Evaluation of pH and total soluble solid (ºbrix) on the cariogenic and erosive potential of milk powder infantile formula

Avaliação do pH e sólidos solúveis totais (ºbrix) no potencial cariogênico e erosivo do leite em pó fórmula infantil

Evaluación de pH y sólidos solubles totales (ºbrix) en el potencial cariogénico y erosivo de fórmulas infantiles de leche en polvo

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Abstract
The objective of this study is to analyze the influence of pH and the amount of Total Soluble Solids on the ºBrix scale on the cariogenic and erosive potential of powdered infant formula. This is a laboratory study with 5 brands of powdered infant formula for children aged 6 to 36 months. Brands with intact packaging, stored in cool places and within the expiry date for consumption were included. Obtaining the Brixº was performed by refractometer and pH with a pHmeter with a cycle of three repetitions. Of the five brands tested, the sugar levels on the ºBrix scale were between 10.67 and 12.0, with all maras having total carbohydrate values between 9.0 and 14.0 g, which are considered healthy. Regarding the pH, four had a pH lower than 7, being considered acidic and all above the critical pH (5.5) for dissolution of dental structures. It was concluded that by measuring pH and sugars on the Brixº scale, the brands analyzed were within acceptable standards for maintaining oral health, making these two factors positive determinants so as not to influence the cariogenic and/or erosive potential of milk powder infant formula.
Keywords: Milk; Dental caries; Dental erosion.

Resumo
O objetivo deste estudo é analisar a influência do pH e a quantidade Sólidos Solúveis Totais na escala ºBrix no potencial cariogênico e erosivo de leite em pó fórmula infantil. Trata-se de estudo laboratorial com 5 marcas de leites em pó fórmula infantil para crianças de 6 à 36 meses de idade. Foram incluídas marcas com embalagens íntegras, armazenadas em locais frescos e dentro do prazo de validade para consumo. A obtenção do Brixº foi realizada por refratômetro e pH com pHmetro com um ciclo de três repetições. Das cinco marcas testadas, aos níveis de açucares na escala ºBrix ficaram entre 10,67 e 12,0, com todas as maras com valores de carboidratos totais entre 9,0 e 14,0 g consideráveis saudáveis. Em relação ao pH, quatro apresentaram pH menor do que 7, sendo considerado ácido e todas acima do pH crítico (5.5) para dissolução das estruturas dentárias. Concluiu-se que através da mensuração do pH e o açucares na escala Brixº, as marcas analisadas apresentaram-se dentro dos padrões aceitáveis para a

manutenção da saúde bucal, tornando esses dois fatores como determinantes positivos para não influenciar o potencial cariogênico e/ou erosivo de leite em pó fórmula infantil.
Palavras-chave: Leite; Cárie dentária; Erosão dentária.

Resumen
El objetivo de este estudio es analizar la influencia del pH y la cantidad de Sólidos Solubles Totales en la escala ºBrix sobre el potencial cariogénico y erosivo de las fórmulas lácteas en polvo. Este es un estudio de laboratorio con 5 marcas de fórmula infantil en polvo para niños de 6 a 36 meses. Se incluyeron marcas con empaque intacto, almacenado en lugares frescos y dentro de la fecha de vencimiento para el consumo. La obtención del Brixº se realizó por refractómetro y pH con un pHmetro con un ciclo de tres repeticiones. De las cinco marcas probadas, los niveles de azúcar en la escala de ºBrix estuvieron entre 10,67 y 12,0, teniendo todas las maras valores de carbohidratos totales entre 9,0 y 14,0 g, que se consideran saludables. En cuanto al pH, cuatro presentaron un pH inferior a 7, siendo considerados ácidos y todos por encima del pH crítico (5,5) para la disolución de las estructuras dentarias. Se concluyó que mediante la medición de pH y azúcares en la escala Brixº, las marcas analizadas se encontraban dentro de los estándares aceptables para el mantenimiento de la salud bucal, siendo estos dos factores determinantes positivos para no influir en el potencial cariogénico y/o erosivo de las fórmulas infantiles de leche en polvo.
Palabras clave: Leche; Caries dental; Erosión dental.

1. Introduction

Current changes in individuals' eating habits due to the large intake of industrialized foods can affect oral health with the appearance of carious and non-carious lesions, causing aesthetic and functional damage (Oliveira et al., 2017; Chi & Scott, 2019). These pathologies are related by the vulnerability of the teeth to the frequent and unrestrained ingestion of sweetened and acidic foods, mainly by children through the influence of biological, psychological, social and environmental factors (Farias et al., 2012; Miranda et al., 2017).

