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CHALLENGES IN SPRAY-DRYING HABANERO RED EXTRACT: A PILOT STUDY ON THE MICROENCAPSULATION WITH MALTODEXTRIN AND GUM ARABIC

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ABSTRACT

This study investigated the microencapsulation of *Capsicum chinense* Jacq. Habanero Red extract (HRE), focusing on the preservation of its bioactive components and physicochemical stability. We analyzed total polyphenol content (TPC), antioxidant activity (AA), capsaicinoid levels, and selected polyphenols in both pure and spray-dried form. Microencapsulation was achieved using a blend of maltodextrin (MD) and gum Arabic (GA) in a 3:2 ratio, with an inlet temperature of 120 °C. Key microencapsulation efficiency parameters were evaluated, including phenolic retention, antioxidant preservation, solids recovery, moisture content (MC), and water activity (a_w) in the microencapsulated powder. Our results showed high phenolic retention (70.37 ± 5.55%) and antioxidant capacity preservation (90.51 ± 0.72%) in the spray-dried HRE powder, even though solids recovery was below 50% (44.46 ± 0.51%). This powder had values for MC (5.50 ± 0.75%) and a_w (0.352 ± 0.004) within the microbiologically stable range. The findings indicate that microencapsulation effectively retains bioactive components, supporting the stability and potential application of HRE in functional food formulations. Essentially, the pilot study generated an initial data focusing on polyphenol and AA preservation under the selected conditions and it can provide a basis for larger-scale or more comprehensive studies utilizing these parameters to enhance the encapsulation efficiency and stability of diverse bioactive compounds.

Keywords: chili pepper, spray drying process, microencapsulation efficiency, bioactive compounds

INTRODUCTION

In addition to capsaicinoids, which are responsible for its characteristic heat, chili peppers (*Capsicum* spp.) are rich in a variety of bioactive compounds, such as phenolic compounds, ascorbic acid, other vitamins, and carotenoids (including apocarotenoids) (**Duranova *et al.*, 2022**). These compounds contribute to their high antioxidant capacity (**Antonious, 2018**), making them beneficial in both nutrition and functional food applications. Habanero chili peppers (*Capsicum chinense* Jacq.), native to Latin America and widely consumed in the Caribbean and tropical regions, are well-known for their intense heat due to high capsaicin content. This makes them attractive to the pharmaceutical industry, particularly for products like pepper sprays (**Aryee *et al.*, 2022**). Furthermore, Habanero peppers are not only valued for their distinct flavor and vibrant color in culinary uses (**Pino *et al.*, 2020**) but also for their potential health benefits. These include antioxidant effects, as they help neutralize free radicals and reactive oxygen species in the human body (**Palma-Orozco *et al.*, 2021**). Additionally, they exhibit a wide range of pharmacological and physiological actions, such as anticancer, anti-inflammatory, pain relief, cardiovascular, gastrointestinal, antidiabetic, and anti-obesity effects, as well as antimicrobial and antifungal properties (**Duranova *et al.*, 2022**).
Using bioactive compounds in industrial processes poses several challenges, including low solubility, poor stability, undesirable taste, and limited bioavailability (**Lu *et al.*, 2021**). For example, phenolic compounds exhibit antioxidant activity due to specific structural elements, such as one or more aromatic rings with attached hydroxyl groups, as seen in phenolic acids and polyphenols (**Minatel *et al.*, 2017**). Despite this, their application is often restricted due to their vulnerability to oxidation, hydrolysis, and thermal degradation, which can compromise antioxidant potential (**de Lima Marsiglia *et al.*, 2023**). To address these stability issues, stabilization technologies have become a focal point in food processing. Among these, spray drying microencapsulation has gained popularity due to its cost-effectiveness, scalability, and short processing time, making it one of the most popular drying methods in the food industry (**Sosnik and Seremeta, 2015; Morales and Ruiz, 2016; Valková *et al.*, 2022; Hoskin *et al.*, 2023a,b**). In the spray drying process, a feed solution is atomized into a high-temperature environment, where the solvent (typically water) quickly evaporates. The dried particles are then collected from the outlet air at a lower temperature (**Champagne and Fustier, 2007; Jana *et al.*, 2017; Cittadini *et al.*, 2022**), forming a stable, free-flowing powder in which bioactive compounds are encapsulated within diverse protective agents (**Lingua *et al.*, 2021**). This protective barrier can enhance particles physicochemical attributes, as well as increased bioavailability, and efficacy of bioactive compounds (**Rigon and Zapata Noreña, 2016; Lu *et al.*, 2021; Hoskin *et al.*, 2023b**). It also enables controlled release, masks unpleasant tastes or odors, and therefore, offers diversified applications in the food and pharmaceutical sectors (**Furuta and Neoh, 2021; Jafari *et al.*, 2023**). The resulting solid particles typically have a narrow size distribution, ranging from the submicron to micron scale (**Sosnik and Seremeta, 2015**), which supports consistent dosing, improved stability, and potential applications in food and pharmaceuticals.
Different wall materials affect spray drying efficiency and outcomes (**Stabrauskiene *et al.*, 2024**), because of their unique emulsifying and film-forming abilities that influence the encapsulation performance (**Cano-Higuita *et al.*, 2015**). Selecting the appropriate wall material is critical since it significantly influences the structural and physicochemical stability and functionality of microcapsules (**Jafari *et al.*, 2023**). Maltodextrins (MD), derived from starch, are extensively used for encapsulation purposes in the food industry due to their cost-effectiveness, neutral taste, and high solubility. Their high molecular weight and glass transition temperature (*Tg*) warrants stability to encapsulated powders with preserved sensitive compounds by providing excellent oxidation protection. Additionally, MD contributes to increasing process efficiency because of their ability to reduce wall deposition and prevent stickiness during the drying process, given their low viscosity in cold water (**Do and Nguyen, 2018; Cittadini *et al.*, 2022**). Gum Arabic (GA), a polysaccharide sourced from the *Acacia senegal* and

