (cell /g)		Coliform bacterial numbers (cells / g)		Spores bacterial numbers (cells / g)		Yeast and mold numbers (cell /g)	
	24 h	10 d	24 h	10 d	24 h	10 d	24 h	10 d
Control 3.5g/50mL milk	Zero	1×10^{2}	Zero	Zero	9	70	Zero	Zero
A (0.1)	Zero	Zero	Zero	Zero	8	80	Zero	Zero
B (0.2)	Zero	Zero	Zero	Zero	5	60	Zero	Zero
C (0.3)	Zero	Zero	Zero	Zero	2	30	Zero	Zero
D (0.4)	Zero	Zero	Zero	Zero	4	40	Zero	Zero
(0.5) E	Zero	10^{-2} heavy	Zero	1×10^{-3}	5	50	Zero	3
(0.6) F	1×10^{-1}	1×10^{-2}	Zero	2×10^{-2}	5	50	Zero	4
LSD value	8.04*	21.47*	0.00 NS	17.41*	5.61*	14.79*	0.00 NS	3.05*

Sensory evaluation

The results of the sensory evaluation of this study showed (Table 6) that the cream is thermally covered and stored at a temperature of 4°C. maintained its sensory characteristics well during 10 d of storage period. The flavor consists of the combination of taste by the tongue and the smell that is perceived by the nose, as there is a sense of smell and the response by the receptors in the oral cavity to chemical stimuli **(Born, 2002)**. (Table 4) shows the flavor and taste scores for control and treated cream with different concentration of stevia. Treated Cream was significantly ($P < 0.05$) different from the control Cream. From the following table, it can be seen that the cream added to it 0.01, 0.02, 0.03 and 0.04 of stevia had the highest values for flavor and taste (43, 43, 43 and 41) while the cream with added stevia at concentrations of 0.05 and 0.06 decreased the flavor and taste values to (38 and 33), respectively.The low score for flavor of treated cream with 0.5 and 0.6% could be due to flavor binding capacity of stevia. The results were identical to what he found **Bourne (2002); Friedeck *et al.* (2003)**. The results were also similar to **Alizadeh *et al.* (2014)** where they found that the complete substitution of sugar with stevia reduces the degree of sweetness, and the reason was attributed to several factors such as concentration, ingredients and the temperature of cream manufacture affecting the sweetening strength and persistence of sweetness by stevioside. The results of the study showed that 0.03 and 0.04% of stevia was added to the cream with the light sweet taste desired in the standard cream without any strange flavors. Based on that, there is the possibility of concluding that the cream supplement with stevia meets the desired taste as well as is rich in active ingredients and is available for people who suffer from many different diseases, especially diabetics. The results were similar to what he found **Skripleva & Arseneva (2015); Mohamad *et al.* (2018)**, but on the yogurt and whipping cream product respectively. The visual appearance of food is very necessary for the consumer to accept any product, and color is the first feeling that the consumer perceives and uses it to accept or reject the product according to **Lyon *et al.* (2006)**. (Table 6) shows the color scores for control and treated

cream with different concentration of stevia. From the following table, it can be seen that the cream added to it 0.01, 0.02, 0.03 and 0.04 of stevia had the highest values for color (8, 9, 9 and 9) while the cream with added stevia at concentrations of 0.05 and 0.06% the colors score decreased significantly (P≤0.05) to 7 and 6, also it was notice the color changed to a bale color, may be due to somewhat increased microbial content especially after 10 d of storage time.

Table (6): Sensory evaluation scores of standard covered cream-fortified stevia samples during production and stored in the refrigerator for 1 and 10 d.

Treatment	Storage time/d	Characters	Standard degree of storage	Given score/d of storage	LSD value
Flavor and taste					
Control sample	After 1 d		45	44	5.62 *
	After 10 d			42	
A = 0.01	After 1 d			43	
	After 10 d			43	
B = 0.02	After 1 d			43	
	After 10 d			42	
C = 0.03	After 1 d			43	
	After 10 d			43	
D = 0.04	After 1 d			41	
	After 10 d			40	
E= 0.05	After 1 d			38	
	After 10 d			37	
F= 0.06	After 1 d			33	
	After 1 0 d			30	
Look and Texture					LSD value
Control sample	After 1 d		30	27	4.07 *
	After 10 d			26	
A = 0.01	After 1 d			25	
	After 10 d			22	
B = 0.02	After 1 d			24	
	After 10 d			23	
C = 0.03	After 1 d			26	
	After 10 d			26	
D = 0.04	After 1 d			26	
	After 10 d			24	
E= 0.05	After 1 d			22	
	After 10 d			20	
F= 0.06	After 1 d			18	
	After 1 0 d			14	
Color					LSD value
Control sample	After 1 d		10	9	1.16 *
	After 1 0 d			8	
A = 0.01	After 1 d			9	
	After 1 0 d			8	
B = 0.02	After 1 d			9	
	After 1 0 d			8	
C = 0.03	After 1 d			9	
	After 1 0 d			8	
D = 0.04	After 1 d			9	
	After 1 0 d			9	
E= 0.05	After 1 d			7	
	After 1 0 d			7	
F= 0.06	After 1 d			6	
	After 1 0 d				
Salty taste					LSD value
Control sample	After 1 d		10	9	1.02 NS
	After 1 0 d			9	
A = 0.01	After 1 d			9	
	After 1 0 d			9	
B = 0.02	After 1 d			9	
	After 1 0 d			9	
C = 0.03	After 1 d			9	
	After 1 0 d			9	
D = 0.04	After 1 d			9	
	After 1 0 d			9	
E= 0.05	After 1 d			9	
	After 1 0 d			9	
F= 0.06	After 1 d			9	
Packing					LSD value
Control sample	After 1 d		5	2	1.05 *
	After 1 0 d			2	
A = 0.01	After 1 d			2	
	After 1 0 d			2	
B = 0.02	After 1 d			2	
	After 1 0 d			2	
C = 0.03	After 1 d			2	
	After 1 0 d			2	
D = 0.04	After 1 d			2	
	After 1 0 d			2	
E= 0.05	After 1 d			2	
	After 1 0 d			2	
F= 0.06	After 1 d			2	

* (P≤0.05).

CONCLUSION

In conclusion, it was possible to make stevia Cream by replacement about 100% of sucrose with natural sweeteners without significant effects on cream quality. Cream has a sweet flavor without foreign tastes and odors with 0.03-0.04 % stevia. Based on these studies it can be concluded that Cream supplemented with such natural sweeteners, is available for people suffering from diabetes, especially children and overweight consumers.

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