

**UTILIZATION OF WHEY FOR THE PREPARATION OF CHOCOLATE****MILKSHAKES WITH CHIA (*Salvia hispanica* L.)<sup>1</sup>**

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**ABSTRACT**

Utilization of whey for the preparation of new food products may be an alternative to minimize environmental problems and reduce the disposal of high nutritional value by-products. This study aimed to prepare chocolate milkshakes with chia by partially replacing milk with whey. Three milkshake formulations were developed: a control formulation (without whey), a formulation containing 36% milk and 24% whey, and a formulation containing 24% milk and 36% whey. Microbiological parameters, proximate composition, energy value, total solids, titratable acidity, pH, and water activity were evaluated after preparation. The data were subjected to Analysis of Variance in a Completely Randomized Design, and means were compared by Tukey's test at the 5% significance level. Milkshakes were found to have good microbiological quality, with low counts of thermotolerant coliforms (determined at 45 °C) (< 3 MNP/g) and yeasts and filamentous fungi (< 10 CFU/g). That replacement of milk by whey resulted in a reduction in ash, lipid and energy values and an increase in carbohydrate values. One portion of the developed milkshakes was estimated to provide between 72.12 and 84.52 Kcal. There were no significant differences in titratable acidity, total solids, or water activity between formulations. It is inferred that chocolate milkshakes with chia prepared with partial replacement of milk by whey are a feasible and innovative alternative to minimize environmental impacts. Complementary studies are recommended on storage stability and sensory quality.

**Keywords:** Functional foods. Ice-cold foods. Agro-industrial wastes.

**APROVEITAMENTO DO SORO DE LEITE NA ELABORAÇÃO DE MILKSHAKES  
DE CHOCOLATE COM CHIA (*SALVIA HISPANICA* L.)****RESUMO**

O aproveitamento do soro de leite na elaboração de novos produtos pode ser uma alternativa para minimizar problemas ambientais e o desperdício de subprodutos de alto valor nutricional. Assim, objetivou-se elaborar *milkshakes* de chocolate com chia, substituindo parcialmente o leite por soro de leite. Foram elaboradas três formulações de *milkshakes*: sem adição de soro – controle, com 36% de leite/24% de soro e 24% de leite/36% de soro. A qualidade

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microbiológica, composição proximal e análises físico-químicas quanto aos parâmetros valor calórico, extrato seco, acidez total, pH e atividade de água foram avaliados, após o processamento. Os dados obtidos foram analisados através de Análise de Variância, em Delineamento Inteiramente Casualizado, comparando-se as médias pelo teste de *Tukey* a nível de 5% de significância. Verificou-se boa qualidade microbiológica, com baixas contagens para coliformes a 45 °C (< 3 NMP/g) e fungos filamentosos e leveduriformes (< 10 UFC/g). A inclusão de soro de leite provocou redução nos teores de cinzas, lipídeos e valores calóricos, bem como aumento nos teores de carboidratos. O consumo dos *milkshakes* indicou que as porções fornecem de 72,12 a 84,52 Kcal. Não houveram diferenças significativas entre os *milkshakes* para os parâmetros acidez total, extrato seco e atividade de água. Infere-se que a elaboração de *milkshakes* de chocolate com chia, substituindo parcialmente o leite por soro de leite, pode ser viável e uma alternativa inovadora para minimizar impactos ambientais, sendo recomendadas análises complementares sobre a estabilidade de armazenamento e a qualidade sensorial.

**Palavras-chave:** Alimentos funcionais. Gelados comestíveis. Resíduos agroindustriais.

## 1 INTRODUCTION

Whey, a by-product of the dairy industry, accounts for 80 to 90% of the total volume of milk used in cheese production. Whey is rich in organic compounds and retains about 55% of the nutrients present in milk, needing adequate treatment before disposal to minimize environmental impacts (ALVES *et al.*, 2014; VILELA *et al.*, 2020). Aiming to prevent the waste of this high nutritional value by-product, food industries and researchers have investigated sustainable alternatives for whey utilization. A major application is in the development of novel food products (ALVES *et al.*, 2014).

In recent years, whey has been extensively used in the production of cheese bread (TESSER *et al.*, 2010), sliced bread (OLIVEIRA *et al.*, 2011), whey powder (PERRONE *et al.*, 2014), dulce de leche (LIMA; ROCHA, 2016), ice creams (VETTORELLO *et al.*, 2017), milk spreads (MENESES *et al.*, 2019), and drink mixes (HOLANDA *et al.*, 2020). However, there are no reports in the literature on the use of whey for the preparation of milkshakes.

