Page 228 to 235

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Effect of the Addition of Probiotics and Sucrose Replacement by Stevia on the Physicochemical, Nutritional and Sensory Properties of Yogurt

Galvis López Elizabeth Bogotá[1], López Ortiz Norma Constanza[2]* and Novoa Castro Carlos Fernando[3]

[1]Galvis López Elizabeth Bogotá, Colombia,

[2]López Ortiz Norma Constanza Department Chemistry, Bogotá, National University, Colombia

[3]Novoa Castro Carlos Fernando Institute of Food Science and Technology ICTA Bogotá, National University, Colombia

***Corresponding Author:** López Ortiz Norma Constanza, Department Chemistry, Bogotá, National University, Colombia.

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Abstract

Background: Two current concerns in human nutrition are obesity and the eating habits of the population. The development of food products that offer health benefits is an important option.

Objective: To determine the influence of sweetening with stevia and the addition of probiotic culture on the nutritional and sensory properties of yogurt.

Materials and Methods: Starter culture composed of Streptococcus thermophilus, Lactobacillus del brueckii subps. bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis and probiotic HOWARU®Bifido was used.

Sweeteners: Sucrose and Stevia rebaudiana. It was determined: pH, titratable acidity, proximal analysis, carbohydrates, energy content, fatty acid profile and sensorial analysis.

Results: The stevia yogurt presented the same physicochemical characteristics when sweetened with sucrose and the caloric intake was reduced by 11.5%. The stevia-sucrose and probiotic blend produced better sensory quality in flavor and taste compared to yogurt sweetened with sucrose alone. In the determination of fatty acids there were significant differences ($p < 0.05$) in acid 9c, 11t Octadecadienoic, there was effect of probiotic culture and stevia.

Conclusion: Stevia represents an alternative as a sweetener in yogurt, offering benefits such as reduced calorie intake, without affecting the sensory quality. The addition of probiotic culture improved sensory properties. yogurt, stevia, probiotic, fatty acids, polyunsaturated fatty acids.

Keywords: *Yogurt; Stevia; Probiotic; Fatty acids; Polyunsaturated fatty acids*

Abbreviations: Flame ionization detector: FID; Institute of Food Science and Technology: ICTA; World Health Organization: WHO; Conjugated linoleic acid: CLA; Metabolic syndrome: MS; High Density Lipoprotein: HDL

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Introduction

One of the areas of research in human nutrition studies milk and dairy products. In fact, many traditional dairy products have physiological activity [1]. Yogurt has been considered a good vehicle for the delivery of viable *Lactobacillus acidophilus* and *Bifidobacterium cells* [2,3]. These probiotics are live microorganisms, which produce beneficial effects on the health of consumers, such as modulation of the immune system, increased lactose digestibility, hypocholesterolemic action and control of colon cancer risk among others [4]. The WHO recommends dietary intake of milk and dairy products at a level of 180 L/person/year, but this recommendation is not met in developing countries [5,6].

Taking into account that one of the recommendations to prevent weight gain is through diets with low energetic density [7,8], stevia was chosen. It is a plant with a sweetening power almost three hundred times greater than cane sugar [9] and has been used for a variety of purposes, specially in the management of diabetes, since it reduces plasma glucose and insulin levels, suggesting that stevia may help regulate glucose [10]. This stevioside has little or no acute toxicity, and its use as a supplement is safe [11] and does not stimulate appetite, therefore there is no risk of weight gain in its consumption [12].

This work aimed to determine the influence of the addition of probiotic culture and total and partial replacement of sucrose by stevia on the physicochemical, nutritional and sensory properties of yogurt.

Materials and Methods

Milk: A single batch of raw fresh milk from the herd (from the Normandy breed) from the Faculty of Veterinary and Animal Science, of the National University of Colombia, was used, from which aliquots were taken for the different treatments in order to eliminate the effect of change of lot. Milk was subjected to platform tests at plant receipt: titratable acidity: 0.14% expressed as lactic acid, pH 6.8, fat content 3.2%, total solids content 11.7% and without presence of antibiotics.

Sweeteners: 99% purity commercial sucrose and Stevia rebaudiana powder, supplied by Laboratorios Vida Stevia Ltda of Bogotá, were used.

The doses (treatments) used to sweeten the yogurt were: A1: stevia 0.8 g/L, A2: stevia 0.8 g/L; probiotic culture, B1: 80 g/L sucrose at 80º Brix, B2: 80 g/L sucrose at 80º Brix; Probiotic cultivation, C1: sucrose 57.1 g/L in dilution at 80º Brix and stevia 0.4 g/L, C2: sucrose 57.1 g/L in dilution at 80º Brix and stevia 0.4 g/L; probiotic culture.

