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CHOCOLATE ENRICHED WITH MICROCAPSULES OF ORANGE ESSENTIAL OIL

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ABSTRACT

The development of functional foods enriched with bioactive compounds has gained increasing importance due to their potential health benefits and growing consumer demand for value- added products. Dark chocolate is considered an ideal food matrix for incorporating functional ingredients because of its natural antioxidant content and high consumer acceptance. However, essential oils are highly volatile and sensitive to environmental conditions, including heat, light, and oxygen, which can degrade their bioactive components during food processing and storage. Microencapsulation techniques, especially spray drying, have been widely used to protect these compounds and improve their stability. This study investigated the incorporation of spray-dried microcapsules containing orange essential oil into 70% cocoa chocolate and evaluated their effects on the functional and physicochemical properties of the resulting product. Microcapsules were prepared using maltodextrin as the wall material and Tween 80 as an emulsifying agent to enhance the retention of bioactive compounds and improve antioxidant potential. The resulting microcapsules were incorporated into chocolate formulations at different concentrations (w/w): 6%, 8% and 10 %. The developed samples were analyzed using an experimental design that included determination of total polyphenol content, antioxidant capacity (DPPH radical scavenging), sensory evaluation, color measurement, and water activity assessment. Results showed that chocolate fortified with orange essential oil microcapsules exhibited improved functional properties such as antioxidant activity and total polyphenol content compared with the control sample. The enriched chocolate presented a higher total polyphenol content (1.83 ± 0.04 mg GA/g) compared with the control formulation (1.56 ± 0.05 mg GA/g). Similarly, antioxidant capacity was significantly higher in the fortified chocolate (69.33 ± 0.13%) than in the control sample (64.08 ± 0.83%), showing statistically significant differences ($p < 0.05$). The incorporation of microcapsules did not negatively affect important physicochemical characteristics, as color parameters and water activity remained within acceptable stability ranges. Sensory evaluation indicated that the formulation containing 10% microcapsules achieved the highest level of panelist acceptance obtaining 5.47 ± 0.20 out of a 9 point hedonic scale. These results show that spray-drying microencapsulation is an effective method for incorporating orange essential oil into chocolate, enabling the development of functional chocolate with enhanced antioxidant properties, while maintaining acceptable physicochemical and sensory characteristics.

Key words: Antioxidant capacity, Chocolate, Microencapsulation, Spray-dried, bioactive compounds, phenols, sensory analysis

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INTRODUCTION

Chocolate is widely consumed worldwide, with annual intake exceeding 8 million metric tons [1]. This sweet treat's popularity is attributed to its hedonic properties and health benefits because of its content of polyphenols, flavonoids, and minerals, among others [2]. Despite its benefits, the consumption of this food and its derivatives is a concern due to their high fat and sugar content, which has been linked to various chronic diseases [3]. In response to these concerns and the growing demand for healthier and functional foods, incorporating essential oils (EOs) is being investigated as an alternative. Among these, orange essential oil (OEO), recognized for its antioxidant properties and is presented as a promising option for the improvement of food products such as chocolate [4]. However, incorporating EOs in their natural state is not a promising method because of the oils' extreme volatility and susceptibility to degradation in ambient conditions [5]. Among the existing alternatives for the preservation of EOs is encapsulation, which allows loading substances into a heterogeneous or homogeneous matrix, thereby protecting active agents from their low stability, susceptibility to oxidation, and evaporation [6]. Nevertheless, the objective is to preserve the beneficial properties of these oils when incorporated into different foods. In addition, if the product obtained has a radius ranging from 1 µm to 1000 µm, it is termed microencapsulation. This variant protects EOs from high temperatures, light, and oxygen, and enhances the oil's long-term preservation [7].

Therefore, microencapsulation of EOs is a promising strategy, as it allows for the protection and controlled release of bioactive compounds [8]. Among different techniques for microencapsulation, spray drying is known for its low cost and applicability for oils [9]. This technology is an efficient, scalable microencapsulation method that transforms liquids into dry microparticles by atomizing them in a chamber heated by hot air. It is also important to mention that the efficiency and functionality of spray-microencapsulated EOs depend on the initial emulsion and the wall material chosen [10]. One of the key aspects of microencapsulation is the encapsulation agent, as its properties–such as chemical stability, solubility-affect the final product. Among them, maltodextrin is widely used as an economical encapsulant [11], with good emulsifying properties and able to stabilize bioactive compounds [12]. It also stands out for its solubility, low cost, low viscosity, chemical stability, and the capacity to reduce the stickiness and hygroscopicity of mixtures [13].

Orange essential oil contains polyphenols, recognized for their antioxidant properties; among these are limonene and β-myrcene, which contribute to their functional properties. In addition, it has demonstrated antidiabetic and antihypertensive effects, as well as antimicrobial activity [14]. Therefore, OEO is a

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viable option to incorporate into chocolate owing to its chemical profile. According to Quispe-Sanchez [15], chocolate with microencapsulated EOs enhances its functional properties and confers additional benefits, including antimicrobial activity. Moreover, this technique enables the controlled release of bioactive compounds beneficial to health. Additionally, it preserves its texture, flavor, and consumer acceptance [16]. Therefore, this research aims to evaluate the effect of incorporating orange essential oil microcapsules into chocolate through measurement of physicochemical parameters, determination of antioxidant activity, quantification of total polyphenol content and assessment of sensory attributes (aroma, taste, texture and overall acceptance).

