

Effect of microencapsulation conditions on phenolic compounds and antioxidant activity of propolis using double emulsion solvent evaporation approach

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ABSTRACT

The aim of this work was to microencapsulate propolis phenolic compounds using polycaprolactone as wall material by double emulsion solvent evaporation ($w_1/o/w_2$). Microencapsulation experiments were carried out by investigating the effect of sample/solvent ratio ($10\text{--}100\text{ mg mL}^{-1}$), poly(ϵ -caprolactone) (PCL) concentrations ($200\text{--}1,000\text{ mg mL}^{-1}$), poly(vinyl alcohol) (PVA) concentrations ($0.5\text{--}2.5\text{ g mL}^{-1}$), and stirring speed ($200\text{--}1,000\text{ r.p.m.}$) on the microencapsulation efficiency of total phenolic content

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(TPC%) and antioxidant activity of propolis. The best microencapsulation conditions were selected according to the total phenolic amount and their antioxidant activity. Experimental results showed that all microencapsulation conditions had significant effects ($P < 0.05$) on total phenolic content and antioxidant activities. The best conditions were: 30 mg mL⁻¹, 600 mg mL⁻¹, 2 g mL⁻¹, and 400 r.p.m. for sample/solvent ratio, PCL concentrations, PVA concentrations, and stirring speed, respectively, with values of $86.98 \pm 0.03\%$ for phenolic encapsulation efficiency, $53.81 \pm 0.50\%$ for free radical scavenging activity (DPPH), and 45,480 Trolox equivalent, mg TE/100 g dry weight for ferric reducing antioxidant power (FRAP). Under all encapsulation conditions, a significant positive correlation was observed between ferric reducing antioxidant power, free radical scavenging activity, and phenolic content.

KEYWORDS

propolis, microencapsulation, double emulsion, phenolic compounds, antioxidant activity

1. INTRODUCTION

Propolis is a natural resinous and adhesive substance produced by bees (*Apis mellifera*). This substance has been used more recently as health food and additive in functional food (Ferreira et al., 2023); although it is a natural remedy that has been used since ancient times (Farre et al., 2004). The chemical composition of propolis varies according to the botanical origin (Mouhoubi-Tafnine et al., 2016). Propolis is composed of resins (50%), waxes (30%), volatile essential oils (10%), organic and inorganic chemical compounds (5%), and pollen (5%) (Farre et al., 2004). Propolis exhibits a wide range of biological and pharmacological activities including antifungal, antibacterial, antiviral, anti-inflammatory, antitumour, and notably, antioxidant activity. This last activity is attributed to its rich content of phenolic compounds (Ramos and Miranda, 2007; Valente et al., 2011).

Microencapsulation is employed to provide protection to the core material against environmental factors such as light, humidity, temperature, or oxygen (Dias et al., 2015). In addition, it contributes to stabilising the core material to increasing shelf life of the products, and to controlling the release of material from the core. Among several methods for encapsulation of the core material, the most widely used is the double emulsion solvent evaporation method due to its high efficiency encapsulation and maintenance of chemical stability and controlled release of encapsulated molecules (Klinkesorn et al., 2005; Bouriche et al., 2019). This method is a complex system consisting of an internal emulsion (w/o) dispersed in a second continuous aqueous phase (w/o).

Double emulsion has great potential for the encapsulation of bioactive compounds, due to its ability to encapsulate hydrophilic and also lipophilic substances (separately and simultaneously) (Iqbal et al., 2015; Castillo and Martinez, 2023). However, the applications are dependent on the polymers used (Khaldia et al., 2020).

Polyphenols are known for their antioxidant properties and their ability to protect intestinal cells from oxidative and inflammatory damage. However, the health benefits of phenolic compounds largely depend on their bioavailability, absorption, and metabolism (Cueva et al., 2017). Therefore, employing encapsulation as a strategy to protect phenolic compounds becomes



crucial, as it can preserve their biological activities, enhance their bioavailability, and improve absorption rates.

In this context, this work aimed to study the effect of microencapsulation conditions (sample/solvent ratio, PCL, PVA, stirring speed time) on phenolic compounds (phenolic encapsulation efficiency) and antioxidant activity (DPPH and FRAP) of propolis using polycaprolactone polymer through the ($w_1/o/w_2$) double emulsion solvent evaporation technique.

