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Development of a microencapsulated cocoa (*Theobroma cacao*) - based product and evaluation of total phenolic compounds and antioxidant capacity

Desenvolvimento de um produto a base de cacau (*Theobroma cacao*) microencapsulado e avaliação de compostos fenólicos totais e capacidade antioxidante

Desarrollo de un producto a base de cacao (*Theobroma cacao*) microencapsulado y evaluación de compuestos fenólicos totales y capacidad antioxidante

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Abstract

Oxidative stress is associated with the pathogenesis of several chronic diseases. Cocoa is a food rich in polyphenols, with high antioxidant properties, and is an important food in its fight. However, most polyphenols have low solubility, which impairs their biological action. Therefore, encapsulation through the spray drying technique can significantly improve these parameters by generating a protective layer using proteins and polysaccharides. Therefore, the objective of this work was to elaborate a cocoa-based product encapsulated with maltodextrin (CM) or goat milk whey (CW) and to evaluate the total phenolic compounds and their antioxidant capacity, as well as the particle size of the encapsulated product. Cocoa-based products were encapsulated using maltodextrin DE20 or goat whey using a B-290 mini spray dryer (Büchi Labortechnik, Flawil, Switzerland) in a 1:1 weight ratio. Subsequently, the antioxidant capacity was analyzed by the 2,2'-azino-bis (3-ethylbenzoatiazoline-6-sulfonic acid) (ABTS) method and total phenolic compounds using the phenol reagent Folin-Ciocalteu by spectrophotometry, as well as the size of the particle. The CW obtained a higher yield (33.11%) when compared to the CM (24.03%) in the spray dryer. Most of the particles (90%) present in the CM and CS had a size of 21.92 and 21.12 µM, respectively. CW had a higher content of phenolic compounds compared to CM (CM: 536 ± 8.0 vs. CW: 818 ± 77.0 mg GAE/100g dw, $p = 0.05$). No significant difference was observed in antioxidant capacity between samples (CM: 545 ± 26.0 vs. CW: 478 ± 18.0 µmol TE/100g dw, $p = 0.114$). The results showed that although CS has a higher content of phenolic compounds, both had the same antioxidant capacity.

Keywords: Food technology; Bioactive compounds; Microencapsulation; Spray-drying.

Resumo

O estresse oxidativo está associado à patogênese de várias doenças crônicas. O cacau é um alimento rico em polifenóis, com altas propriedades antioxidantes, sendo um alimento importante no seu combate. No entanto, a maioria dos polifenóis apresenta baixa solubilidade, o que prejudica sua ação biológica. Portanto, o encapsulamento através da técnica de spray dryer pode melhorar significativamente esses parâmetros ao gerar uma camada protetora utilizando proteínas e polissacarídeos. Portanto, o objetivo deste trabalho foi elaborar um produto à base de cacau encapsulado com maltodextrina (CM) ou soro de leite de cabra (CW) e avaliar os compostos fenólicos totais e sua capacidade

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antioxidante, bem como o tamanho de partícula do produto encapsulado. Os produtos à base de cacau foram encapsulados usando maltodextrina DE20 ou soro de leite de cabra usando um mini spray dryer B-290 (Büchi Labortechnik, Flawil, Switzerland) na proporção de peso de 1:1. Posteriormente, a capacidade antioxidante foi analisada pelo método 2,2'-azino-bis (ácido 3-etilbenzoatiazolina-6-sulfônico) (ABTS) e os compostos fenólicos totais utilizando o reagente fenol Folin-Ciocalteu por espectrofotometria, bem como o tamanho da partícula. O CW obteve maior rendimento (33,11%) quando comparado ao CM (24,03%) no spray dryer. A maioria das partículas (90%) presentes no CM e CW tinham um tamanho de 21,92 e 21,12 µM, respectivamente. O CW apresentou maior teor de compostos fenólicos em relação ao CM (CM: 536 ± 8,0 vs. CW: 818 ± 77,0 mg GAE/100g dw, p = 0,05). Não foi observada diferença significativa na capacidade antioxidante entre as amostras (CM: 545 ± 26,0 vs. CW: 478 ± 18,0 µmol TE/100g dw, p = 0,114). Os resultados mostraram que embora o CW tenha maior teor de compostos fenólicos, ambos apresentaram a mesma capacidade antioxidante.
Palavras-chave: Tecnologia de alimentos; Compostos bioativos; Microencapsulação; Spray-drying.