Acacia seyal trees, is well-suited for encapsulating bioactive compounds, due to its non-digestibility, low solution viscosity, and generally recognized safe status (**Patel and Goyal, 2015**). However, its higher cost, limited supply, and quality variations can restrict its broader use. Consequently, it is often used as a co-carrier, i.e., it is combined with other encapsulating agents (**Bajac *et al*., 2022**) to balance cost, quality, and availability. Combining MD and GA has shown to significantly enhance the quality of the final spray-dried powder by improving stability and encapsulation efficiency (**Fazaeli *et al*., 2012; Do and Nguyen, 2018**).

Spray drying has been extensively utilized for the microencapsulation of bioactive compounds derived from chili peppers. Examples include capsaicin (**Liu *et al*., 2022**), volatile compounds from Habanero chili (**Pino *et al*., 2020**), oleoresins extracted from *Capsicum* species (**Krithika *et al*., 2014**), capsaicinoids from red pepper oleoresin (**Razavizadeh *et al*., 2015**), and emulsions of paprika and cinnamon oleoresins (**Ferraz *et al*., 2022**). Microencapsulation of Habanero pepper (*Capsicum chinense* Jacq.) extracts utilizing MD as a standalone coating material has been previously reported (**Avilés-Betanzos *et al*., 2023; Marín Castro *et al*., 2023**), highlighting the expanding application of this technique. However, these investigations have employed diverse extraction methods, such as methanolic extraction, and have examined various coating agents depending on the intended application.

The objective of the present study was to evaluate the stability of phenolic compounds present in Habanero Red extract (HRE) after spray drying microencapsulation with a blend of MD and GA (3:2) and inlet temperature of 120 °C. The goal was to verify the initial viability of microencapsulation for chili extract and to provide a starting point for further refinement and optimization in future research. The important parameters including solids recovery, moisture content, water activity, phenolic retention, antioxidant capacity preservation, as well as total phenolic content (TPC), antioxidant activity (AA), major capsaicinoids and individual phenolic compounds were evaluated using various analytical tools. Furthermore, this study lays the groundwork for our future research where microencapsulated HRE will be evaluated for its anticancer potential on human colorectal cancer cell line. It will also be incorporated into medium-chain triglyceride (MCT) oil to assess its acceptability among consumers sensitive to capsaicin, broadening its potential applications in both health and food science.