2. MATERIALS AND METHODS

2.1. Preparation of propolis extracts

Propolis samples were collected from hive colonies in the region of El Mhir, Bourdj Bou Arreridj city, in August 2022. A quantity of 10 g of powder samples were extracted with 100 mL of ethanol (70%) following the method reported by [Ydjedd et al. \(2017\)](#).

2.2. Preparation of microparticles

The microencapsulation technique of the propolis extracts was carried out following the protocol described by [Ydjedd et al. \(2017\)](#).

2.3. Effect of different parameters

The effect of encapsulation conditions including, sample/solvent ratios (10, 30, 50, 70, and 100 mg mL⁻¹), the PCL concentration (200, 400, 600, 800, and 1,000 mg mL⁻¹), the PVA concentration (0.5, 1, 1.5, 2, and 2.5 g mL⁻¹), and the stirring speeds (200, 400, 600, 800, and 1,000 r.p.m.) were optimised. The best conditions were identified based on the microencapsulation efficiency of TPC% and antioxidant activities measured (DPPH and FRAP).

2.4. Efficiency of phenolic microencapsulation

The total phenolic content of propolis extracts was determined using the spectrophotometric method as reported by [Chaalal et al. \(2018\)](#). Likewise, the encapsulation rate was calculated following the method reported by [Ydjedd et al. \(2017\)](#).

2.5. Antioxidant activity

The free radical scavenging activity DPPH was evaluated according to the method described by [Chaalal et al. \(2018\)](#). Likewise, the ferric reducing antioxidant power (FRAP) assay was carried out according to method described by [Ydjedd et al. \(2017\)](#). The results of FRAP were expressed as milligrams of Trolox equivalents per 100 g of dry weight (mg TE/100 g DW).

2.6. Statistical analysis

All experiments were performed in triplicate. The results are presented as mean $\pm$ standard deviation. The analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$).



3. RESULTS AND DISCUSSION

3.1. Phenolic content and antioxidant activity

Table 1 presents the total phenolic content and the antioxidant activities of propolis extracts before encapsulation. The results showed that the total phenolic content was 58,510 mg gallic acid equivalent, GAE/100 g dry weight. The TPC values of propolis extract obtained were higher than those reported by Mouhoubi-Tafnine et al. (2016) on Algerian propolis samples (5,351 mg GAE/100 g). This variation may be assigned to the botanical origin, environmental hives, and type of extraction methods (Ouchemoukh et al., 2007).

However, a higher free radical scavenging activity DPPH was obtained with value of $80.37 \pm 1.26\%$. This result was higher compared to those reported by Soltani et al. (2021). Furthermore, as the result of FRAP assay, the extracts exhibited an activity of 55,940 mg TE/100 g dry weight of propolis. The impact of geographic variation on the chemical composition and antioxidant activity of Algerian propolis was studied by Kouadri et al. (2021).

3.2. Effect of sample/solvent ratio

The results of total phenolic encapsulation efficiency and antioxidant activity under the effect of different sample to solvent ratios are presented in (Fig. 1). According to the results, the sample/solvent ratio of 30 mg mL^{-1} showed the highest encapsulation phenolic efficiency with values of $86.93 \pm 0.03\%$. This value decreased from $79.79 \pm 0.22\%$ to $65.81 \pm 0.47\%$ with the increase of sample/solvent ratio from 50 mg mL^{-1} – 100 mg mL^{-1} . Similarly, the highest antioxidant activities, DPPH and FRAP, were also obtained with the ratio of 30 mg mL^{-1} with values of $63.02 \pm 0.97\%$ and $52,320 \text{ mg TE/100 g DW}$, respectively. Likewise, the ratio of 100 mg mL^{-1} gave the lowest rate of phenolic encapsulation and antioxidant activity.

The sample/solvent ratio in the inner aqueous layer plays a crucial role in determining the encapsulation efficiency. The encapsulation efficiency of microparticles increased with an extract concentration of up to 30 mg mL^{-1} . However, concentrations exceeding 30 mg mL^{-1} led to lower encapsulation efficiency and reduced antioxidant activities. This may be attributed to viscosity property. The entrapped substance with low viscosity could facilitate the emulsion droplet to break up, as well as reduce the loading efficiency of the sample (Li et al., 2017). Moreover, the higher concentration of sample not only elevated the viscosity but also promoted the precipitation of sample.

The study of Paulo et al. (2021) showed that the phenolic encapsulation efficiency of propolis extracts-loaded microparticles formulated using ethyl cellulose polymer was $99.0 \pm 1.0\%$. This indicates a minimal phenolic loss of only $1.0 \pm 1.0\%$ when using a sample/polymer ratio of 3/4.