Crops: To inoculate the milk, the YO-MIX® 205 LYO 250 DCU was used as the starter culture, which is composed of *Streptococcus thermophilus, Lactobacillus delbrueckii subps. bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis* and HOWARU®Bifido probiotic culture were donated by Danisco (now DUPONT).

Physicochemical characterization

Tests for proximal analysis were performed by triplicate following the methodology established by the Association of Analitycal Chemistry [13]. Carbohydrates were determined by difference and calories were calculated with the conversion factors.

Determination of fatty acids

It was used the method suggested by Osorio JA, Ramírez C, Novoa CF, Gutiérrez LF [14] the extraction, derivatization and chromatographic analysis of the milk fat. The fatty acid methyl esters (FAME) were obtained by alkaline catalysis of the fat samples (20 mg) using 0.5M sodium methoxide in methanol. The FAME were analyzed using an Agilent 7890A gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector (FID). The analysis conditions were as follows: Helium (2.0 mL/min) was used as entrainment gas; the temperatures of the injector and detector were set at 250°C; the oven temperature was initially 60°C (1 min) and then increased to 190°C at 20°C/min, remaining at this latter temperature for 12.5 min. Samples (1.0 µl) were injected into a BPX-70 capillary column (60m 0.25 mm 0.25m; SGE, Melbourne, Australia) using a 1:20 split.

Sensory Analysis

It was performed by means of a descriptive analytic test of scores. With the collaboration of 6 trained panelists, who make up the Institute of Food Sience and Technology (ICTA) sensory evaluation panel of the National University of Colombia, which qualified the color, aroma and flavor, acidity and texture of the yogurt, on an interval response scale. The quality factor scores have been developed in the dairy plant of ICTA based on the suggested scores for sensory evaluation of yogurt, the score or rating goes according to a predicate in which the characteristics of the optimum product are described which is given the maximum score and the rating is lowered according to the defect presented according to the severity of the defect [15]. The quality scores are framed in intervals of a maximum and a minimum of quality. The scores obtained are assigned ranges according to the order they occupy in the total data set. [16]. The higher order range indicates higher sensorial quality in the evaluated characteristic. The tasters rated the samples simultaneously, in order to evaluate the effect of the sweetener and the effect of the probiotic on the sensory properties of yogurt.

Experimental Design

A completely randomized design with factorial arrangement of 2*3 was proposed, two cultures: starter culture and starter culture with probiotic and three sweeteners: stevia, sucrose and stevia-sucrose. The experimental unit consisted of lots of 20 liters of milk, all from the same tank. Total: 6 treatments with 3 replicas each, for a total of 18 trials. Elaboration of yogurt

The procedure suggested by Novoa CF and Osorio DP [17] was followed, which consists on the standardization of the fat content of the milk, homogenization at 65°C, 150 bar, heat treatment at 90°C for 5 min, inoculation with starter culture and probiotic cultures and fermentation until reaching a pH close to 4.6.

Statistic Analysis

The data of the physicochemical characterization were processed using the statistical package SAS, version 9.0. The Wilk and Shapiro test were applied to the variables evaluated to verify if they fit a normal distribution, then a Bartlett test in the case of normality and the Levene test if it did not present it ($p < 0.05$). For the variables that presented significant differences, an LSD test was done to determine the minimum significant difference. For the results of the sensorial analysis, Kruskal Wallis nonparametric test was applied with a level of significance of 0.05. The effect of the sweetener and probiotic effect on each of the sensory properties were evaluated separately. For the variables that presented significant differences, the test was performed for multiple comparisons between the averages of the sum of the grading ranges and the value found according to the calculation of the Kruskal Wallis equation [16].

Results and Discussion

Physicochemical characterization

Table 1 shows the results in percentage, obtained from the determinations of the physico-chemical analysis for the 6 treatments:

Treatments A1 and A2 were the ones that presented the highest percentages of humidity with statistically significant differences ($p < 0.05$). The A1 treatment had the lowest carbohydrate intake and the highest B2, with significant statistical differences in relation to the other treatments ($p < 0.05$).

As for protein, there were significant differences ($p < 0.05$). Treatments A1 and A2 presented the highest percentages of protein. As for pH, acidity and fat percentage, no significant differences were found ($p < 0.05$). Finally, the highest energy intake was presented in treatment B2 and the lowest in treatments A1 and A2, the differences were statistically significant ($p < 0.05$), see table 1.