MATERIALS AND METHODS

Materials

Orange essential oil (food grade) was bought from NUA Perú; Tween® 80 (polysorbate), 2, 2 – diphenyl - 1- picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, and methanol were bought from Merck; chocolate (70% cocoa) from the National Museum of Chocolate; and food-grade maltodextrin (Qinhuangdao Lihua Starch Co. Ltd) purchased from Mitu Alimentaria.

Experimental design

The present work was carried out by a multilevel factorial statistical design, as shown in Table 1, which presents the specific concentrations of Tween 80 and OEO used. This design allows for assessing the concentration effects on microcapsules' properties, optimizing the formulation for better encapsulation and bioactive properties.

Preparation of chocolate with the microcapsules loaded with Orange Essential Oil

Previously, an emulsion was prepared following the method described by Herman-Lara [17] with slight modifications. 6.5 mL of Tween 80 was added to 90 mL of distilled water. Subsequently, 3.5 mL of OEO was added dropwise. Finally, 8 g of maltodextrin (used as wall material) was added, and the mixture was stirred until a homogeneous dispersion was obtained. This procedure was followed in all three tests of the six formulations.

On the other hand, microcapsules were prepared according to the method of Hadnađev [18], with some modifications: wall and core materials were prepared immediately prior to emulsification, core to wall ratios (v:w) were: 3.5:8, 5.0:8, and 6.5:8 respectively. Emulsification was carried out using a magnetic stirrer at 450-650 rpm for 90 min. First, 100 mL of the total dispersion was introduced into the spray-drying equipment (ATM/3000, PIGNAT), with the heating temperature set to 150°C, the pump power to 30%, and the fan speed to 50%. Each run lasted until the volume

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was consumed, typically requiring 12–15 minutes. Thus, having the encapsulated orange essential oil with an approximate weight of 4.56g. The 70% cocoa chocolate was melted at 45°C in a double boiler with constant stirring. It was left to stand for approximately 3-4 min until the chocolate melted, then stirred for 4 min for rapid melting. The orange essential oil microcapsules were added, stirred until homogenized (1-2 min), and immediately poured into 3 cm x3 cm silicone molds, which were temporarily stored at 4°C until analysis or consumption, usually within 48 h [19].

Quantification of Total Polyphenolic Content

The determination of total polyphenolic content (TPC) was carried out using the Folin- Ciocalteu method described by Nikolaeva [20] with slight modifications (Folin-Ciocalteu reagent dilution, adjusted reaction volumes, extraction and incubation times). Initially, a 0.188 g mL^{-1} sodium carbonate solution was prepared, and a 500 mg L^{-1} gallic acid solution was used as the stock solution. For the total polyphenolic content determination in microcapsules, 500 mg of the six samples were weighed, and 10 mL of ethanol was added to each sample, followed by magnetic stirring for 60 minutes. Then, for the quantification of total polyphenolic content in chocolate, samples were defatted with n-hexane by magnetic stirring, followed by centrifugation, then covered in aluminum foil and placed in a desiccator for 24 hours. The phenolic compounds were then extracted from the samples by shaking with 10 mL of 80% methanol for 30 minutes. Finally, 1 mL was extracted using a micropipette for the sample preparation. Subsequently, total polyphenolic content was calculated using a calibration curve in the range from 2 to 12 mg L^{-1} (R^2 = 0.993) with gallic acid as standard. The curve was prepared by taking 1 mL of each standard, adding 1 mL of Folin-Ciocalteu reagent, and allowing it to stand for 5 minutes; then, 4 mL of 20% calcium carbonate and 2 mL of water were added. All samples were left to stand for one hour and analyzed using UV-Visible spectroscopy at 760 nm. Similarly, the TPC of the sample formulations was determined using 0.5 mL of sample, and performed in triplicate.

Antioxidant capacity

Antioxidant capacity for microcapsules was determined following the 2,2-Diphenyl-1- picrylhydrazyl (DPPH) assay as described by Baliyan [21]. A 0.2 mg mL^{-1} DPPH solution in methanol was prepared by magnetic stirring for 20 minutes. Subsequently, 50 mg of each formulation was weighed, and 5 mL of methanol was added; then the mixture was stirred for 30 minutes. Afterwards, the samples were centrifuged for 15 minutes; then, 0.5 mL of the resulting supernatant was transferred to a new tube, 0.5 mL of DPPH solution and 4 mL of methanol were added, the mixture was stirred for 1 minute, and allowed to stand in the dark for 1 hour. Finally,

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the samples were analyzed by UV–Visible spectrophotometry at 516 nm, using 1 mL of methanol mixed with 9 mL of DPPH solution as a blank.