MATERIAL AND METHODS

Materials

Capsicum chinense Jacq. Habanero Red fruits were sourced from experimental plots at the Botanical Garden of the Slovak University of Agriculture in Nitra, Slovakia (167 m above sea level). Maltodextrin (dextrose equivalent in the range of 4.0–7.0) and gum Arabic (from acacia tree) were obtained from Sigma-Aldrich (SaintLouis, Missouri, USA). Other chemicals were purchased from Merck KGaA (Darmstadt, Germany), or Centralchem, Ltd. (Bratislava, Slovakia) and were of analytical grade.

Production of Habanero concentrated extract

Fully ripe Habanero Red (*Capsicum chinense* L.) fruits were harvested, destemmed, and their pulp, including seeds, was sliced into approximately 1 cm pieces. The samples were then dried in a forced-air drying chamber (Memmert UF160, Memmert GmbH + Co. KG, Büchenbach, Germany) at 40 °C for 72 to 96 h, with the duration adjusted to accommodate the inherent variability in the drying behavior of the materials. Once thoroughly dried, the pepper samples were homogenized using a grinder with chopping blades (ETA Gratus 0028 90030, ETA-Slovakia Ltd., Bratislava, Slovakia). The resulting habanero powder was stored in sealable polyethylene bags within a desiccator until further use.

A habanero concentrated extract was produced following a modified method based on our previously published protocol (**Hoskin *et al*., 2023a,b**). Briefly, the habanero powder was homogenized with a 50% ethanol solution (1:3 extraction ratio [w/v]) and transferred to a laboratory water bath set at 80°C for 2 h and subsequently filtered under vacuum. After filtration, the mixture was centrifuged at 4000 rpm for 20 min (Rotina 420, Hettich, Germany). The ethanol was removed using a rotary evaporator in a water bath at 40 °C (RVO 400, Ingos, Czech Republic). The extract was divided into two portions: one was used for spray drying microencapsulation, while the second portion was freeze-dried (Lyoquest - 55, Azbil Telstar Technologies, Spain) to covert the extract into powder. The resulting HRE powder was stored in sealable polyethylene bags within a desiccator until further analysis.

Spray drying microencapsulation

It was carried out following the methodology of **Hoskin *et al*. (2023a,b)**. Three independent batches were prepared using a lab-scale spray dryer B-290 (BÜCHI Labortechnik AG, Switzerland), with a concurrent flow system and a nozzle diameter of 0.7 mm. Based on preliminary experiments (data not shown), the feed flow rate was maintained at 10 mL/min using a peristaltic pump, the air flow rate was set to 32.5 m^3/h, and the drying air was heated to an inlet temperature of 120 °C, with the outlet temperature ranging from 56 to 66 °C. Prior to each batch, a blend of MD and GA at concentrations of 4.5% and 3% (w/v), respectively, in a 3:2 (w/w) ratio, was dispersed directly into the habanero concentrated extract and stirred for 2 h until fully dissolved. The prepared feed solution was kept under constant magnetic stirring throughout the drying process. The resulting spray-dried habanero particles were collected from the collection chamber, weighed, sealed, and stored in a refrigerator (4 °C) until further use.

Solids recovery, phenolic retention, and retention rate of antioxidant capacity

The solids recovery was calculated as a percentage (%) using the following formula: [total solids content of resulting spray-dried habanero particles / total solids content of the feeding mixture (before spray drying)] × 100, following the protocol described in previous studies (**Hoskin *et al*., 2021; Hoskin *et al*., 2023a,b**).

The percentage (%) total phenolic (TP) retention was determined according to the method described by many authors (**Zhang *et al*., 2020; Grace *et al*., 2021; Hoskin *et al*., 2023a**) as the ratio [TP of spray-dried habanero powder, mg/g) × (solids in powder, g)] / (TP of feed mixture, mg/g) × (solids in feed mix, g) × 100. The same principle was applied in the calculation of retention rate (%) of antioxidant capacity (AC) (**Kasapoğlu *et al*., 2024**), which was calculated as the ratio [AC of spray-dried habanero powder, mg/g) × (solids in powder, g)] / (AC of feed mixture, mg/g) × (solids in feed mix, g) × 100.

Physicochemical analysis – Water activity (a_w) and moisture content (MC)

The a_w was measured using a LabMaster aw Standard (Novasina, Lachen, Switzerland). For this measurement, 1.0 g of each sample was placed into a sample pan, and the a_w value was automatically recorded at 25 °C over a period of 10–15 min, as described by **Valková *et al*. (2022)**. The MC was determined using a moisture analyzer DBS 60-3 (Kern & Sohn, Balingen, Germany). For this measurement, 1.0 g of each sample was placed on the sample plate, and the analysis was conducted at 120 °C for 5–10 min, as previously described (**Valková *et al*., 2022**).