Sample	Moisture	Total solid	Protein	Ash	pH	Acidity	Fat	Carbohydrates	Energy
	%	%	%	%		%	%	%	kcal
Treatment A1	87,6	12,4	3,6	0,5	4,4	0,8	3,2	5,1	63,6
Treatment B1	84,8	15,2	3,1	0,5	4,4	0,8	3,2	8,5	75,2
Treatment C1	84,5	15,5	3,1	0,7	4,4	0,8	3,2	8,5	75,2
Treatment A2	87,6	12,4	3,8	0,5	4,4	0,8	3,2	4,9	63,6
Treatment B2	83,1	17,0	3,3	0,5	4,4	0,8	3,2	9,9	81,7
Treatment C2	84,7	15,2	3,4	0,7	4,4	0,8	3,2	7,9	74,1

The percentages are average of three samples of each treatment.

Table 1: *Physicochemical characterization of yogurt.*

Determination of fatty Acids

The results by treatment obtained from the determination of fatty acids are listed in Table 2.

In the determination of fatty acids there were only significant differences in the content of cis-diphenadecenoic acid (9c, 11t), in which there was interaction of culture and sweetener in treatment A2 (with probiotic and stevia).

	Treatment					
	A1	B1	C1	A2	B2	C2
fatty acid	%	%	%	%	%	%
C4:0 (Butíric)	1,90	2,17	2,06	1,93	2,06	2,02
C6:0 (Caproic)	2,58	2,52	3,00	2,43	2,64	2,71
C8:0 (Caprílic)	0,97	1,07	1,02	0,97	1,07	0,98
C10:0 (Cápric)	2,15	2,34	2,20	2,13	2,37	2,15
C12:0 (Láuric)	2,71	2,86	2,72	2,70	2,94	2,67
C13:0 (Tridecanoic)	0,09	0,08	0,10	0,08	0,09	0,07
C14:0 (Mirístic)	10,49	10,73	10,47	10,46	11,08	10,33
C14:1 (Miristoleic)	0,85	0,88	0,81	0,85	0,91	0,79
C15:0 (Pentadecanoic)	1,37	1,34	1,30	1,35	1,37	1,31
C15:1 (Cis-10-pentadecenoic)	0,44	0,43	0,41	0,43	0,43	0,43
C16:0 (Palmitic)	29,68	29,67	29,55	29,67	30,23	29,39
C16:1 (Palmitoleic)	2,04	2,03	2,05	2,03	2,00	2,10
C17:0 (Heptadecanoic)	0,93	0,92	0,94	0,93	0,92	0,95
C17:1 (Cis-10-Heptadecenoic)	0,53	0,53	0,55	0,53	0,52	0,54
C18:0 (Esteáric)	12,35	12,09	12,47	12,41	11,81	12,55
C18:1n9t (Elaidic)	4,13	4,15	4,03	4,20	3,84	4,16
C18:1n9c (Oleic)	23,41	22,86	22,99	23,47	22,52	23,28
C18:3n6 (Linolelaidic)	0,46	0,46	0,45	0,46	0,45	0,50
C18:2n6 (Linoleic)	0,72	0,71	0,67	0,68	0,66	0,74
C18:3n3 (Linolénic)	0,34	0,33	0,35	0,40	0,33	0,38

C18:2 (9c,11t Octadecadienoic)	1,55	1,50	1,48	1,41	1,50	1,53
C20:1 (Cis-11 Eicosenoic)	0,32	0,32	0,39	0,51	0,26	0,41
%: corresponds to mg/100g of fat						

Table 2: *Fatty acid composition of different yogurt samples.*

Sensory Analysis

Table 3 shows the results of the sensorial properties of the different treatments made to yogurt:

	Depending on the Sweetener			Depending on the Probiotic	
	Without Stevia	**With Stevia**	**Stevia-Sucrose**	**Without Probiotic**	**With Probiotic**
Color	18,5a	18,5a	18,5a	18,5a	18,5a
Flavor	13,6a	17,2ab	24,6b	20,9a	16,1a
Acidity	19,0a	17,5a	19,0a	19,0a	18,0a
Texture	18,0a	19,6a	17,4a	18,4a	18,5a

*Different letters within each column denote significant differences ($p < 0,05$).

Table 3: *Average ranks of order, obtained from the scores assigned by the panelists.*

The characteristics of color, aroma and flavor and texture were preserved; there was no significant difference ($p < 0.05$) due to the probiotic effect; in the case of the sweetener, it did present significant differences for aroma and flavor characteristics, being better (higher ranking range) in the treatment with probiotic and stevia.

In the case of the probiotic effect on the color, flavor and flavor, acidity and body and consistency, there was no significant difference ($p < 0.05$), in the case of the sweetener, it did present significant differences for the characteristic of aroma and flavor, the test statistic was 6.9991, the null hypothesis was rejected according to which all three types of sweetener also value this characteristic ($p = 0.0302$).