Brazilian legislation (RDC No. 266/2005) defines ice-cold foods as frozen food products obtained from an emulsion of fats and proteins or from a mixture of water and sugar (BRAZIL, 2005a). The development of new ice-cold food products (such as popsicles, ice creams, and milkshakes) containing whey represents an innovative strategy (VETTORELLO *et al.*, 2017).

There is an increasing demand for food products containing functional ingredients. According to Silva *et al.* (2014), functional foods provide benefits that go beyond the role of nutrition. Fortified, enriched, and enhanced foods have beneficial health effects when consumed as part of a varied diet (SOUZA *et al.*, 2013).

Chia (*Salvia hispanica* L.) seeds are widely known for their functional properties and high contents of polyunsaturated fats, essential amino acids, fibers and antioxidants (CAPITTANI *et al.*, 2012; COELHO; SALAS-MELLADO, 2014). Chia consumption promotes satiety, improves intestinal motility, and reduces the risk of diabetes (MADRUGA; ROCHA; FERNANDES, 2020).

Given the need to find novel applications for whey and vehicles for functional ingredients, this study aimed to develop chocolate chia milkshakes by partially replacing milk with whey and determine the effects of milk replacement on microbiological and physicochemical parameters.

## 2 MATERIAL AND METHODS

This study was conducted at the Federal Institute of Education, Science and Technology of Rio Grande do Norte, Pau dos Ferros-RN campus, Rio Grande do Norte State, Brazil. Ultrahigh temperature (UHT)-treated milk, powdered milk, light cream, 100% cocoa powder, emulsifier (Emustab<sup>®</sup>), table sugar, and chia flour were purchased from a local market. The whey resulting from the manufacture of *Coalho cheese* was purchased from a cheese factory in Pau dos Ferros-RN. After collection, whey samples were transported to the laboratory in a cool box with ice and then treated at 85 °C for 15 min to promote enzyme inactivation and fat separation.

Three chocolate milkshake with chia formulations were developed on the basis of preliminary tests: M<sub>1</sub>, a control formulation (without whey); M<sub>2</sub>, milkshake containing 36% milk and 24% whey; and M<sub>3</sub>, milkshake containing 24% milk and 36% whey. Ingredient proportions are presented in Table 1.

Table 1 – Ingredient composition of chocolate chia milkshake formulations

Ingredient (%)	Formulation		
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
UHT milk	60	36	24
Whey	0	24	36
Powderedmilk	10	10	10
Light cream	10	10	10
Cocoapowder	8	8	8
Emulsifyingagent	5	5	5
Table sugar	5	5	5
Chia flour	2	2	2

Source: Authors (2020).

The first step in the preparation of milkshakes was to mix the liquid ingredients (UHT milk, whey, and light cream). Then, the solid ingredients (milk powder, cocoa powder, table

sugar, and chia flour) were incorporated using an industrial blender. The resulting mixtures were stored at -18 °C for 3 h, until the outer surface began to freeze. Homogenization and gradual addition of emulsifier were performed for 15 min in an industrial mixer. Milkshakes were stored in polyethylene containers (200 mL) at -18 °C until analysis.

Microbiological quality was assessed after processing by determination of total coliforms (at 35 °C), thermotolerant coliforms (at 45 °C), and yeasts and filamentous fungi, according to the methods described by Silva *et al.* (2017).

Physicochemical analysis were performed after processing in triplicate. Water content and total solids were determined by oven-drying samples at 105 °C for 24 h, ash contents by heating in a muffle furnace at 550 °C for 6 h, titratable acidity (expressed as lactic acid) by titration with a standard solution of 0.1 N NaOH, and pH by using a portable pH meter calibrated with pH 4.0 and 7.0 buffer solutions, according to Adolfo Lutz Institute methods (ALI, 2008). Lipids (FOLCH; LESS; STANLEY, 1957) and proteins (AOAC, 2016) were also quantified. Carbohydrate contents were then calculated by subtracting the sum of moisture, ash, lipid, and protein contents from 100. Energy values were estimated in accordance with national legislation (BRAZIL, 2005b). Water activity was measured at 25 °C using a water activity meter (LabStart®, Novasina).

Physicochemical data were subjected to analysis of variance in a Completely Randomized Design, followed by Tukey’s test at  $p < 0.05$  for comparison of means. Statistical analysis were performed using Assistat software version 7.7 beta (SILVA; AZEVEDO, 2016).

### 3 RESULTS AND DISCUSSION

Table 2 shows the microbiological properties of chocolate milkshakes with chia.

Table 2 – Microbiological quality of chocolate milkshakes with chia formulated with different concentrations of whey

Parameter	Formulation		
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
Total coliforms (MPN/g)	$1.10 \times 10^3$	$1.10 \times 10^3$	$1.10 \times 10^3$
Thermotolerant coliforms (MPN/g)	< 3	< 3	< 3
Yeasts and filamentous fungi (CFU/g)	< 10	< 10	< 10

M<sub>1</sub>, milkshake formulated without the addition of whey; M<sub>2</sub>, milkshake containing 36% milk and 24% whey; M<sub>3</sub>, milkshak econtaining 24% milk and 36% whey; MPN, most probable number; CFU, colony-forming units. Source: Authors (2020).

