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Análise *in vitro* do potencial erosivo de bebidas *Whey Protein*

In vitro analysis of the erosive potential of Whey Protein

Análisis in vitro del potencial erosivo de las bebidas de *Whey Protein*

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Resumo

O objetivo do estudo foi avaliar o pH e o teor de sólidos solúveis totais (SST) de bebidas com whey protein. Nove produtos de três sabores (baunilha, morango e chocolate) das marcas

Muke®, Best Whey® e Essencial Nutrition Whey®, tiveram seu pH avaliado por potenciometria e o SST por meio de refratometria. O menor pH registrado foi do Muke® Chocolate (5,75), e o maior foi do Essencial Nutrition Whey® Cacao (6,91). Quanto aos SST, a menor média foi do Essencial Nutrition Whey® Vanilla (10,17), e a maior do Essencial Nutrition Whey® Red Berry (16,66). Não houve diferença estatisticamente significativa do pH e SST entre os sabores e marcas avaliadas. Concluiu-se que as bebidas analisadas apresentaram pH superior ao pH crítico do esmalte dentário e um teor de SST considerado alto.

Palavras-chave: Erosão dentária; Suplementos nutricionais; Bebidas; Técnicas in vitro.

Abstract

The objective of this study was to evaluate the pH and total soluble solids (TSS) content of whey protein beverages. Nine products of three different flavors (vanilla, strawberry and chocolate) of the following brands Muke®, Best Whey®, and Essencial Nutrition Whey®, had their pH evaluated by potentiometry, and their TSS evaluated by refractometry. The lowest pH recorded was for Muke® Chocolate (5.75), and the highest was for Essencial Nutrition Whey® Cacao (6.91). Regarding TSS, the lowest average was for Essencial Nutrition Whey® Vanilla (10.17), and the highest was for Essencial Nutrition Whey® Red Berry (16.66). There was no statistically significant difference in pH and TSS among the flavors and brands evaluated. It was concluded that the analyzed beverages had a higher pH than the pH that is critical to dental enamel and the TSS content was considered high.

Keywords: Dental erosion; Nutritional supplements; Beverages; In vitro techniques.

Resumen

El objetivo del estudio fue evaluar el pH y el contenido de sólidos solubles totales (SST) de las bebidas de whey protein. Nueve productos de tres sabores (vainilla, fresa y chocolate) de las marcas Muke®, Best Whey® y Essential Nutrition Whey®, los valores de pH fueron evaluados por potenciometría y los SST por refractometría. El pH más bajo registrado fue para la muestra de Muke® Chocolate (5.75), y el más alto fue para la muestra de Essential Nutrition Whey® Cacao (6.91). En cuanto a la SST, el promedio más bajo fue para la bebida de Essential Nutrition Whey® sabor Vanilla (10.17), y el más alto para el sabor Red Berry (16.66). No hubo diferencias estadísticamente significativas en el pH y los SST entre los sabores y las marcas evaluadas. Se concluyó que las bebidas analizadas tenían un pH más alto que el pH crítico del esmalte dental y un contenido de SST demasiado alto.

Palabras clave: Erosión de los dientes; Suplementos dietéticos; Bebidas; Técnicas in vitro.

1. Introduction

Dental erosion is a current problem initiated when acids reach the dental surface, of multifactorial origin - intrinsic (related to digestive system acids) or extrinsic (originating from acidic substances found in the external environment) (Maltarollo et al., 2020), such as the ingestion of drinks with acid pH, which can lead to loss of dental tissue (Diniz & Lussi, 2017).

The amount and frequency of consumption of acidic foods and beverages has increased in recent years, mainly due to changes in the lifestyle of the population (Almenara et al., 2016). Changes in the eating habits of the Brazilian population have also been reflected in the high consumption of nutritional supplements, especially by people who practice physical activities and go to the gym (Hallak et al., 2007). Additionally, the technology applied by the food industry in order to increase the shelf life of products has raised questions as to the use of food additives, given the possibility of the harmful effects on consumers (Polonio & Peres, 2009).