The dose of stevia was 100 times lower than commercial sucrose, therefore the total solids intake decreased significantly in treatments containing Stevia (A1, A2, C1, C2).

Differences in protein values are possibly due to the fact that in the formulation in which sucrose is added, a dilution effect occurs due to the higher volume of the sweetener occupied compared to that occupied by stevia, reflecting a higher total protein content in stevia treatments.

As for pH, acidity and fat percentage, no significant differences were found between the treatments, which indicates that neither the sweeteners nor the culture used are involved in chemical reactions that change these characteristics.

The values of pH, acidity and fat percentage are within the limits allowed by Colombian legislation for whole yogurt [18].

There are significant differences ($P < 0.05$) in carbohydrate content, because stevia does not contribute these compounds which are the main source of energy in the formulation of yogurt.

Stevia is a non-caloric sweetener, therefore, there is a considerable decrease in energy intake in yogurt sweetened with this compound. This is important because it is possible to develop an unsweetened beverage such as the nutritional recommendation based on evidence for the prevention and treatment of overweight and obesity in adults [19]. As well as the concern was informed in the Guideline of the World Health Organization (WHO) on the intake of sugars in adults and children [20].

It has been quantified the caloric contribution of fat in the diet, but it has also been seen that the quality of its components is very important and specifically fatty acids. Until recently, the fat derived from milk and its products had been questioned. However, recent research has been identifying important functions of some fatty acids contained in these foods. In a variety of studies, conjugated linoleic acid (CLA) has been shown to have protective effects against cancer, obesity, diabetes and atherosclerosis in animals and in different cell lines [21], its effects on patients with metabolic syndrome (MS) improvement in weight and body mass index, with no effect on blood glucose, insulin resistance index and triglycerides in patients with MS. However, ingestion of CLA could significantly impair High Density Lipoprotein (HDL) in patients with MS [22]. The attraction effects of butyric acid for the removal of cancer cells in the colon [23], experimental studies with laboratory animals find this short chain acid as a potential inhibitor of the carcinogenesis process. The results obtained showed that the supplementation with butyric acid in the diet modifies the growth of colonic neoplasias causing less development of the tumor size and less frequency of aggressive behavioral neoplasias. These facts corroborate that the development and biological behavior of intestinal cancer can be modified through the manipulation of the diet [24], these are some positive properties that have been contributing information about the milk lipids. This is why it is important to identify and quantify their presence in food.

Although the composition of the dairy fat in the Bogota savanna may vary [25], the results of 22 fatty acids that were quantified in this study, are comparable with the results of other studies, having differences because the composition of the fat Milk varies according to genetic factors [26,27] and feeding the animals [28-30] among others.

In the determination of fatty acids there were only significant differences in the ALC, (C18: 2 c9t 11), in which there was effect of the addition of the culture and the sweetener in the treatment with probiotic and stevia. This indicates that under the conditions studied most of the lipids of the milk fat are not affected by the presence of the lactic cultures used (*Streptococcus thermophilus, Lactobacillus delbrueckii subps*), neither by the sucrose and stevia sweeteners as suggested in [1]. According to the authors, some additives such as sucrose, lactose, fructose and sodium chloride negatively affect the content of conjugated linoleic acid in fermented skim milk [1].

The fatty acid C18: 2 (9c, 11t Octadecadienoic) also known as linoleic acid isomer was found at levels of 1.55%. Since the first publication on the biological activities of CLA, there are many scientific publications that report on properties attributed to that fatty acid. Currently it is considered a "metabolic regulator" [31]. Studies on the effect of the change in milk matrices on the lipid profile of yogurt [32,33] are still being carried out. The results of the present study provide information on the lipid profile with the possibility of developing dairy products while maintaining sensory quality.

According to the results it can be observed that the probiotic and the stevia influence the taste and aroma characteristic of the yogurt, improving them. This is important because probiotics are a future target for the symptomatic treatment of many diseases [34] and are used by dairy companies as an innovative option to add health functionality to regular milk without significant changes in their sensory characteristics [35].

Conclusion

It is possible to obtain yogurt sweetened with stevia, with the same physicochemical characteristics as traditionally sweetened with sucrose and to reduce by 11.5% the caloric intake of this food. The addition of stevia as a sweetener, at the doses studied, does not change the characteristics of pH and acidity. The stevia-sucrose mixture produced better sensory quality in terms of the aroma and taste of yogurt. The addition of probiotic affects the sensory characteristics studied in terms of color, aroma and taste, acidity and texture.

From the above, it can be concluded that the use of stevia represents an alternative as a sweetener in yogurt. This type of product can be an option for both consumers since their consumption can help those who carry a strict calorie diet. As well as to reduce free sugar consumption, as recommended to reduce the risk of noncommunicable diseases like overweight and obesity.

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