Capsaicin, dihydrocapsaicin, and phenolic compounds determination by HPLC

Initially, spray-dried habanero particles (1 g) were blended with 20 mL of 80% ethanol (v/v) at laboratory temperature for 4 h by horizontal shaker Unimax 2010 (Heidolph Instruments, GmbH, Germany), filtered through the PVDF syringe filter (0.45 µm, 13 mm diameter) (Frisenette ApS, Knebel, Denmark) into the HPLC vials with PTFE/RS septa caps, and stored in the freezer (-12 °C) until further analyses.

Capsaicin (CAP) and dihydrocapsaicin (DHC) contents were determined using an Agilent 1260 Infinity II HPLC (Agilent Technologies GmbH, Wäldbronn, Germany) according to **Golian *et al*. (2024)**.

Selected phenolic compounds were determined by HPLC-DAD Agilent 1260 (Agilent Technologies, Waldbronn, Germany) according to **Kulig *et al*. (2019)** with modification in HPLC C18 column (Accucore 2.6 µm; 100 x 4.6 mm; Fisher Scientific, New Hampshire, USA).

Total phenolic content (TPC) and antioxidant activity (AA) analyses

Approximately 20 mg of spray-dried habanero particles were mixed with 1 mL of MetOH: H_2O: acetic acid (80:18:2) extraction solution and homogenized. The mixture was sonicated for 5 min at 50 °C followed by centrifugation (Hettich Rotina 380) at 12,000 rpm for 10 min. The supernatant was transferred to clean microtubes. The elution process was repeated once again to ensure complete extraction.

TPC was determined spectrophotometrically using the Folin-Ciocalteu procedure in a microplate format with slight modifications, as described by **Xiong *et al*. (2020)**. In a 96-well plate, 50 µL of distilled water was added, followed by 25 µL of the sample or standard (in triplicate), and 25 µL of Folin-Ciocalteu reagent into each well. After 6 min, 100 µL of 7.5% sodium carbonate solution were added to all wells. The plate was left in the dark for 90 min at room temperature (RT), and absorbance was measured at 765 nm using a microplate reader (Glomax Multi+, Promega Corp., Madison, USA). The photometric measurements were calibrated against a gallic acid standard curve (0–500 mg/L; R2 = 0.972), and the results were expressed as gallic acid equivalents (GAE) in mg per g of dry weight (DW). AA was determined photometrically using the DPPH assay described by **Brand-Williams *et al*. (1995)**, adapted to a microplate protocol. Briefly, to prepare the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, 25 mg was dissolved in 100 mL of 96% ethanol and adjusted to an absorbance of 0.7. In a 96-well plate, 25 µL of the sample, blank (DPPH), or standard (in triplicate) were added, followed by 180 µL of DPPH solution. The plate was then left in the dark for 10 minutes at RT, while shaking at 500 rpm. Absorbance was measured using a microplate reader (Glomax Multi+, Promega Corp., Madison, USA) at 515 nm. AA was calculated as the percentage of DPPH inhibition (%), expressed by the ratio [Absorbance of the control solution (blank) – Absorbance of the sample solution] / Absorbance of the control solution (blank) x 100. Additionally, the ability to scavenge DPPH radicals was calculated using a Trolox standard curve (0 – 100,242 mg/L; $R^2 = 0.9982$) and expressed as Trolox equivalent antioxidant capacity (TEAC) in mg per g DW.