The growing interest in healthier, more nutritious food products is also noteworthy. Dairy beverages are good examples of such products, since they have proteins, fats, lactose, minerals, and vitamins in their composition, which are indispensable for the proper functioning of the body (Silva et al., 2017).

Therefore, the growing market investment in the industrialization of foods with guaranteed functional properties is evident. Besides their nutritional functions, these foods also produce metabolic and physiological effects in the organism (Carvalho et al., 2013). Thus, the commercial demand for nutritional supplements, especially for protein energy sources, is increasing, especially among physical exercise practitioners, who are motivated by the desire for physical improvement (Gomes et al., 2008).

In this context, whey protein is well known and widely consumed as a high quality protein source that is especially incorporated in sports and nutritional products (West et al., 2017). It has an excellent profile of essential amino acids, characterized by its high biological value, and therefore it is being used as a raw material in protein supplements for athletes and in the food industry as an additive in the manufacturing of a series of products (Scarlato et al., 2016).

These products act as an aid in skeletal muscle protein synthesis, body fat reduction and increased physical performance (Haraguchi et al., 2006). In addition, they have bioactive peptides that act as an antimicrobial, antihypertensive, and regulator of immune function agents, as well as a growth factor (Aimutis, 2004).

Given the above, and in view of the increase in sales of sports and energy beverages, the objective is to evaluate, in vitro, the endogenous pH and total soluble solids content of commercial beverages that are enriched with powdered hydrolyzed and isolated whey protein in the form of sachets. The hypothesis adopted in this study is that there are statistically significant differences between hydrogen potential (pH) and total soluble solids (TSS) content in relation to flavor and brand variation.

2. Methods

The study was characterized as being *in vitro* experimental, and the research technique was direct observation in laboratory. Nine commercial beverages with isolated and hydrolyzed powdered whey protein in sachets purchased from commercial establishments were evaluated. The experiments were carried out by a previously trained examiner at the Oral Pathology Laboratory of Paraíba State University.

The Table 1 presents data from products according to the type and flavor of the beverage.

Table 1. Distribution of the products according to the type and flavor of the beverage.

Groups	Beverages	Flavor	Manufacturer
G1	Muke®	Vanilla	Mais Mu Com. Alimentos e Bebidas Ltda.
G2	Muke®	Strawberry	Mais Mu Com. Alimentos e Bebidas Ltda.
G3	Muke®	Chocolate	Mais Mu Com. Alimentos e Bebidas Ltda.
G4	Best Whey®	Vanilla	ADS Laboratorio Nutricional Ltda.
G5	Best Whey®	Strawberry	ADS Laboratorio Nutricional Ltda.
G6	Best Whey®	Brigadeiro	ADS Laboratorio Nutricional Ltda.
G7	Essencial Nutrition Whey®	Vanilla	INP Indústria de Alimentos Ltda.
G8	Essencial Nutrition Whey®	Red Berry	INP Indústria de Alimentos Ltda.
G9	Essencial Nutrition Whey®	Cacao	INP Indústria de Alimentos Ltda.

Source: Authors.

The analyzed drinks had three different flavors, vanilla, strawberry and chocolate, and three different commercial brands, Muke®, Best Whey® and Essential Nutrition Whey®.

Sample

For the experiment, each beverage sample was produced in a Becker by adding 60 ml of mineral water (Indaiá®, pH ± 5.67, at room temperature ± 27^0C) to the sachet powder, which was previously weighed by electronic analytical scales (AUY220, Shimadzu Philippines Manufacturing Inc., Philippines), according to the manufacturer's instructions.

Endogenous pH measurement

For pH measurement, a digital pH meter Q400-AS (Quimis Aparelhos Científicos Ltda., Diadema, SP) was used. After calibration of the device, 20 ml of the sample was transferred to a test tube, followed by immersion of the electrode and subsequent reading and recording of the values obtained. For each sample, the same procedure was performed three

times, discarding the volume in the test tube and washing the electrode with distilled water at each check.