Dental caries is a bacterial disease of multifactorial and chronic condition of the relationship of dental biofilm, carbohydrates and saliva, being caused by acid-producing chemical reactions that lead to the dissolution of enamel and dentin (Miranda et al., 2017). It stands out as determinants for the development of this pathology: the tooth surface, bacterial plaque, carbohydrates and exposure time, and for cariogenicity it mainly includes food processing, pH and carbohydrate content (Frydrych, et al., 2022).

The non-carious lesion of the dental erosion type is defined by the physical/chemical result of the loss of hard tissue from the dental surface caused by acid and/or chelators through the chemical dissolution of mineralized dental tissues without bacterial involvement (Lima et al, 2011; Chan et al., 2020). Its etiology is multifactorial with intrinsic and extrinsic factors with biological, behavioral and mainly chemical involvement, through the low pH of food acids (Maharani et al., 2019; Philip et al., 2019). Determination methods evaluate the titratable acidity or provide the concentration of free hydrogen ions, through pH, which can be given at a given temperature, the acidity or alkalinity of a solution in which it is indicated by the pH value or by hydrogen ions (Lutz A, 1985). A pH equal to or less than 5.5 is considered critical for enamel dissolution.

A high concentration of Total Soluble Solids (TSS) added to the low pH, can contribute to the development of caries and dental erosion in the excessive intake of dairy beverages by children (Lima et al., 2011). Studies support the justification that these products contain lactose, an important sugar in the rapid decrease of oral pH (Aarthi et al., 2013).

The large intake of acidic drinks contributes to the demineralization of the enamel with the critical pH ≤5.5 through the reduction of the pH of the saliva, affecting the buffer system that is responsible for the protection of the teeth in relation to the demineralization and remineralization of the dental structures (Cavalcanti et al., 2006; Lima et al., 2011).

Thus, Brazilian studies have sought to show the percentage concentrations of TSS contained in a sample (water solution) on the ºBrix scale through the number of grams of sugar (sucrose, fructose and glucose) contained in 100g. Thus, considering the percentage that high concentrations of sugars present in the diet directly imply as one of the factors for the cariogenic potential of food products (Cavalcanti et al., 2006).

To determine the titratable acidity, the methods occur through the concentration of free hydrogen ions, through the pH, which can be given at a given temperature, the acidity or alkalinity of a solution in which it is indicated by the pH value or by hydrogen ions (Lutz A, 1985).

Understanding this relationship between the large amount of sugar and the low pH as two factors that contribute to the cariogenic and erosive potential of foods, a gap was found in the literature regarding the problem involving powdered infant formula consumed during the two first years of life. Considering that the data collected will elucidate the relationship between these two factors on the implications of milk consumption on oral health in early childhood, the present study aims to influence the pH and the amount of Total Soluble Solids in the ºBrix scale on the cariogenic and erosive potential of infant formula milk powder.

2. Methodology

This in vitro laboratory study is characterized in a quantitative and analytical way with infant formula milk powder recommended for children from 6 months to 36 months of age, justified in the emergence of the first teeth in the children's oral cavity. 5 brands NanLac® (Nestlé, Brazil), Enfanutri ® (MeadJohnson, Brazil), Nestogeno® (Nestlé, Brazil), Ninho® (Nestlé, Brazil) and Aptamil® (Danone, Brazil) were used in 3 different batches with intact, closed cans, stored in a cool and ventilated place, within the expiration date and with adequate quality for consumption (Table 1).

Table 1 - Batches corresponding to each brand.

Brand	Batch 1	Batch 2	Batch 3
Nanlac®	1160046041 0548	1218046041 1257	1308046041 1442
Enfanutri®	DE0HRN6D	DE0HTO2D	DE0HTN1D
Nestogeno®	1259046041 04 16	1258046041 21 22	1214046041 05 04
Ninho®	1230046012 1000	1283046011 2363	1262046011 1504
Aptamil®	101602180	101579655	101602442

Source: Research Data (2021).

Samples from each milk can were taken according to Resolution n. 44 (Brasil, 1976), milks must be prepared with boiled water and subsequently cooled to a temperature not lower than 70°C. Pursuant to the clear instructions of the manufacturers on the labels of infant formulas, drinking water was boiled and a 1-minute bubbling boil was maintained to check the temperature of 100ºC. Then, it was waited 15 minutes until the water reached a temperature of 70ºC, being measured 210 ml of water and inserted in disposable pots of 350 ml for the dilution of 7 measuring spoons produced by each manufacturer contained inside each can for the experimental units.