Statistical analysis

All analytical determinations, including total polyphenol content, selected polyphenolic compounds, capsaicinoids, and antioxidant capacity in HRE and its microencapsulated spray-dried form, were expressed as mean values ± standard deviation. Statistical analysis was performed using an unpaired Student's t-test with Welch's correction to account for unequal variances between groups. Calculations and data processing were carried out using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). A p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Solids recovery

In general, solids recovery, also known as spray drying yield, is a critical parameter for evaluating the feasibility and efficiency of the spray drying process (**Hoskin *et al*., 2019; Grace *et al*., 2021; de Medeiros *et al*., 2025**). This metric expresses the percentage of total solids recovered in the final dried product relative to the original solids content in the feed solution (**Hoskin *et al*., 2023a**). In our study, the solids recovery was approximately 44.46 ± 0.51%. This value reflects the efficiency of the spray drying process and is influenced by factors such as feed solution properties, inlet/outlet temperatures, and the nature of the encapsulating materials used (i.e, MD and GA). According to **Sosnik and Seremeta (2015)**, spray drying yield at the laboratory scale with conventional equipment often falls short of optimal levels, typically ranging between 20–70%. This is primarily due to product losses on the walls of the drying chamber and the low efficiency of the cyclone in separating fine particles (< 2 μm). Additionally, uncollected residues and manual handling losses contribute to reduced efficiency (**Fang and Bhandari, 2011**). Nonetheless, a solids recovery rate of approximately 50% is commonly considered satisfactory for lab-scale operations (**Bhandari *et al*., 1997; Bazaria and Kumar, 2016; Hoskin *et al*., 2023a,b; de Medeiros et al., 2025**). In our study, the relatively low solids recovery (44.46 ± 0.51%) could be attributed to the uncontrolled stickiness of the powder particles during drying. This indicates the need to optimize the microencapsulation process to improve the yield without compromising particle characteristics. One potential approach to achieve this could be adjusting the ratio of microencapsulating agents. However, our preliminary experiments with MD:GA ratios of 2:1, 3:2, and 4:3 (w/w) showed that the 3:2 ratio achieved the highest solids recovery, with the recovery rates following the order: 3:2 > 4:3 > 2:1 (data not shown). This trend can suggest that while the higher MD content in the 2:1 ratio enhanced film-forming properties (due to its higher *Tg* reducing stickiness), the reduced GA proportion might have negatively impacted recovery efficiency. Conversely, the increased GA content in the 4:3 ratio likely introduced the stickiness of the mixture that negatively impacted solids recovery. Additionally, excessive GA might affect the balance needed for efficient drying. The 3:2 ratio provided the best compromise, balancing the film-forming ability of MD with the stabilizing and emulsifying properties of GA. However, achieving the desired solids recovery rate of 50% will require further optimization of the formulation and processing conditions. Generally, the interplay between wall materials and feed composition critically influences yield. While increasing MD concentrations can improve yield in some cases (e.g., bayberry juice) by raising the *Tg* and reducing stickiness (**Fang and Bhandari, 2012**), other studies have observed opposite effects. For example, for açaí (*Euterpe oleraceae* Mart.), higher MD concentrations resulted in decreased yields, possibly due to the exponential increase in feed mixture viscosity, which hampers the spray drying process (**Tonon *et al*., 2008**). Similarly, an increase in the proportion of GA in the coating material has been reported to reduce the yield of spray-dried grape polyphenols, suggesting contrasting effects of MD and GA on yield optimization (**Tolun *et al*., 2016**). Moreover, increasing the wall/core ratio (**Jafari *et al*., 2023**) would potentially increase the solids recovery. However, this approach leads to lower core material content, which might reduce the phytochemical content and AA in the final spray-dried product (dilution effect) (**Mishra *et al*., 2014; Tolun *et al*., 2016**). Additionally, increasing inlet air temperatures can lead to a higher process yield. This can be attributed to the greater efficiency of heat and mass transfer that occurs at higher temperatures (**Tonon *et al*., 2008**), as well as the potential for insufficient water evaporation in microcapsules at lower temperatures. However, higher temperatures can have a negative impact on the preservation of bioactive compounds, potentially leading to their degradation due to the increased heat exposure during the spray drying process (**Tolun *et al*., 2016**). As a consequence, a decrease in DPPH radical scavenging activity can be observed (**Mishra *et al*., 2014**). Therefore, further studies are needed to find the best combination of wall/core ratio and coating material composition, to optimize both the spray drying performance and the bioactivity of the final microencapsulated product.