Determination of total soluble solids content (TSS)

The determination of total soluble solids (TSS) content was by refractometry, using the Abbé refractometer (PZO-RL1, Warsawa, Polônia), calibrated at room temperature with distilled water (refractive index = 1.3330 and 0° Brix at 20°C).

Statistical analysis

Data were analyzed descriptively expressed as means and standard deviations. Since data presented non-normal distribution (Shapiro-Wilk), and inferentially through the Kruskal-Wallis test, using the SPSS 20 software (*StatisticalPackage for theSocial Sciences*), the significance used was 5% ($p < 0.05$), with 95% of confidence interval.

3. Results and Discussion

Means and respective standard deviations of endogenous pH and TSS of beverages are expressed in Table 2.

Table 2. Mean and standard deviation (SD) of pH and TSS of commercial Whey Protein beverages.

GROUPS	**pH**	**TSS**
	Mean (SD)	Mean (SD)
G_1	5.95 (±0.05)	12.58 (±0.38)
G_2	5.83 (±0.04)	12.50 (±0.50)
G_3	5.75 (±0.08)	12.00 (±0.43)
G_4	6.87 (±0.02)	12.25 (±0.00)
G_5	6.77 (±0.04)	12.25 (±0.43)
G_6	6.74 (±0.11)	11.66 (±0.28)
G_7	6.39 (±0.33)	10.16 (±0.28)
G_8	5.85 (±0.09)	16.66 (±0.38)
G_9	6.91 (±0.03)	10.41 (±0.14)
TOTAL	**6.34 (±0.49)**	**12.27 (±1.81)**

Source: Authors.

For pH, the lowest value was recorded for G3 (5.75), while the highest value was for G9 (6.91). Regarding total soluble solids, G7 presented the lowest average (10.16), while G8 revealed the highest (16.66).

The Table 3 presents data from Comparison between same brand and same flavor groups for pH and TSS variables.

Table 3. Comparison between same brand and same flavor groups for pH and TSS variables.

Variables	pH		TSS	
	Mean (SD)	p-value	Mean (SD)	p-value
Brand				
Muke	5.84 (±0.05)	0.09	12.36 (±0.18)	0.55
Best Whey	6.79 (±0.03)		12.05 (±0.19)	
Essencial Nutrition Whey	6.38 (±0.30)		12.41 (±2.12)	
Flavor				
Vanilla	6.40 (±0.26)	0.73	11.66 (±0.75)	0.17
Strawberry	6.15 (±0.31)		13.80 (±1.43)	
Chocolate	6.47 (±0.36)		11.36 (±0.48)	

Source: Authors.

No statistically significant difference was observed between pH values and total soluble solids (TSS) according to brand and flavors.

The practice of physical exercises in association with proper nutrition is extremely important to prevent atrophy and to favor the process of muscle hypertrophy (Haraguchi et al., 2006). In this context, bovine whey is a good example of the application of proteins as functional ingredients, since it is a byproduct of the dairy industry, and is rich in nutritional components (Haraguchi et al., 2006; West et al., 2017).

Thus, the industrial valorization of this product is clear, since it enables the enrichment and the development of new food articles, such as the preparation of dairy beverages added to whey protein (Santos et al., 2015). However, differences in processing systems, especially in relation to protein concentration, may cause alterations in functional properties, interfering with enzymatic, antioxidant and antimicrobial hydrolysis activities (Iltchenco et al., 2018; Souza et al., 2019).

Whey protein concentrate (WPC) is one of the commercially available whey products obtained by removing non-protein constituents from the whey so that the final dried product generally contains between 35% and 80% whey content and the whey protein isolate (WPI), which is the purest commercial form of whey protein, contains between 80 and 95% protein (Brans, 2006).