On the other hand, to determine the antioxidant capacity of a chocolate, each sample of 2 grams of finely chopped chocolate was placed in a beaker with 10 mL of n-hexane and shaken at 150 rpm for 40 minutes, then centrifuged for 10 minutes at 4000 rpm, the supernatant was discarded, and the residual solid was subjected again to the same defatting step (shaking with n-hexane, centrifugation, and supernatant removal). Finally, the solid was covered with aluminum foil and kept in a desiccator for 24 hours [22]. Consequently, 1g of the solid sample was stirred in 5 mL of 80% (v/v) methanol for 30 minutes. Next, a test tube was prepared for measurement, containing 0.5 mL of sample, 0.5 mL of DPPH, and 4 mL of 80% (v/v) methanol. Finally, the samples were left to stand in the dark for 45 minutes and then measured in the UV- Visible spectrophotometer [22]. A mixture of 0.5 mL of DPPH and 4.5 mL of 80% methanol was used as the blank, and the antioxidant capacity was determined using the following equation:

$$\text{Antioxidant capacity } (\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \text{ X } 100\% \quad (1)$$

Where: AC is the absorbance of the control solution, and A_S is the absorbance of the sample solution.

Statistical analysis

The formulated microcapsules were used to determine the optimal conditions of the process parameters on the measured responses using response surface methodology (RSM).

The independent factors were the volume of Tween 80 (X1) and the volume of OEO (X2). An analysis of variance (ANOVA) was performed to examine the model fit, considering the significance of the regression coefficients, probability values (P), and the lack of fit for the variables: total polyphenolic content and antioxidant capacity. All statistical processing and multiple response optimization were executed using Statgraphics Centurion® 19 software at a 95% confidence level ($P < 0.05$). The experimental data were fitted to a second- degree polynomial model, equation (2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \quad (2)$$

Where: Y represents the response variable, β_0 is the intercept, and coefficients β_1 to β_5 correspond to the linear effects, interaction, and quadratic terms.

The raw data gathered from the untrained consumer panel were filtered and grouped by formulation code (178, 285, and 303) using data segregation criteria to isolate individual scores. To evaluate the precision of the response variability an overlap

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analysis of the standard error intervals was conducted at a 95% confidence level (α = 0.05)

The descriptive statistics for overall acceptability were expressed as the arithmetic mean accompanied by the Standard Error of the Mean (SEM).

Sensory analysis

A hedonic sensory test was conducted to evaluate the acceptability of chocolates formulated with different percentages of OEO microcapsules. The evaluation was carried out with 100 untrained consumers who participated voluntarily [23]. Each participant was given three chocolate samples, corresponding to different formulations, coded with random three-digit numbers to avoid bias. The samples evaluated were: code 178, containing 6% OEO microcapsules; code 285, with 8% OEO microcapsules; and code 303, with 10% OEO microcapsules. The samples were presented in random order and under controlled conditions.

On the other hand, sensory acceptability was evaluated using a nine-point hedonic scale, where 1 corresponded to "I dont like it at all" and 9 to "I like it very much." The attributes analyzed included the color (hue, uniformity, and intensity), aroma, texture (balance between firmness and smoothness), flavor (balance between sweet and bitter), and overall product (chocolate) acceptability. The data obtained were subjected to statistical analysis to determine significant differences between the formulations and to establish the sample with the highest level of sensory acceptance [24].

Color analysis

Color measurement was performed using a colorimeter (model CR-20, Konica Minolta, Japan). The sample holder was half-filled with the chocolate sample, then the instrument was inverted so that the lens faced the upper plate [25]. Chromaticity values were obtained in the CIELAB color space, where L*, a*, and b* correspond to lightness/brightness, green to red, and blue to yellow, respectively. A comparison of the chocolate samples with and without microcapsules was also performed using the CIELAB color difference. For the analysis, 0.5 g of the sample was placed on the plate and compacted to ensure complete coverage of the base, where the color difference (ΔE^*) was determined using Equation (3).

$$\Delta \mathrm{E} * = \frac{\sqrt{(\Delta \mathrm{L}*)^2+(\Delta a*)^2+(\Delta \mathrm{b}*)^2}}{1 \quad 0 \quad 1} = \frac{\sqrt{(\mathrm{L}*-\mathrm{L})^2+(a*-a)^2+(\mathrm{b}*-\mathrm{b})^2}}{0 \quad 1 \quad 0} \tag{3}$$

Where L^*, a^* and b^* were the color parameters of the chocolate without microcapsule, while L^*, a^* and b^* were the measurements of the chocolate sample with microcapsule. The whiteness index (WI) of each sample was calculated using equation (4) [18].

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Volume 26 No. 6
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$$WI = 100 - \sqrt{(100 - L*)^2 + a^{*2} + b^* - b^2} \quad (4)$$

Determination of water activity

The water activity (aw) of the chocolate was determined using a water activity measuring device (CH8853, Novasina, Switzerland). This test was carried out by following steps: first, a piece of chocolate was grated to increase the exposure surface, then the equipment was allowed to reach equilibrium temperature, the grated sample was placed on a clean plate until half capacity, the chamber was closed, and aw measurement was initiated until the final reading was obtained [26].