The values of Total Soluble Solids (SST) on the Brixº scale were obtained and the pH of these samples was determined to establish cariogenic and erosive potential (Lutz A, 1985).

Obtaining °BRIX

To obtain the values of Total Soluble Solids (°Brix) a refractometer calibrated before the experiment with water with a refractive index at 20°C of 1.333 was used. Then, 2 drops of each milk sample were placed between the focused prisms and the refractive index was obtained by reading the scale carried out at a temperature of 25±2°. An experimental design with repetitions of three times is necessary for the arithmetic mean of the three refractive indices, obtaining the result of each sample.

As for the value of Brixº, a content of measuring sugar concentration in infant milk powder was used, since total carbohydrate values must be between 9.0 and 14.0 g/100 kcal (3.3 g/100 Kj), according to Art. 19 of the Resolution of the Collegiate Board of Directors RDC nº 44, of September 19, 2011 (Brazil, 2011), and values above 14g/100kcal may be related to the cariogenic potential. Taking into account that 1g of sugar corresponds to 1º Brix, the reference value of 9º to 14º Brix was used.

Obtaining pH values

To determine the pH, a calibrated pHmeter was used with the use of a combined glass electrode as indicated in the device manual. First, buffer solutions of pH 4 (acid), pH 7 (neutral) and pH 10 (basic) were used to calibrate the reference values of the device. These solutions were kept in a refrigerator to avoid contamination by fungi and about one hour before use, about 20 ml were transferred to 50 ml beakers to reach room temperature. Thus, the linearity of the electrode with insertion in each solution with gentle, constant agitation was verified and the temperature of the solution was read. After measuring each solution, the electrode was washed with distilled/deionized water and gently dried with disposable paper.

After the device calibration step, the electrode was inserted into each sample contained in the 350ml disposable cups with agitation to perform the reading at a temperature of 25±2°. The reading was allowed to remain constant and the pH value of the sample was recorded. It was necessary that the readings were taken three times in each milk, with the final pH obtained by the arithmetic mean. After measuring each sample, the electrode was washed with distilled/deionized water and gently dried with disposable paper.

The pH comparison values were considered the value of 5.5, considered critical by scientific evidence, since a pH value equal or lower causes enamel dissolution.

Statistical analysis

All data were organized and distributed in the Excel program (2019) and comparative analyzes were performed in SPSS Statistics (Version 28.0) and R (statistical and graphic programming language) software. In order to meet the research objectives, the Anova Kruskall-Wallis and Mann-Whitney tests were performed, with a significance level of 5% ($p < 0.05$).

3. Results and Discussion

In an initial superficial analysis, the concentrations of total soluble solids (°Brix) presented values within the acceptable limits between 9 to 14 ºBrix; and the pH data showed values close to the value of a substance considered neutral, pH = 7 (Table 2).

Table 2 – Infant milk brands with their respective values in relation to pH and °Brix.

Brands	Batch 1		Batch 2		Batch 3	
	pH	°Brix	pH	°Brix	pH	°Brix
Nanlac®	6,8	11º	7	11º	7	11º
Enfanutri®	6,8	11º	6,7	11º	6,7	11º
Nestogeno®	6,8	12º	6,8	12º	6,8	11º
Ninho®	7	11º	7,1	10º	6,9	11º
Aptamil®	6,6	12º	6,6	12º	6,7	12º

*pH: Acid < 7; Neutro = 7; Alkaline > 7; *°Brix: ≤9 and ≥14. Source: Research data.

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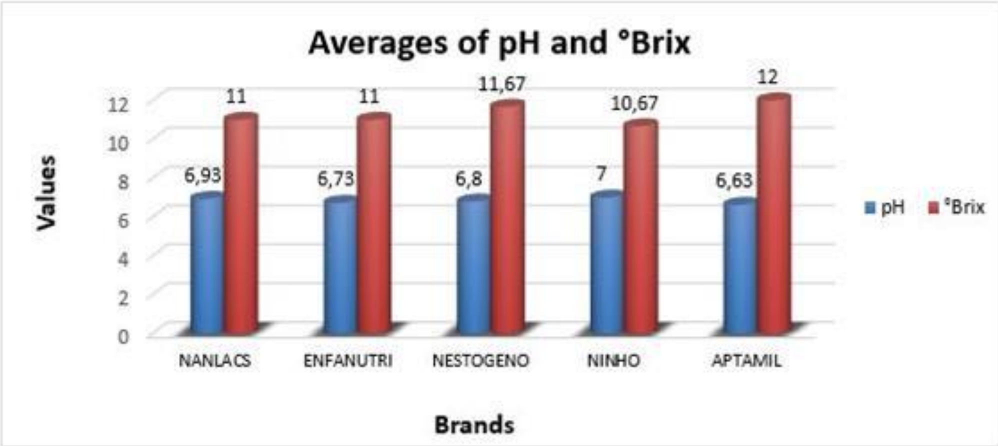
After the descriptive analysis of the data, the averages and standard deviations of the values referring to °Brix and pH were obtained (Figure 1).