Resumen
El estrés oxidativo está asociado con la patogénesis de varias enfermedades crónicas. El cacao es un alimento rico en polifenoles, con altas propiedades antioxidantes, y es un alimento importante en su lucha. Sin embargo, la mayoría de los polifenoles tienen una baja solubilidad, lo que perjudica su acción biológica. Por lo tanto, la encapsulación mediante la técnica de secado por aspersión puede mejorar significativamente estos parámetros al generar una capa protectora utilizando proteínas y polisacáridos. Por tanto, el objetivo de este trabajo fue elaborar un producto a base de cacao encapsulado con maltodextrina (CM) o suero de leche de cabra (CW) y evaluar los compuestos fenólicos totales y su capacidad antioxidante, así como el tamaño de partícula del producto encapsulado. Los productos a base de cacao se encapsularon usando maltodextrina DE20 o suero de cabra usando un mini secador por aspersión B-290 (Büchi Labortechnik, Flawil, Switzerland) en una proporción de peso de 1:1. Posteriormente se analizó la capacidad antioxidante por el método del 2,2'-azino-bis(3-etilbenzoatiazolina-6-sulfónico) (ABTS) y compuestos fenólicos totales utilizando el reactivo fenólico Folin-Ciocalteu por espectrofotometría, así como el tamaño de la partícula. El CW obtuvo un mayor rendimiento (33,11%) al compararlo con el CM (24,03%) en el atomizador. La mayoría de las partículas (90%) presentes en el CM y CS tenían un tamaño de 21,92 y 21,12 µM, respectivamente. CW tuvo un mayor contenido de compuestos fenólicos en comparación con CM (CM: 536 ± 8,0 vs CW: 818 ± 77,0 mg GAE/100g dw, p = 0,05). No se observaron diferencias significativas en la capacidad antioxidante entre las muestras (CM: 545 ± 26,0 frente a CW: 478 ± 18,0 µmol TE/100 g dw, p = 0,114). Los resultados mostraron que aunque el CS tiene un mayor contenido de compuestos fenólicos, ambos tienen la misma capacidad antioxidante.
Palabras clave: Tecnología de los Alimentos; Compuestos bioactivos; Microencapsulación; Spray-drying.

1. Introduction

Oxidative stress is discerned as the imbalance between the production of antioxidant and oxidant molecules, which has been linked to the pathogenesis of several chronic diseases such as cardiovascular disease, neurodegenerative diseases, cancer, and even aging process. There is robust evidence showing that high consumption of foods rich in phenolic compounds can reduce the risk of these diseases, with cocoa being one of the richest in phenolic compounds (Martins et al., 2020; Oliveira et al., 2021). Cocoa is a food rich in polyphenols, which have antioxidant, anti-inflammatory, antidiabetic, and antihypertensive properties (Ciumarnean et al., 2020). Previous studies have shown that cocoa powder has a significant amount of phenolic compounds (approximately 3900 mg GAE/100g dw) and its antioxidant capacity has been compared between different brands sold in the market (Oliveira et al., 2021). The main phenolic compounds present in cocoa are flavonoids and tannins, such as flavanols and anthocyanins (Efraim et al., 2011). In this context, cocoa intake emerges as a food capable of preventing and delaying the progression of various diseases (Martín & Ramos, 2016). However, most of the polyphenols present in cocoa (and other sources of polyphenols) have low solubility, which impairs their absorption and biological action (Sorrenti et al., 2020).

The encapsulation of these food-derived bioactive compounds (e.g., polyphenols) using polysaccharides and proteins through the spray drying technique, a food technology, can significantly improve the bioavailability of polyphenols (Oliveira et al., 2021). Polyphenols are integrated into a matrix in the encapsulation process, generating a microparticle with an outer layer (capsule) and a core (bioactive compounds). Therefore, encapsulation has been utilized to protect bioactive components against elevated temperatures, exposure to light, pH, and high humidity. In addition, spray drying is a methodology considered simple and economical, becoming viable due to its numerous benefits for hydrophilic and hydrophobic food ingredients, high encapsulation efficiency, and extended shelf life after obtaining (Assadpour & Jafari, 2019).