Microbiological analysis

Microbiological analyses were performed at the Laboratory of Microbial Ecology and Biotechnology of the Facultad de Ciencias de la Universidad Nacional Agraria La Molina. Chocolate samples were evaluated for total coliform enumeration (MPN/g), lactic acid bacteria count (CFU/g), and yeast and mold count (CFU/g) using standardized microbiological procedures commonly applied for the assessment of microbial quality and safety in chocolate products [27]. Results were expressed as Most Probable Number per gram (MPN/g) for total coliforms and colony-forming units per gram (CFU/g) for lactic acid bacteria, yeasts, and molds.

Ethical Considerations

The sensory evaluation conducted in this study involved voluntary participation of consumers, who were previously informed about the purpose of the research and the characteristics of the evaluated chocolate samples. All participants provided informed consent prior to participation, and the collected information was treated confidentially and used exclusively for academic and research purposes. Furthermore, the study did not involve any procedures that could pose risks to the health or well-being of the participants. The development and evaluation of the chocolate samples complied with food safety and hygiene standards established for products intended for human consumption.

RESULTS AND DISCUSSION

Emulsion Stability and Microencapsulation of Orange Essential Oil

The emulsion used for the preparation of the OEO microcapsules exhibited a slightly yellowish-white color, possibly due to light scattering caused by the size of the oil droplets dispersed in the continuous phase. The emulsion remained stable for 20 minutes, which limits system stability, probably due to insufficient dispersion of the oil droplets, as evidenced by noticeable phase separation (aqueous and oily), as explained by Ma [28]. This phase separation was evidenced by the formation of distinct oily and aqueous layers, probably due to the immiscibility between both phases, differences in polarity, and insufficient dispersion of the oil droplets within the emulsified system. In addition, the amount of Tween 80 used in the emulsion

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was insufficient to completely disperse the EO; so, the droplets' attraction overcame the emulsifier, leading to coalescence and phase separation [29]. The preparation of the emulsion and subsequent microencapsulation of the EO allowed the protection of volatile compounds and those susceptible to degradation, such as the polyphenols and antioxidants present in the EO [30]. In this study, the stability of the microcapsules was associated with the use of maltodextrin as the encapsulating material, whose structure favored the protection of the essential oil against factors such as moisture, light, and oxidation [29]. Furthermore, during the incorporation of the microcapsules into the chocolate formulation, the processing temperature was a determining factor in maintaining the stability of the final product and achieving desirable characteristics such as texture and gloss. Therefore, a temperature of 45 °C was used, as it allowed the preservation of properties suitable for consumption and was consistent with the findings reported by González [31], who, under similar conditions, obtained favorable rheological behavior in chocolate.

Polyphenol Content and Antioxidant Capacity of Microencapsulated Orange Essential Oil and Functional Chocolate

Figure 1A shows the total polyphenolic content, where formulation F4 exhibited the highest value (0.163 mg GA/100 g sample) and F6 the lowest (0.093 mg GA/ 100 g sample), which may be due to the ratio of components. Moreover, when comparing formulation F3 with the maximum value obtained for F4, a similar behavior was observed, with a content of approximately 0.157 mg GA/100 g, indicating that both concentrations achieved optimal stabilization of the emulsion used for the preparation of the microcapsules. This behavior can be attributed to the proportion of the components, which allowed an adequate interaction with Tween 80, a crucial factor for the formation of an elastic interfacial film that enhanced emulsion stability [32]. In contrast, the drastic decrease observed in F6 suggests that the EO level exceeded the emulsifier's dispersion capacity. According to Sukardi [33], maltodextrin not only dilutes polyphenols but also can create a physical barrier that limits the extractability of active compounds during analysis. This interference suggests that while the system protects the oil, there is also a trade-off between microcapsule stability and the final availability of antioxidants for quantification.

On the other hand, the Folin–Ciocalteu assay to estimate total phenolic compounds in matrices such as chocolate should be interpreted with caution, since this reagent is not specific only to phenols but can also react with other reducing species such as sugars, amino acids, proteins, or metal complexes [34]. For instance, in matrices with a high sugar content, such as chocolate formulations, it has been found that total polyphenol content values can be overestimated by up to 40% when proper matrix cleanup is not performed. Table 2 shows that the total polyphenol content values for chocolate with the optimal formulation (1.56 ± 0.05 mg GA/g for the sample

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SCHOLARLY, PEER REVIEWED
Volume 26 No. 6
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without microcapsules and 1.83 ± 0.04 mg GA/g with microcapsules) were lower than those reported for chocolates with higher cocoa content or phenolic extracts (~2.5–3.0 mg GAE/g in milk chocolate and ~8–13 mg GAE/g in dark chocolate). These differences may be attributed to both the formulation composition and the method sensitivity, which was observed after the incorporation of microcapsules, as reported in the literature, although the magnitude of the change could also be partially influenced by the nonspecific nature of the assay.