Phenolic retention and antioxidant retention

The assessment of phenolic retention provides insights into how well the microencapsulation process preserves these bioactive compounds and determines the efficiency of encapsulation in protecting and retaining these compounds from degradation due to heat, oxidation, or other environmental factors. High phenolic retention (often considered >70 %) is a key goal, as it reflects not only the preservation of valuable compounds, but also the overall success of the encapsulation method in maintaining their functional integrity (**Hoskin *et al*., 2023a**). This result suggests that the microencapsulation process and chosen parameters (temperature, carrier materials, their ratio) were effective in protecting phenolic compounds from degradation.
In effect, the operating temperatures in spray drying are crucial for preserving heat-sensitive materials, as they play a key role in minimizing thermal degradation (**Fang and Bhandari, 2011; Tolun *et al*., 2016**). Also, it was found that the MD:GA blend offers superior protection compared to using MD alone (**Tolun *et al*., 2016**). This improvement is due to the excellent emulsifying and film-forming properties of GA, which help form a more stable encapsulation matrix. Both GA and MD exhibit high solubility (**Sarabandi *et al*., 2019**), facilitating the effective encapsulation of phenolic compounds as the feed solution is atomized during spray drying. As the mixture passes through the spray dryer, hydrocolloids boasting intricate molecular frameworks, such as GA, can support a more resilient coating layer that better shields sensitive compounds against degradation, ensuring greater retention and stability of these bioactive ingredients in the final product (**Nascimento *et al*., 2023**).
Additionally, the remarkable antioxidant activity retention (90.51 ± 0.72 %) is a desirable result, indicating that the microencapsulation process was very effective also at maintaining the bioactive properties of the HRE. This signalizes effective protection of the coating blend and spray drying operational conditions in protecting not only the polyphenols (with >70% retention) but also other bioactive compounds, like capsaicinoids (**Palma-Orozco *et al*., 2021; Thongin *et al*., 2022**). The greater preservation of AA suggests that the drying process was particularly effective in safeguarding non-polyphenolic antioxidants, which are often more susceptible to degradation from heat, light, and oxidation. This result emphasizes the comprehensive protective capability of the microencapsulation approach, extending beyond polyphenols to the broader spectrum of antioxidant compounds. Anyway, both these findings (high phenolic retention and excellent AA preservation) underscore the success of the microencapsulation method with selected type and ratio of MD and GA, as well as an inlet temperature and its potential for use in functional foods or supplements where high antioxidant preservation is desirable.

Water activity and moisture content

Water is a fundamental component of food, significantly influencing its physical, chemical, and biological properties. Key metrics for evaluating the role of water in food include MC and aw, both of which are critical determinants of food stability and shelf life (**Zhang *et al*., 2020**). In the current study, the MC and a_w of the spray-dried HRE powder were measured immediately after the sample collection. These parameters reached the values of 5.50 ± 0.75%, and 0.352 ± 0.004, respectively. Beyond its importance as a parameter for predicting the stability and shelf life of a product, the MC which reflects the residual water in powders, also serves as a key indicator of the drying process efficiency in spray drying (**Avilés-Betanzos *et al*., 2023**). Considering that MC requirements of a food powder suitable for long-term storage supposes values of around 4–6% (**Bajac *et al*., 2022**), our microencapsulated powder can provide a longer shelf life and fewer degradations of the encapsulated Habanero Red bioactive compounds. Similar values for MC were also recorded in microencapsulated Habanero pepper (*Capsicum chinense* Jacq.) extracts (**Avilés-Betanzos *et al*., 2023**) and volatile compounds (**Pino *et al*., 2020**) using MD alone and MD in combination with GA, respectively, as coating materials. Generally, a decreased MC is typically observed with the increase in drying air temperature due to a greater temperature gradient between the atomized feed and the drying air, leading to a greater energy transferred to the mixture and the evaporation of greater amounts of water (**Tonon *et al*., 2008; Fazaeli *et al*., 2012; Lingua *et al*., 2021**). Also, it was found that a decrease in the MC of microencapsulated powder can be achieved by increased concentration of MD (**Abadio *et al*., 2004; Kha et al., 2010; Tolun *et al*., 2016; Lingua *et al*., 2021**). This finding can be associated with the fact that the increase in MD concentration is accompanied by an increase in solid matter in the feed solution and a decrease in total moisture to be evaporated may result in a decrease in total MC of the end product. Moreover, the addition of GA into MD decreased the MC of the microencapsulated particles (**Tolun *et al*., 2016**). In a study conducted by **Pino *et al*. (2020)**, the MC of microencapsulated Habanero chili pepper volatile compounds revealed a reduction with an increase in carrier (MD:GA) content. This behavior could be again explained by the fact that an additional amount of biopolymer resulted in an increase in feed solids and a reduction in total moisture. Essentially, reducing the moisture level can minimize the agglomeration and caking in spray-dried particles, which are undesirable phenomena that can jeopardize the proper storage of the final product (**Ravichandran *et al*., 2023**).