The most used encapsulating ingredient in the literature is maltodextrin (capsule component of microencapsulated product). It has high water solubility, lack of color, low viscosity, and mild flavor (Bakowska-Barczak & Kolodziejczy, 2011; Alves et al., 2017). Its use has been linked to increasing the polyphenol content in products and improving bitterness and astringency in a chocolate bar enriched with microencapsulated cocoa powder (Grassia et al., 2021). In addition, better protection of phenolic compounds is demonstrated by a better formation of microcapsules, providing positive storage and culinary use (Papillo et al., 2018). However, protein can also be an interesting encapsulating ingredient due to many nutritional aspects, such as protein content, elevated levels of specific fatty acids, vitamins, etc. In this sense, whey protein is a very nutritious by-product generated in the manufacture of cheeses, which are usually discarded in the environment. In this context, whey protein may be involved in increased pollution by being released into the water without any treatment and having a high consumption of water in the process of production and sanitation, in which approximately 40% of the whey is discarded during its manufacture (Silva et al., 2016).

Therefore, this study aimed to elaborate on a cocoa-based product microencapsulated with maltodextrin (CM) or goat milk whey (CW) and evaluate the total phenolic compounds and their antioxidant capacity and the particle size of the product encapsulated.

2. Methodology

Sample acquisition

The cocoa powder product used in this study was from the Garoto® brand, maltodextrin was used from the Adicel® brand and the goat milk whey was purchased from a local agricultural cooperative in the city of Macaé, Rio de Janeiro, Brazil (22º 22’ 18” S, 41º 47’ 9” W).

Cocoa-based products were encapsulated using maltodextrin or goat whey using a B-290 mini spray dryer (Büchi, Labortechnik, Flawil, Switzerland) with a 1.0 mm standard diameter nozzle and evaporation capacity of 1.0 L/h. Cocoa was mixed with maltodextrin (DE20) or goat whey in a 1:1 weight ratio in 200 mL water and subjected to spray drying. The conditions used in the spray dryer were air inlet temperature at 160°C and sample feed flow rate into the system at 30%. The product formed was stored in amber opaque packaging and placed inside a desiccator until the analysis time.

Determination of total polyphenols

As previously described, total polyphenols and flavonoids of cocoa-based products were determined using the Folin-Ciocalteu (F-C) reagent (Deng et al., 2013). Briefly, samples (4 g) were homogenized with 8 mL of methanol following centrifugation to 10 000 g for 15 min. Afterward, 500 µL of the supernatant was mixed with 300 µL of 1.5N hydrogen peroxide to oxidize the interfering compounds. The sample was vortexed and underwent the F-C assay by diluting 15 µL of the sample mixture with 240 µL of distilled water and 15 µL of 0.25 N F-C reagent. After 4 min, 30 µL of 1 N sodium carbonate was added. The mixture was incubated for 2 h in the dark, and the absorbance values were determined at 765 nm. The outcome data were expressed as gallic acid equivalents in mg/100g of dry weight (mg GAE/100g dry weight) and the analyzes were performed in triplicate.

Determination of total antioxidant capacity

Total antioxidant capacity was evaluated using the Trolox equivalent antioxidant capacity (TEAC) assay described by Singleton and Rossi (1965). The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) for the stock solution was prepared from 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate in a volume ratio of 1:1, and then incubated in the dark at room temperature for 16 h and used within 2 days. A 100 mL sample was mixed with a 3.8 mL ABTS working solution, and the absorbance was taken at 734 nm after 6 min of incubation at room temperature. The percent of inhibition of absorbance at 734 nm was calculated, and the results were expressed as μmol of Trolox equivalents (μmol TE/100 g dry weight) and the analyzes were performed in triplicate.

Determination of particle size

The diameter measurement was evaluated by the laser diffraction method (Mastersizer 2000®, Malvern Instruments, UK), using water as a dispersant. The data were analyzed by Mastersizer 2000 E Ver. 6.01 software and determined particle size ranging from 0.100 to 1.000.000 µm. The software's span values were determined by dividing the difference between D0.1 and D0.9 by D0.5.