As shown in Figure 1 B, the antioxidant capacity of the microencapsulated OEO varied significantly among the six formulations. Formulation F4 showed the highest antioxidant activity (17.8%), followed closely by F5. This suggests that in these two samples, the system reached its maximum protection level, and that increasing polyphenol levels in F5 did not enhance the antioxidant effect. This behavior can be explained by the fact that antioxidant activity depends not only on the total concentration of polyphenols but also on their chemical structure, stability, and availability within the microencapsulated matrix. In formulation F5, a fraction of the phenolic compounds may have been strongly retained or trapped in the wall material, limiting their interaction with free radicals during the antioxidant assay. Furthermore, interactions between phenolic compounds and encapsulating agents can decrease their radical scavenging efficiency, so a higher phenolic content does not necessarily imply a greater measurable antioxidant capacity. In contrast, formulation F2 showed a relatively high phenolic content (0.142 mg AG/ 100 g sample) but a lower antioxidant capacity (5.45%), suggesting that the presence of phenolic compounds does not necessarily translate into greater antioxidant activity. This discrepancy may be associated with less efficient microencapsulation or reduced accessibility of phenolic compounds, a phenomenon previously reported in studies on spray-dried EO microcapsules. Moreover, these differences can be mainly attributed to the Tween 80/OEO ratio, as appropriate surfactant levels promote emulsion stability, enhance protection of phenolic compounds against oxidative degradation, and improve their functional performance. Overall, the results confirm that Tween 80 concentration is a key factor influencing both polyphenol retention and the antioxidant capacity of microencapsulated OEO, as reported for similar encapsulation systems.

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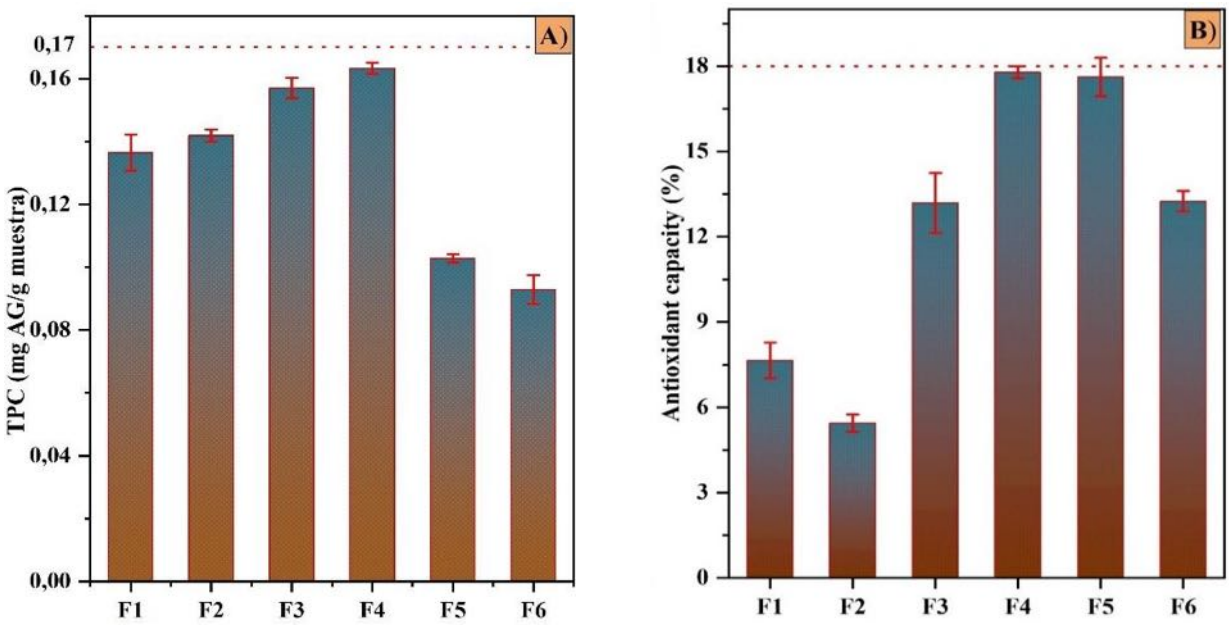

Figure 1: A) TPC and B) Antioxidant capacity of microencapsulated orange essential oil

In addition, factors such as radical solubility, sample turbidity, or lack of standardization in experimental conditions can generate variability among studies. In this study, the antioxidant capacity of the chocolate increased significantly from 64.08 ± 0.83% (without microcapsules) to 69.33 ± 0.13% after their addition (Table 3). Although this increase was statistically significant ($p < 0.05$), its magnitude (~8.2%) was lower than in other studies reporting 20–30% increases when enriching chocolates with encapsulated phenolic extracts. This suggests that the characteristics of the encapsulation system or the dispersion of the compounds within the chocolate matrix may have limited their reactive availability toward the DPPH radical.