Whey protein is well known and widely consumed because it functions as a high quality protein source, especially incorporated in sports and nutritional products (West et al.,

2017). Thus, whey protein consumption, especially by athletes, ostensibly benefits the increase in whole body anabolism by maximizing lean mass growth and post-exercise recovery (West et al., 2017), which has led to a rise in commercial demand for nutritional supplements, especially protein-energy (Gomes et al., 2008).

However, processed products need to have preservatives and other substances added to them to extend shelf life and flavor, which in turn makes them sweeter and more acid (Malik et al., 2006). Thus, these characteristics of some processed foods and beverages may modulate the development of certain dental problems, such as dental caries and erosion (Salas et al., 2018). When acidic beverages are ingested, saliva pH decreases and drastic increase in enamel apatite solubility occurs (Sobral et al., 2000), which can start the demineralization process of dental surface without bacterial involvement, that is, dental erosion, which is a multifaceted process whose etiology depends on the combination of several factors (Diniz & Lussi, 2017).

Thus, diet is naturally considered an extrinsic factor that causes dental erosion, since fruit juices, soft drinks and other industrialized beverages usually have low pH, and a pH around 5.5 is capable of weakening and demineralizing enamel surface (Branco et al., 2008; Souza, 2017). This value may vary, depending on concentrations of calcium and phosphate in saliva (Sobral et al., 2000), as well as the buffering capacity of the saliva and the availability of fluoride in the oral cavity (Souza, 2017).

In this research, nine protein beverages of different flavors and different brands were evaluated. All had a pH above the value considered critical (5.5), with the lowest value at 5.75 (*Muke® chocolate*) and the highest value at 6.91(*Essencial Nutrition Whey® cacao*). Although the strawberry flavor had the lowest pH average (6.15), no significant difference was found among the three flavors evaluated with respect to pH. In addition, there was no significant difference among the brands evaluated in terms of pH. However, it is noteworthy that Muke® had the lowest average pH at 5.8.

Considering only the pH, the studied beverages have no ability to produce erosive effect on the surface of the tooth enamel (Branco et al., 2008). However, in addition to pH, other properties, such as the presence of sugars, can influence the acidity of foods through biochemical modification of bacterial biofilm, increasing acid production and hastening the onset of demineralization (Assad et al., 2010; Cardoso et al., 2013).

Refractometry on the Brix scale is a method accepted by the scientific community to measure values equivalent to total soluble solids (TSS) of beverages, and the percentage of the reading is expected to be similar to the existing sugar concentration in the solutions

analyzed (Cardoso et al., 2013). TSS content is also directly related to product viscosity, which may favor the retention of dietary components in dental structures (Cavalcanti et al., 2010), and influence the erosive potential.

When evaluated for TSS content, no significant difference of soluble solids was observed regarding the investigated brands and flavors. However, the G8 group (*Essencial Nutrition Whey® redberry*) had the highest average (16.66), and group G7 (*Essencial Nutrition Whey® Vanilla*) had the lowest mean for TSS (10.17). In addition, among the flavors considered, strawberry had the highest percentage average (13.80) and the *Essential Nutrition Whey*® brand had the highest TSS average (12.41).

The TSS values of the studied beverages are higher than recommended, possibly to ensure better taste acceptability by consumers (Castro et al., 2016). Thus, the results of this study suggest that the evaluated beverages have little erosive potential, due to the endogenous pH values found, which are above the critical value for enamel demineralization (Branco et al., 2008). In turn, the verification of the high content of total soluble solids in beverages imposes the need for caution regarding the consumption of these products, especially as to the frequency of ingestion and the adoption of correct oral hygiene.

Thus, knowing that early diagnosis of dental erosion is necessary to prevent its progression (Assad et al., 2010), attention should be paid to the importance of incorporating regular oral health check-ups by frequent users of whey protein drinks. In addition, the need for further *in situ* studies is worth emphasizing so that the erosive potential of these beverages is also understood under clinical conditions.

4. Conclusion

The analyzed beverages had a pH higher than the critical pH for tooth enamel, and high total soluble solids content. Given these findings, these drinks should be consumed in moderation.

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