Figure 1 – Avagares of pH and ºBrix.



Standard Deviation: *NANLACS – pH (Dp ± 0,16) e °Brix (Dp ± 0); *ENFANUTRI – pH (Dp ± 0,06) e °Brix (Dp ± 0); *NESTOGENO – pH (Dp ± 0) e °Brix (Dp ± 0,58); *NINHO – pH (Dp ± 0,1) e °Brix (Dp ± 0,58); *APTAMIL – pH (Dp ± 0,06) e °Brix (Dp ± 0).
Source: Research data.

The comparative analyzes provided an assessment of the possible existence of statistically significant differences in the values of °Brix and pH in the powdered infant formula milk brands, where the sample characteristics, small size and non-normality of the data were taken into account. Thus, the Anova Kruskall-Wallis test was applied to the descriptive data of infant milk brands (pH and °Brix), obtaining the chi-square (X2) and p-value, in addition to the degree of freedom (gl). Thus, in our results (Table 3), statistically significant differences were obtained in relation to pH values (X2 = 11.92; p-value = 0.02) and °Brix (X2 = 10.71; p -value = 0.03), inside the marks.

Table 3 – Mean Ranks and Kruskal-Wallis Test applied to infant milk brand data.

Brands	Mean Ranks	
	pH	°Brix
Nanlac®	11,33	6,00
Enfanutri®	5,33	6,00
Nestogeno®	8,00	10,67
Ninho®	13,00	4,33
Aptamil®	2,33	13,00
Chi-square (X^2)	11,92	10,71
Degree of Freedom (df)	4	4
P-value (ref. < 0,05)	0,02*	0,03*

* p-value below 0.05; thus indicating statistical significance. Source: Own elaboration.

And finally, comparisons were made between groups, of infant milk brands, specific from the performance of Mann-Whitney tests in pairs, in relation to °Brix and pH. Where the results showed that, in relation to °Brix, there were statistically significant differences between the groups: Nanlac x Aptamil, Enfanutri x Aptamil and Ninho x Aptamil (Table 4). Regarding the pH, there were statistically significant differences between the groups: Nanlac x Aptamil, Nestogeno x Ninho and

Nestogeno x Aptamil (Table 5). In the other comparisons, there were no statistically significant results, due to the p-value being equal to or greater than 0.05.

Table 4 – P-value (statistical significance) in the comparison between pairs of groups regarding the ºBrix value of infant milk brands.

	Nanlac®	**Enfanutri®**	**Nestogeno®**	**Ninho®**	**Aptamil®**
Nanlac®	-----	1,00	0,11	0,32	**0,03***
Enfanutri®	-----	-----	0,11	0,32	**0,03***
Nestogeno®	-----	-----	-----	0,10	0,32
Ninho®	-----	-----	-----	-----	**0,03***
Aptamil®	-----	-----	-----	-----	-----

*Statistically significant values (ref. – p-value < 0.05). Source: Research data.

Table 5 – P-value (statistical significance) in the comparison between pairs of groups regarding the pH value of infant milk brands.

	Nanlac®	**Enfanutri®**	**Nestogeno®**	**Ninho®**	**Aptamil®**
Nanlac®	-----	0,68	0,11	0,49	**0,04***
Enfanutri®	-----	-----	0,11	0,05	0,10
Nestogeno®	-----	-----	-----	**0,04***	**0,03***
Ninho®	-----	-----	-----	-----	0,05
Aptamil®	-----	-----	-----	-----	-----

*Statistically significant values (ref. – p-value < 0.05).Source: Research data.