Response Surface Optimization of Microencapsulated Orange Essential Oil Formulations

Table 4 shows the regression coefficients for the fitted quadratic polynomial model. The response surfaces show the dependence between X1 (Tween 80) and X2 (OEO). The fitted quadratic model and response surface analysis indicate that the optimal conditions were obtained through a multiple-response optimization using the desirability function, which simultaneously maximizes total polyphenolic content and antioxidant capacity. The global desirability value of 0.87391 reflects a satisfactory compromise between both responses. Under these conditions, Tween 80 (X1) was set at 6.5 mL, while the orange essential oil (OEO, X2) was an optimal intermediate value of approximately 5.14 mL. These values do not correspond to the individual maxima of either response but represent the combination that optimizes the functional properties. The results confirm that Tween 80 contributes to the stability

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of the microencapsulation system, and the OEO concentration governs the antioxidant and polyphenolic responses, enabling the definition of suitable conditions for microcapsule development. In addition, analysis of the regression coefficients and corresponding p-values (Table 4) indicates that the amount of orange essential oil (OEO, X2) significantly influences ($p < 0.05$) both antioxidant capacity and total polyphenolic content. The quadratic term of OEO (X2) influence also significantly ($p < 0.05$), suggesting a non-linear effect of orange essential oil concentration on this response, and a linear effect of X1. On the contrary, the interaction term (X1X2) was not significant ($p > 0.05$) for any response variable. For total polyphenol content, OEO showed a strong statistical significance ($p < 0.05$) for the linear and quadratic terms, confirming its dominant role in determining polyphenolic content. Moreover, the model's accuracy was supported by the coefficient of determination ($R^2 = 0.967$) for both total polyphenol content and antioxidant capacity ($R^2 = 0.830$), indicating that the models explain a high proportion of the experimental variability, particularly for total polyphenol content.

The response surfaces reveal the dynamics of the microencapsulation system, where increasing factor X2 directly raises the phenolic content and antioxidant capacity, a result that is corroborated by the standardized Pareto analysis [35]. Figure 2B shows an optimal value of 0.163324 mg GA/g sample of total polyphenols, a result consistent with that reported in recent studies on orange essential oil microcapsules, which demonstrate that encapsulation favors the preservation and concentration of phenolic compounds in the final matrix [36]. This behavior is consistent with models where an increase in the primary active component enhances the concentration of bioactive compounds in the mixture. On the other hand, the influence of factor X1 is fundamental for stability and yield (Figures 2A and C), as adequate levels of this parameter are essential to protect the lipid core of the system. Particularly, Figure 2D shows an optimal antioxidant capacity of approximately 17.2%, a value aligned with research by Mohammed [37] about microencapsulation of *Citrus aurantium* L. essential oil. Finally, the overall desirability surface (Figure 2E) exhibits a parabolic shape, demonstrating that the system's success does not depend on the individual maximization of variables but on an optimal balance; the maximum point is reached at medium to high levels of both parameters, achieving a synergistic combination that ensures the highest functionality and stability, in agreement with multiple response statistical optimization models.

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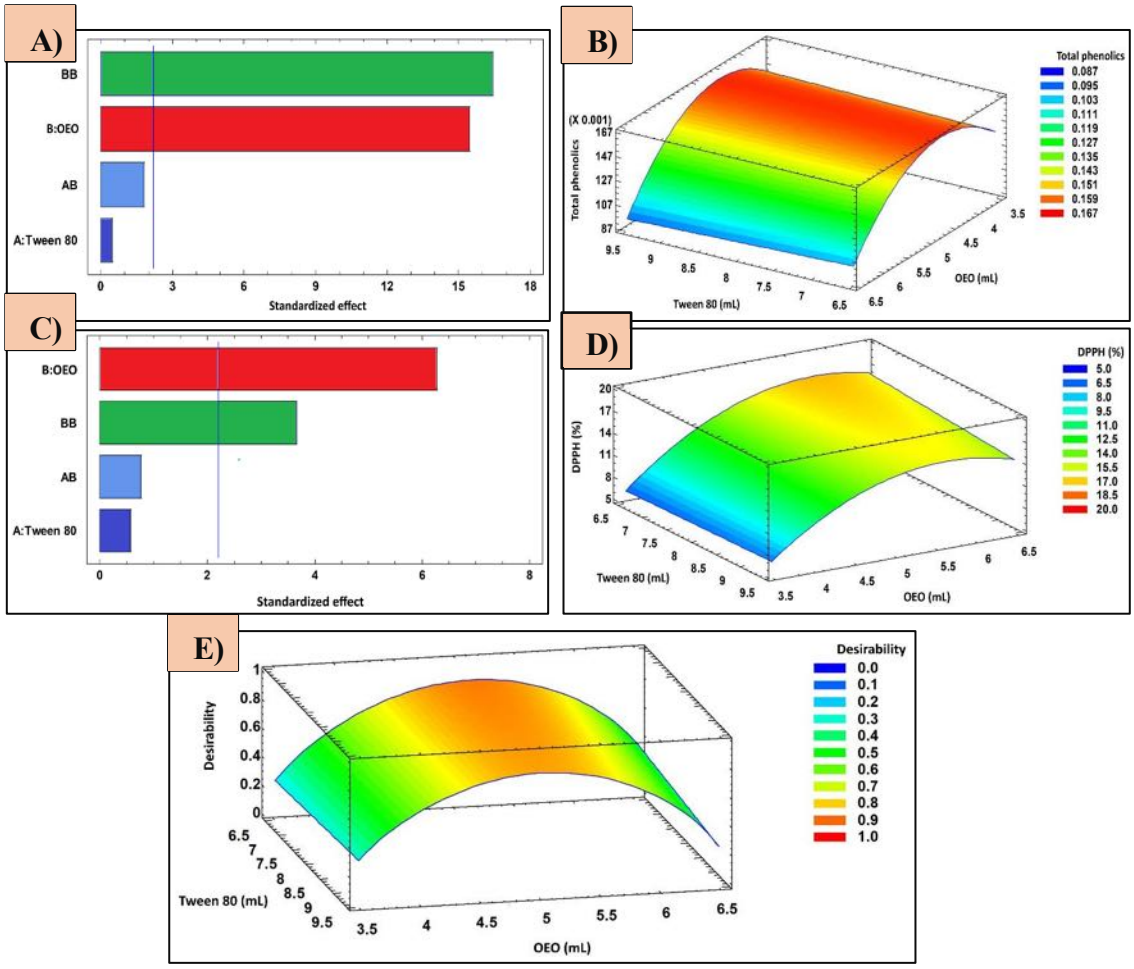