Water activity measures the availability of free water responsible for deteriorative reactions and provides an important index to estimate the shelf life of particulate products (**Hoskin *et al*., 2023b; Ravichandran *et al*., 2023**). The aw of our microencapsulated powder fell within the range considered as safe against microbial deterioration (lower than 0.6) (**Eroğlu *et al*., 2018; Aguilera-Chávez *et al*., 2022; Valková *et al*., 2022; Moraes *et al*., 2024**).The measured aw value for our spray-dried habanero powder was below the critical aw (aw C) of 0.49, as determined for MD in spray-dried habanero extracts (**Marín Castro *et al*., 2023**). This indicates that the powder is less prone to undesirable transformations such as collapse, stickiness, or caking, compromising product quality.

Anyway, both the MC and a_w of spray-dried particles play a crucial role in determining the flowability, stickiness, and storage stability of powdered food products due to their influence on the *Tg* and crystallization properties of food materials (**Ravichandran *et al*., 2023**).

Phytochemical composition and antioxidant activity

Table 1 summarizes the TPC, AA, capsaicinoids concentrations, and selected polyphenol profiles of concentrated HRE. A portion of the extract was lyophilized to produce a powdered form for further analyses, while the second portion was used for microencapsulation.

Table 1 Total polyphenol content, selected polyphenols, capsaicinoids, and antioxidant capacity in the Habanero Red extract and its microencapsulated spray-dried form

Sample	TPC	RSV	Myricetin	trans-p-coumaric acid	rutin trihydrate	CAP	DHC	AA
	mg GAE/g DW	ug/g DW						mg TEAC/g DW
HRE	6.28 ± 0.35^{a}	4.53 ± 0.17^{a}	58.26 ± 0.56^{a}	2.75 ± 0.12	130.37 ± 2.55	18 759.3 ± 142.8^{a}	5 004.62 ± 67.01^{a}	12.54 ± 1.31^{a}
Spray-dried HRE	1.58 ± 0.12^{b}	3.42 ± 0.05^{b}	2.24 ± 0.18^{b}	ND	ND	2 301.12 ± 18.0^{b}	830.77 ± 9.43^{b}	2.69 ± 0.08^{b}

Legend: HRE - concentrated Habanero Red extract; GAE - gallic acid equivalent; TPC - total phenolic content; CAP - capsaicin; DHC - dihydrocapsaicin; AA - antioxidant activity; TEAC - Trolox equivalent antioxidant capacity. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

When comparing the data with the literature values, our pure HRE displayed a higher content of total phenolics than dried Habanero Red fruits (3.53 ± 0.451 mg GAE/g DW) investigated in the study by **Hudáková *et al*. (2023)**. Similarly, lower TPC values (1.166 - 5.493 mg GAE/g DW; 0.0083 mg GAE/g; 0.3297 ± 0.0008 mg GAE/g DW; 0.3961 - 0.7934 mg GAE/g DW) were observed in immature *Capsicum chinense* (habanero) pepper extracts from Mexico, as reported by **Jattar-Santiago *et al*. (2022)**, **Pérez-Ambrocio *et al*. (2018)**, **Avilés-Betanzos *et al*. (2023)**, and **Oney-Montalvo *et al*. (2020a)**, respectively. Additionally, mature habanero peppers cultivated in black soil had a lower content (1.2278 ± 0.1260 mg GAE/g DW) of total polyphenols **(Oney-Montalvo *et al*., 2020b)** than that of our sample. Also, the contents of CAP and DHC in our pure HRE were found to be higher than those reported for Mexican habanero pepper extract derived from immature fruits, which contained 7 770 ± 20 µg/g of CAP and 1 180 ± 20 µg/g of DHC (**Avilés-Betanzos *et al*., 2023**). On the other hand, the closest values for both capsaicinoids (16 179 µg/g and 6 280 µg/g of CAP and DHC, respectively) as identified in our study were detected by **Jattar-Santiago *et al*. (2022)**. Regarding selected polyphenols, the content of myricetin in our extract was similar to that (56.4 - 59.8 µg/g DW) found in red sweet peppers (*Capsicum annuum* L.) identified in the study **Guilherme *et al*. (2020)**; however, their samples exhibited higher values for resveratrol (59.7 - 92.8 µg/g DW) as compared to our study. In red sweet pepper (*Capsicum annuum*), higher values for p-coumaric acid (9.96 ± 0.08 µg/g DW) were observed (**Thuphairo *et al*., 2019**). These comparisons highlight that various factors can significantly influence the content of bioactive compounds in habanero peppers. In effect, factors such as species, variety, the stage of maturity, climatic and storage conditions, and processing practices all play crucial roles in the amount and composition of the *Capsicum* nutrients and phytochemicals (**Duranova *et al*., 2022**).