Chocolate milkshakes with chia contained  $1.10 \times 10^3$  MPN/g total coliforms. The presence of coliforms is likely associated with the use of dairy ingredients. Lactic acid

bacteria are commonly found in dairy products and can multiply in a similar manner to that of coliform bacteria. The concentrations of thermotolerant coliforms (< 3 MPN/g) and yeasts and filamentous fungi (< 10 CFU/g) were low, indicating that raw materials were of good quality and that processing methods were adequate. Partial replacement of milk by whey did not influence the microbiological quality of milkshakes. This result shows that the heat treatment applied to whey was effective in eliminating pathogenic and spoilage microorganisms.

In Brazil, microbiological standards for foods are described in Normative Instruction No. 60/2019. However, the legislation does not determine limits for total and thermotolerant coliforms or yeasts and filamentous fungi in milkshakes (categorized as iced-cold foods) (BRAZIL, 2019). Teixeira *et al.* (2019) assessed the microbiological properties of three biofortified functional ice cream formulations and found that food items were microbiologically safe for consumption. The concentration of thermotolerant coliforms (< 3 MPN/g) was similar to that observed in chocolate chia milkshakes.

The proximate composition and energy value of chocolate chia milkshakes are described in Table 3.

Table 3 – Proximate composition and energy value of chocolate chia milkshakes formulated with different concentrations of whey

Parameter	Formulation			Mean	LSD
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>		
Water (%)	76.27 <sup>a</sup> ± 0.58	77.11 <sup>a</sup> ± 0.89	76.84 <sup>a</sup> ± 0.75	76.74	2.82
Ash (%)	1.92 <sup>a</sup> ± 0.09	1.52 <sup>b</sup> ± 0.10	1.38 <sup>b</sup> ± 0.03	1.61	0.21
Protein (%)	8.28 <sup>a</sup> ± 0.35	8.44 <sup>a</sup> ± 0.12	8.83 <sup>a</sup> ± 0.30	8.52	1.35
Lipid (%)	10.72 <sup>a</sup> ± 0.41	8.53 <sup>b</sup> ± 0.28	6.61 <sup>c</sup> ± 0.33	8.62	0.68
Carbohydrate (%)	2.81 <sup>b</sup> ± 0.88	4.39 <sup>ab</sup> ± 0.91	6.34 <sup>a</sup> ± 0.89	4.51	3.05
Energy value (Kcal/100 g)	140.87 <sup>a</sup> ± 0.90	128.12 <sup>b</sup> ± 0.96	120.20 <sup>b</sup> ± 0.93	129.73	10.53

M<sub>1</sub>, milkshake formulated without the addition of whey; M<sub>2</sub>, milkshake containing 36% milk and 24% whey; M<sub>3</sub>, milkshake containing 24% milk and 36% whey; LSD, least significant difference. Means followed by the same letter do not differ at  $p < 0.05$  by Tukey's test  
Source: Authors (2020).

Partial substitution of milk by whey did not influence water content ( $p > 0.05$ ), but M<sub>1</sub> contained higher levels (76.27%) than M<sub>2</sub> (77.11%), resulting from differences in composition. The water content of chocolate ice cream with orange peel fiber (63-70%) (BOFF *et al.*, 2013) was lower than that of chocolate milkshakes with chia prepared in this study.

Ash content was highest ( $p < 0.05$ ) in M<sub>1</sub> (1.92%), differing from that in M<sub>2</sub> and M<sub>3</sub>, which were prepared by partial substitution of milk by whey. This finding indicates that milk has a higher ash content than whey. The use of chia flour probably increased the content of

minerals, vitamins, and natural antioxidants such as tocopherols and polyphenols, compounds that are commonly found in chia seeds (IXTAINA *et al.*, 2011).

The protein content of milkshakes ranged from 8.28% in M<sub>1</sub> to 8.83% in M<sub>3</sub>. It is likely that chia flour contributed to the high protein content of milkshakes. According to Olivos-Lugo, Valdivia-López e Tecante (2010), chia seeds are an important source of protein, with protein contents ranging from 19 to 23%. Rodrigues *et al.* (2018) reported that the protein content of acai pulp and whey protein ice cream ranged from 3.99 to 10.25% according to the content of whey; the higher the whey concentration, the higher the protein content. In a study by Barcelos *et al.* (2019), chocolate ice cream was found to have a protein content of 1.70-3.69%, lower than that of chocolate chia milkshake.