Figure 2: Pareto and response surface graphs of total phenolic content (A-B), antioxidant activity by DPPH (C-D), and desirability response surface plot (E)

Sensory Evaluation of Chocolate Formulated with Orange Essential Oil Microcapsules

The results of the sensory analysis, conducted with consumer participation using a nine- point hedonic scale, showed that sample F303, corresponding to microcapsules containing 10% w/w EO, achieved the highest overall acceptability, with an average score of 5.47, compared to formulations F285 (5.45) and F178 (5.29). Likewise, F303 obtained higher scores for chocolate flavor (5.37), while color and texture evaluations showed similar values among the formulations, with average scores of 6.75 and 6.19, respectively. These results indicate that the incorporation of EO at this concentration allowed the maintenance of organoleptic characteristics acceptable to consumers without generating significant negative sensory perceptions. This sensory performance is directly related to the functional characteristics previously observed, since the addition of OEO increased the total phenolic content to 1.83 ± 0.04 mg GA/g and the antioxidant capacity to 69.33 ±

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0.13%, while the microencapsulation process effectively protected these bioactive compounds. Furthermore, encapsulation contributed to stabilizing polyphenols and masking the intense flavors of the EO, allowing the chocolate to preserve its characteristic profile. However, the concentration of EO significantly influenced the evaluated sensory properties, especially flavor and texture, due to changes in the release of aromatic compounds and the interaction of the encapsulated oil with the product matrix [38]. These results highlight the importance of optimizing the essential oil concentration to maximize the sensory acceptance of the final product.

Table 5 summarizes the overall acceptability of the chocolate samples, with scores ranging from 5.29 to 5.47, indicating generally favorable sensory acceptance. Sample 303 obtained the highest mean score (5.47 ± 0.20), followed by 285 (5.45 ± 0.20), while 178 showed the lowest value (5.29 ± 0.18). However, all samples belonged to the same statistical group ($p > 0.05$), indicating no significant differences among formulations. These results suggest that the incorporation of the evaluated formulations did not adversely affect sensory attributes such as flavor, texture, and overall appearance, maintaining acceptable consumer perception across all samples.

Color Characteristics of Chocolate Samples

Table 6 depicts the color values obtained, indicating slight differences between chocolate with and without microcapsules. The whiteness index (WI) slightly decreased from 26.22 in the chocolate without microcapsules to 25.63 in the chocolate with microcapsules, suggesting a slightly darker hue after microcapsule incorporation. Furthermore, the total color difference ($\Delta E = 1.22$) indicates that the chromatic variations are not visually perceptible to the human eye, $\Delta E < 3$ [18]. These results are consistent with those reported by Hadnađev [18], who observed that the addition of bioactive compounds or encapsulated matrices may slightly modify color parameters without affecting the appearance of the product. Moreover, the colorimetric values obtained showed slight variations between the chocolate without microcapsules (SM) and the chocolate with microcapsules (CM). The L* parameter increased from 26 to 26.7, while the a* and b* values increased from 6.2 to 7 and from 4.1 to 4.7, respectively. Likewise, the whiteness index (WI) showed a slight variation from 25.63 to 26.22. The obtained ΔE^*ab value was 1.22, indicating a minimal color difference between both samples, which suggests that the incorporation of microcapsules did not significantly affect the visual appearance of the chocolate and allowed it to maintain its characteristic color and homogeneity.