Process yield

The drying yield was determined as the ratio of the mass of total solids in the powder to the mass of total solids in the feed solution. The measurements were made in duplicate.

Statistical analysis

An independent T-test was performed to detect statistical differences in total polyphenols content and total antioxidant capacity (IBM SPSS Statistics version 26 for Windows, Armonk, NY, USA). Statistical significance was set at a P-value ≤ 0.05, and the results were expressed as mean ± standard deviation (SD).

3. Results and Discussion

The spray dryer obtained a higher yield value of CW (33.11%) compared to CM (24.03%). These findings are in agreement with previous studies showing that cocoa aroma with Hi-Cap (32.65-58.77%), pequi pulp with gum arabic (24.24-49.80%), and Uncaria tomentosa with maltodextrin (9.88-40.87%) and acacia gum (14.37-63.94%) (Santana et al., 2014; Sanchez-Reinoso et al., 2017; Rodrigues et al., 2020). Yield is the part recovered from the product and a demonstration of efficiency in its collection. Therefore, some conditions chosen during the microencapsulation process, such as inlet temperature, the proportion with the encapsulating agent, and the feed rate can directly affect both the quality of the powder and its yield (Vu et al., 2020). Therefore, different conditions and proportions are necessary to minimize losses to the equipment, but without losing its quality. Although the yield appears to be somewhat low, microencapsulation technology can preserve bioactive compounds present in food, which can improve its benefice to human health.

Most particles (90%) in CM and CW present sizes of 21.92 and 21.12 µM, respectively (Table 1). This parameter is of paramount importance to demonstrate the influence of this product on the segregation of the mixture and its mixture with different compaction components. Small particle size is crucial to increase the solubility, absorption, and bioavailability of the microencapsulated products, improving the action of the bioactive compounds after ingestion. For example, nanoscale particle size, compared with microscale particle size, has better absorption and release in the body by reaching all cells and crossing different barriers in the body, thus becoming more effective (Kohane, 2006). Thus, the smaller the particle size can enhance its solubility and fluidity, which influences the acceptability of the product, demonstrating that there are particles of this kind that do not have the action of the encapsulating agent. On the other hand, when there is a more significant presence of large particles, it is assumed that there is an agglomeration of particles, making connections among themselves (Santana et al., 2014). The values obtained in this study are in line with those in the literature, such as *Nigella sativa* oil with different wall agents (13.88-50.38

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µM) and maca leaf polyphenol extract with maltodextrin (23.17 µM) and maltodextrin with gum Arabic (18.84 µM) (Lee & Chang, 2020; Mohammed et al., 2020).

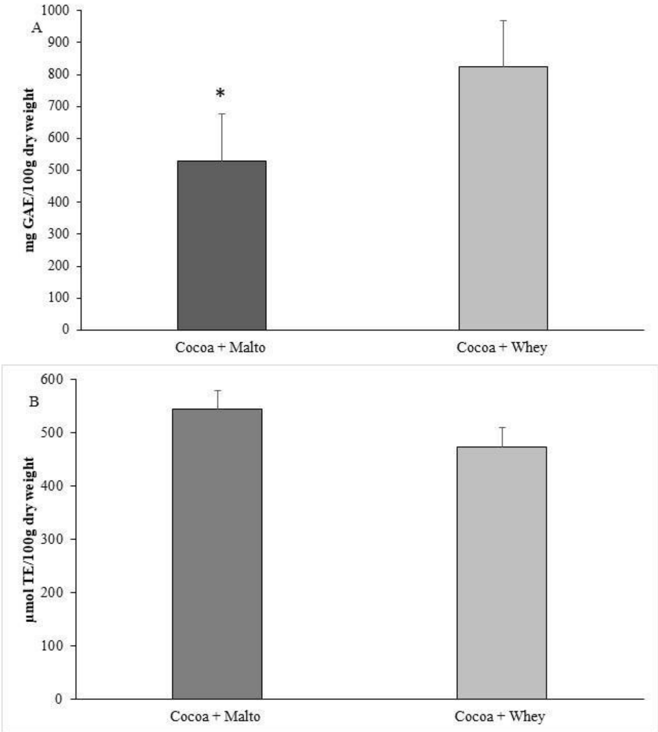
Table 1. *Average particle size diameter of Cocoa + Goat Milk Whey (CW) and Cocoa + Maltodextrin (CM) products.*

Volume (%)	Particle Size (µm)	
	CW	CM
10	1.991	2.315
50	8.569	8.341
90	21.129	21.922

Font: Authors (2022).