Lipid content was highest ( $p < 0.05$ ) in M<sub>1</sub> (10.72%). The higher the substitution of milk by whey, the lower the lipid content. According to Santos *et al.* (2013) and Punia and Dhull (2019), healthy foods have high nutritional quality and low fat contents, contributing to body weight control, reduced energy intake, and decreased risk of cardiovascular diseases.

No differences ( $p > 0.05$ ) in carbohydrate content were observed between M<sub>2</sub> and the other formulations, although M<sub>1</sub> and M<sub>3</sub> differed from each other. The parameter was highest in M<sub>3</sub> (6.34%), which had the highest whey concentration (24% milk and 36% whey). Whey is composed of about 5% lactose, its second major component (PESCUMA *et al.*, 2010; BARUKČIĆ *et al.*, 2015). Risner *et al.* (2019) stated that lactose is the main carbohydrate in milk and is present at high concentrations in whey. Vettorello *et al.* (2017) prepared ice cream using whey powder and found the food to contain 15.38–16.93% carbohydrates.

M<sub>2</sub> and M<sub>3</sub> had a lower ( $p < 0.05$ ) energy value than M<sub>1</sub>. The reduction in energy value with the addition of whey was probably associated with the concomitant reduction in lipid content. Although RDC No. 359/2003 (BRAZIL, 2003) does not determine a serving size for milkshake, it can be considered as 60 g, the same serving size of ice cream, which is the food item that most resembles milkshake. On the basis of this value, it was estimated that one portion of chocolate milkshake with chia provides 72.12 (M<sub>3</sub>) to 84.52 Kcal (M<sub>1</sub>), corresponding to 3.61 and 7.04% of the total energy value of a 2000 Kcal/day diet.

The physicochemical properties of chocolate milkshakes with chia are presented in Table 4.

Table 4 – Physico chemical parameters of chocolate chia milkshakes formulated with different concentrations of whey

Parameter	Formulation			Mean	LSD
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>		
Total solids (%)	23.73 <sup>a</sup> ± 0.58	22.89 <sup>a</sup> ± 0.89	23.16 <sup>a</sup> ± 0.75	23.26	2.82
Titratable acidity (%)	0.44 <sup>a</sup> ± 0.03	0.44 <sup>a</sup> ± 0.01	0.44 <sup>a</sup> ± 0.01	0.44	0.05
pH	6.74 <sup>a</sup> ± 0.03	6.69 <sup>a</sup> ± 0.02	6.65 <sup>b</sup> ± 0.02	0.88	0.03
Water activity	0.88 <sup>a</sup> ± 0.01	0.88 <sup>a</sup> ± 0.01	0.87 <sup>a</sup> ± 0.01	6.69	0.06

M<sub>1</sub>, milkshake formulated without the addition of whey; M<sub>2</sub>, milkshake containing 36% milk and 24% whey; M<sub>3</sub>, milkshake containing 24% milk and 36% whey; LSD, least significant difference. Means followed by the same letter do not differ at  $p < 0.05$  by Tukey's test.

Source: Authors (2020).

Total solids ranged from 22.89% in M<sub>2</sub> to 23.73% in M<sub>1</sub>. According to Kozłowicz *et al.* (2019), low concentrations of total solids may negatively impact the formation of ice crystals, decreasing product quality. Vettorello *et al.* (2017), in assessing the properties of ice cream composed of whey powder, found that total solids varied from 21.14 to 25.85%, similar to the range observed in the present study.

All milkshakes had a titratable acidity of 0.44%. The pH was close to neutrality, ranging from 6.65 in M<sub>3</sub> to 6.74 in M<sub>1</sub>; the pH of M<sub>3</sub> was significantly lower ( $p < 0.05$ ) than that of the other formulations. Campidelli *et al.* (2015) reported blueberry chia ice cream to have a pH of 5.55, lower than that of chocolate chia milkshakes.

Water activity also did not differ between milkshakes ( $p > 0.05$ ). Values ranged from 0.87 (M<sub>3</sub>) to 0.88 (M<sub>1</sub> and M<sub>2</sub>). Water activity values were high, which, according to Sandulachi (2012), increases susceptibility to microbial growth and biochemical reactions responsible for spoilage.

#### 4 CONCLUSIONS

Chocolate milkshakes with chia had good microbiological quality. Partial replacement of milk by whey exerted significant effects on ash, lipid, and carbohydrate contents as well as energy value and pH. Milkshake containing 24% milk and 36% whey had the lowest lipid content and energy value and the highest carbohydrate content. The use of whey as a partial substitute for milk is a feasible and innovative strategy to minimize environmental impacts. Further studies should investigate the storage stability and sensory quality of chocolate chia milkshakes.

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