Water Activity and Microbiological Stability of Chocolate

The water activity (aw) value obtained for the chocolate sample was 0.45, indicating that it remained below 0.61, thereby preventing the proliferation of pathogenic or spoilage microorganisms and fulfilling a fundamental food safety criterion. This result

is consistent with the findings of Verde [39], who reported that most commercial chocolates exhibit aw values between 0.40 and 0.60. According to Sun [26], maintaining water activity within this range not only contributes to product safety but also enhances stability against physical defects such as fat bloom. Similarly, Tolve [40] highlighted that a aw below 0.61 is directly associated with longer shelf life in confectionery products, as it limits the growth of molds and yeasts. On the other hand, the microbiological tests (total coliforms, lactic acid bacteria, molds, and yeasts) carried out at the Laboratory of Microbial Ecology and Biotechnology of the Facultad de Ciencias de la Universidad Nacional Agraria La Molina confirmed this finding, with a total coliform count (MPN/g) < 3, and lactic acid bacteria (CFU/g) as well as molds and yeasts (CFU/g) counts equal to 0, demonstrating the effectiveness of water activity control as the main barrier to ensure the safety and stability of the evaluated chocolate.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The analysis of microcapsule properties, evaluated through organoleptic attributes, DPPH, and Folin-Ciocalteu assays, demonstrated that the emulsifier Tween 80 is a critical factor in determining the overall quality and antioxidant stability of the encapsulated product. Furthermore, incorporating these optimized microcapsules into the chocolate formulation, significantly increased ($p < 0.05$) the total phenolic content (1.83 ± 0.04 mg GA/g) and antioxidant capacity (69.33 ± 0.13%) compared to the control (1.56 ± 0.05 mg GA/g and 64.08± 0.83%, respectively), indicating that the microencapsulation process not only preserved but effectively enhanced the bioactive profile of the system, providing superior stability and protection against oxidative conditions during processing.

The color analysis showed that the incorporation of microcapsules did not significantly affect the appearance of the chocolate, maintaining ΔE*ab values within the range not perceptible to the human eye (ΔE < 3). The slight decrease in the whiteness index (WI) indicates minimal darkening that does not compromise the product's uniformity or visual acceptability, confirming that the addition of microcapsules preserves the characteristic tone and homogeneity of chocolate. On the other hand, the evaluated chocolate exhibited a water activity (aw) below 0.61, ensuring its microbiological and physicochemical stability, which guarantees the inhibition of mold and yeast growth, prevents defects such as fat bloom, and extends the product's shelf life, confirming that controlling aw is a critical parameter for maintaining safety and quality in functional confectionery products.

The results of this study suggest that spray-dried microencapsulation of OEO represents a promising strategy for the development of functional chocolate products with enhanced antioxidant properties. Nonetheless, future research should explore the optimization of microencapsulation parameters, including different wall materials

and encapsulation ratios, to further improve the stability and controlled release of bioactive compounds. In addition, evaluating the shelf life, storage stability, and bioavailability of the encapsulated compounds in chocolate matrices would provide valuable information for industrial applications. Further investigations could also assess consumer acceptance in larger and more diverse populations, as well as explore the incorporation of other natural bioactive ingredients to develop innovative functional chocolate products with potential nutritional and health benefits.

Conflict of interest

The authors declare no conflicts of interest

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Table 1: Multilevel factorial design with Tween 80 and orange essential oil (OEO)

Code	Block	Tween 80 (mL)	OEO (mL)
F11	1	6.5	3.5
F21	1	9.5	3.5
F31	1	6.5	5
F41	1	9.5	5
F51	1	6.5	6.5
F61	1	9.5	6.5
F12	2	6.5	3.5
F22	2	9.5	3.5
F32	2	6.5	5
F42	2	9.5	5
F52	2	6.5	6.5
F62	2	9.5	6.5
F13	3	6.5	3.5
F23	3	9.5	3.5
F33	3	6.5	5
F44	3	9.5	5
F53	3	6.5	6.5
F63	3	9.5	6.5

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Table 2: Total polyphenol content (TPC) for chocolate with and without microcapsules from the optimal formulation

Treatment	TPC (mg GA/g sample)
Without microcapsules	1.56 ± 0.05[b]
With microcapsules	1.83 ± 0.04[a]

Different superscript letters (a, b) indicate significant differences ($p < 0.05$)

Table 3: DPPH for chocolate with and without microcapsules from the optimal formulation

Treatment	Antioxidant capacity (%)
Without microcapsules	64.08 ± 0.83[b]
With microcapsules	69.33 ± 0.13[a]

*a, b: indicate significant differences ($p < 0.05$).

Table 4: Estimated regression coefficients for the model with the response variables

Regression Coefficients	Antioxidant capacity		Total Polyphenolic Content	
	Effect	P_Value	Effect	P_Value
β_0	-57.1122	0.0000*	-0.258419	0.0000*
$\beta_1(X1)$	0.983348	0.5800	0.0053963	0.6475
$\beta_2(X2)$	24.8544	0.0001	0.17398	0.0000
$\beta_3(X1X2)$	-0.240644	0.4608	-0.00115185	0.0950
$\beta_4(X2^2)$	-1.9967	0.0037	-0.019637	0.0000
R^2	0.830015	0.9743	0.967493	0.9341

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Table 5: Overall acceptability results

Sample code	Overall acceptability (Mean ± SEM)	Statistical acceptance
Code 178	5.29 ± 0.18	a
Code 285	5.45 ± 0.20	a
Code 303	5.47 ± 0.20	a

Table 6: Color parameters (CIELAB color system) for chocolate samples without and with microcapsules

Samples	L*	a*	b*	WI	ΔE^*ab
SM	26	6.2	4.1	25.63	-
CM	26.7	7	4.7	26.22	1.22

SM: Chocolate without microcapsules; CM: Chocolate with microcapsules

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