There was a higher statistical content of phenolic compounds in CW compared to MC (CM: 536 ± 8.0 vs. CW: 818 ± 77.0 mg GAE / 100g dry weight, p = 0.05) (Figure 1.A). Such a difference in the phenolic compound can be explained by the higher phenolic content (approximately 200%) found in whey compared to maltodextrin (data not shown). Gallegos-Infante et al. (2013) evaluated different physicochemical properties and antioxidant capacity of infusions of the oak leaf (Quercus resinona) encapsulated by spray-drying with maltodextrin (10 DE) and k-carrageenan. The authors observed that the highest polyphenol retention value was for maltodextrin at 1.6 L/h and the k-carrageenan mixture with maltodextrin at 1.6 L/h and 1.7 L/h, which was explained by the fact that maltodextrin has a more water-soluble composition, reducing, therefore, its viscosity and favoring its drying (Gallegos-Infante et al., 2013). Similarly, Norkaew et al. (2019) observed the effect of different encapsulating agents on physicochemical parameters of microcapsules produced from anthocyanin from black rice (plant with antioxidant capacity) through the spray dryer technique. It was reported that the use of whey protein obtained higher values of phenolic compounds in its composition, but the use of maltodextrin obtained a greater efficiency in the capsules and retention of anthocyanins in the product, due to the structural differences of the materials of wall and their respective different reactions with the phenolic compounds present in the product (Norkaew et al. 2019).

Figure 1. *Total polyphenols content (A) and antioxidant capacity (B) of the cocoa-based products.*



Subtitle: TE = Trolox Equivalent; GAE = Gallic Acid Equivalent. Data are shown as mean ± standard deviation. * Significant difference from microencapsulated cocoa-based products ($P < 0.05$).Font: Authors (2022).

Although a greater phenolic compound was found in CW than in CM, no significant difference was observed in the antioxidant capacity between the samples (CM: 545 ± 26.0 vs. CW: 478 ± 18.0 µmol TE / 100 g of weight dry, $p = 0.114$) (Figure 1. B). The interaction between the proteins present in the CW can likely reduce the analysis of the antioxidant capacity. This protein binds to the polyphenols present in cocoa, depending on the environment in which it is found. It has been previously shown that adding milk to black tea did not affect the antioxidant capacity (Reddy et al., 2005). Furthermore, Arts et al. (2002) demonstrated through the interaction between flavonoids and proteins with the TEAC test that this relationship between them negatively affects the antioxidant capacity, reducing it both in products and in vivo. The study by Papoutsis et al. (2018) carried out the encapsulation of extracts of lemon by-products by the spray drying technique using different combinations of maltodextrin with soy protein and ι-Carrageenan. They were able to observe that the mixture of a protein wall agent with polysaccharide obtained a greater antioxidant capacity for the products, as well as the efficiency of the coating of the aqueous extracts, demonstrating that this union can become effective in protecting against the high temperatures that are subjected in the process, in addition to performing a better interaction between the encapsulating agents and the matrix to be protected (Papoutsis et al., 2018).

A limitation of the present study was not included phenolic and antioxidant analysis of cocoa powder before spray drying. However, a previous study from our laboratory has investigated the content of phenolic and antioxidant capacity in different brands of cocoa (Oliveira et al., 2021). It was shown that cocoa exhibited very higher phenolic compounds (approximately 3-fold) compared to microencapsulated cocoa. It comes not as a surprise given that cocoa was microencapsulated with maltodextrin or goat's whey (1:1). In addition, it should be noted that our microencapsulated product presented a low yield (approximately 30%). Futures studies investigating different encapsulating materials and/or utilizing other temperatures could improve the product yield.

4. Conclusion

The results showed that although CW has a higher content of phenolic compounds, both have the same antioxidant capacity, demonstrating that both have equivalent effects. In addition, the CS produced by spray-drying showed a higher yield. However, changes in the conditions used in the spray dryer can lead to a better performance in the reintegration of the product.

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