Nowadays, spray drying microencapsulation of bioactive compounds has gained increased attention in the food and pharmaceutical industries. As a pilot study, we evaluated the stability and preservation of phytochemical compounds present in HRE after microencapsulation using a blend of MD and GA (3:2 ratio) as coating materials and a 120 °C inlet temperature to enhance their stability, protect against degradation caused by heat and oxidation, and ensure high AA retention for potential use in functional foods or nutraceutical applications. Importantly, the final spray-dried Habanero Red powder was collected from the collection vessel only, and particles deposited on the dryer chamber and connection pieces were discarded, because the amounts of the manually swept and collected particles from these parts are variable to each run and might cause unnecessary calculation errors (**Fang and Bhandari, 2012**). The spray-dried habanero particles exhibited higher TPC and CAP levels (Table 1) compared to values reported by **Avilés-Betanzos *et al*. (2023)** (0.0664 ± 0.0008 mg/g DW, 120 ug/g DW, respectively). Their study utilized MD alone (M:E = 0.8:1 w/w) for spray drying Habanero pepper (*Capsicum chinense* Jacq.) extract at an inlet temperature of 148 °C. However, their findings indicated successful preservation of coumaric acid in the final product. As demonstrated in Table 1, the TPC, capsaicinoids levels, selected polyphenols, and AA in our microencapsulated form of HRE were markedly reduced ($P < 0.0001$; except for resveratrol, where $P < 0.01$) compared to the pure HRE. These findings align with similar trends documented in the study by **Avilés-Betanzos *et al*. (2023)**, where notable decreases in these bioactive compounds were also observed after the microencapsulation process. This reduction was expected, as the coating material did not contain bioactive compounds (**Tolun *et al*., 2016**) but reduced the active ingredient, contributing to the overall weight of the microencapsulated product, which resulted in lowering the relative content of bioactive compounds per g of the final product. Moreover, some bioactive compounds might be lost during the spray drying process (**Tavares *et al*., 2019**) due to exposure to factors like heat, oxidation, or chemical degradation (**Kuck and Noreña, 2016**). This likely explains the complete absence of trans-p-coumaric acid and rutin trihydrate in our spray-dried HRE sample, suggesting their more susceptibility to degradation induced by the drying process. Thus, to evaluate the effectiveness of the microencapsulation process, it is essential to consider the measurement of phenolic retention and AA preservation, which are assessed solely from the feed solution and the microencapsulated powder.

CONCLUSION

Based on the findings of our study, we can conclude that the microencapsulation of HRE using MD and GA as coating materials via spray drying can effectively preserve polyphenols and AA, with satisfactory physical characteristics of the resulting powder. Specifically, the ratio of MD to GA (3:2) showed optimal retention of polyphenols (>70%) and impressive antioxidant preservation (>90%), making it a promising method for functional food or supplement applications. While the spray drying yield was around 45%, further optimization of the process, particularly by increasing the wall/core ratio, may help improve yield without compromising the retention of bioactive compounds. However, increasing the proportion of coating material may pose risks to AA, as excessive coating material could dilute bioactive components. Therefore, additional studies are required to identify the ideal balance between the wall/core ratio and coating material composition to achieve both higher yields and enhanced bioactive compound retention, ensuring the stability and effectiveness of the final microencapsulated product. Our pilot study provides a valuable framework for further research in the field of microencapsulation, offering insight into the optimization of spray drying parameters and coating material selection for bioactive compound preservation.

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