Food is directly linked to the oral health of individuals, especially with new eating practices due to the diversity and quality of the daily menu. In this context, there are incentives from the food industry for a greater consumption of foods with high concentrations of sugar and low pH by children due to the ease of consuming sugary foods (Lima et al., 2011; Chi & Scott, 2019; Matos et al., 2019). These changes cause an increase in caries and dental erosion rates, which directly affects children's quality of life. Therefore, it is necessary to understand and identify the risk factors for these pathologies (Maharani et al., 2019).

With these dietary changes, the possibility arose for newborn children to consume a nutritional supplement called infant formulas produced based on cow's milk with additional carbohydrates, such as corn syrup, sucrose, lactose and other compounds, which may increase the cariogenic potential. and erosive of these compounds (Aarthi et al., 2013).

Through this problem, our study analyzes two risk factors related to the etiology of caries and dental erosion through an in vitro experiment with pre-established methodologies in the literature to obtain sugar concentrations by SST in the ºBrix scale and acidity through the pH of five brands of these infant formulas contributing to the scientific gap.

For the ºBrix analysis, the brands obtained between 10.67º and 12ºBrix, meaning that they were among the standards established by Brazilian government agencies for ideal sugar concentrations between 9º and 14ºBrix for infant formulas (Brazil, 2011). These results are considered positive because these compounds with fermentable carbohydrates feed acid-producing bacteria responsible for the demineralization of tooth structure, contributing to the development of caries. This result was similar to other studies with the same approach and other dairy beverages widely consumed by children, with emphasis on fermented milk obtaining variable indices between 5.93º and 11.2º Brix and that of chocolate milk between 10.23º and 13, 53º Brix (Moreira et al., 2017; Matos et al., 2019).

In measuring the pH, of the five brands used in this research, four had a pH lower than 7, being acidic and all above the considered critical pH of 5.5 for dissolution of dental structures. On the other hand, the literature shows that fermented milk has a pH between 3.13 and 3.68, causing a drop in oral pH, affecting the saliva protection system (Maziero et al., 2011; Miranda et al., 2017). Our positive results are similar to others with the study of the pH of chocolate milk, obtaining results ranging from 6.38 to 6.71 (Matos et al., 2019;).

Among the two factors analyzed, the pH is the one that has a direct relationship with dental erosion because it is caused by exposure to acids, whether or not they come from the human body, to dental tissues with a critical pH lower than 5.5 (Assis et al., 2010; Singana & Suma, 2020). Studies claim that daily intake of dairy beverages with an acidic pH contributes to the development of dental erosion, as it is a continuous exposure and in a short period of time (Matos et al., 2019).

The comparative analysis between the brands showed that the pH and level of fermentable carbohydrates had the best balance in the Ninho® brand, followed by Nanlac®, Enfanutri®, Nestogeno® and Aptamil®. The relationship between the two factors may contribute to the development of caries or dental erosion, as a high sugar content, usually sucrose or fructose, or a combination of the two, affects the pH of the dental biofilm and, consequently, of the entire oral cavity (Jung & Jun, 2021).

Due to the fact that milk has a diverse composition with calcium, phosphorus, proteins, vitamins and fat, the present study has limitations for the exact determination of the cariogenic and erosive potential (Aarthi et al., 2013). Justified in the agenda that these two isolated factors are not enough to determine the appearance of these oral diseases, since both have multifactorial causes (Assis et al., 2010; Lodi et al. 2010).

In the case of caries, it depends on the type and content of sugars, the frequency of consumption and the time spent in the oral cavity related to hygiene. Erosive wear, on the other hand, has a direct relationship with behavioral determinants such as excessive or erroneous oral hygiene and emotional disorders (Maharani et al., 2019; (Frydrych, et al., 2022). Furthermore, both depend on increased salivary viscosity, contributing to greater food exposure in the oral cavity, increasing the risk of developing caries and dental erosion (Almeida et al., 2016; Woodward & Rugg-Gunn, 2020; Wang et al., 2021).

However, these results support possible new studies on the issue of powdered milk and infant formulas with an approach to other factors and new methodologies so that knowledge of applicability in the clinical routine of dentists can be offered. This information is necessary for these professionals to be aware of the patients' food base as a crucial factor for oral health in addition to good oral hygiene, thus not contributing to the development of caries and dental erosion (Matos et al., 2019).

4. Conclusion

Considering the measurement of values referring to pH and concentrations of total soluble solids through the °Brix scale, in all brands analyzed these factors remained within acceptable standards for maintaining oral health, thus considered positive factors not to influence the cariogenic and/or erosive potential of powdered infant formula.

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