Table 1. Total phenolic content and antioxidant activity of phenolic propolis extracts before encapsulation

Parameter, unit	Average $\pm$ SD
TPC content, mg GAE/100 g DW	58,500 $\pm$ 150
DPPH, %	80.37 $\pm$ 1.26
FRAP, mg TE/100 g DW	55,944 $\pm$ 543



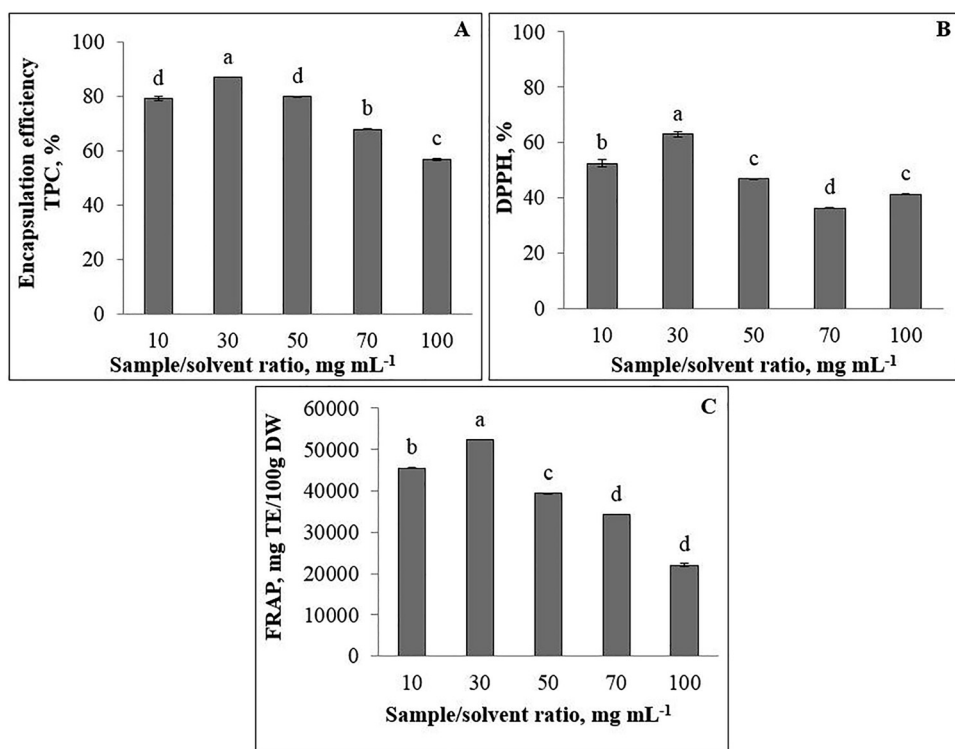


Fig. 1. Effect of sample/solvent ratio on the phenolic compounds' encapsulation efficiency (A) and antioxidant activities DPPH (B) and FRAP (C). a–d: different lowercase letters indicate significant differences at $P < 0.05$

Furthermore, according to the findings of [Marquele et al. \(2006\)](#), propolis microencapsulated using a spray dryer exhibited significant antioxidant activity in terms of free radical scavenging. The samples in their study were within the range of 2.5–5 mg mL⁻¹.

3.3. Effect of PCL concentration in the oily phase

The results of the phenolic encapsulation efficiency and the antioxidant activity under the effect of different PCL concentrations are shown in [Fig. 2](#). The results show that the highest efficiency was obtained with the concentration of 600 mg mL⁻¹ with a rate of $90.08 \pm 0.02\%$ for phenolic efficiency, $58.69 \pm 0.08\%$ for free radical scavenging activity DPPH, and $47,165 \pm 11$ mg TE/100 g DW for ferric reducing antioxidant power FRAP. Based on the results, it can be recognised that the increasing PCL concentration has a positive effect on encapsulation efficiency. [Avanço and Bruschi \(2008\)](#) showed that the phenolic encapsulation efficiency of formulated microparticles of ethyl cellulose loaded with propolis was $62.99 \pm 0.52\%$ using the double emulsion method. A higher polymer concentration in the oily phase affected the encapsulation efficiency. This effect may be attributed to the interactions between the polymer and the active compound, as suggested by [Sahlan and Supardi \(2013\)](#).



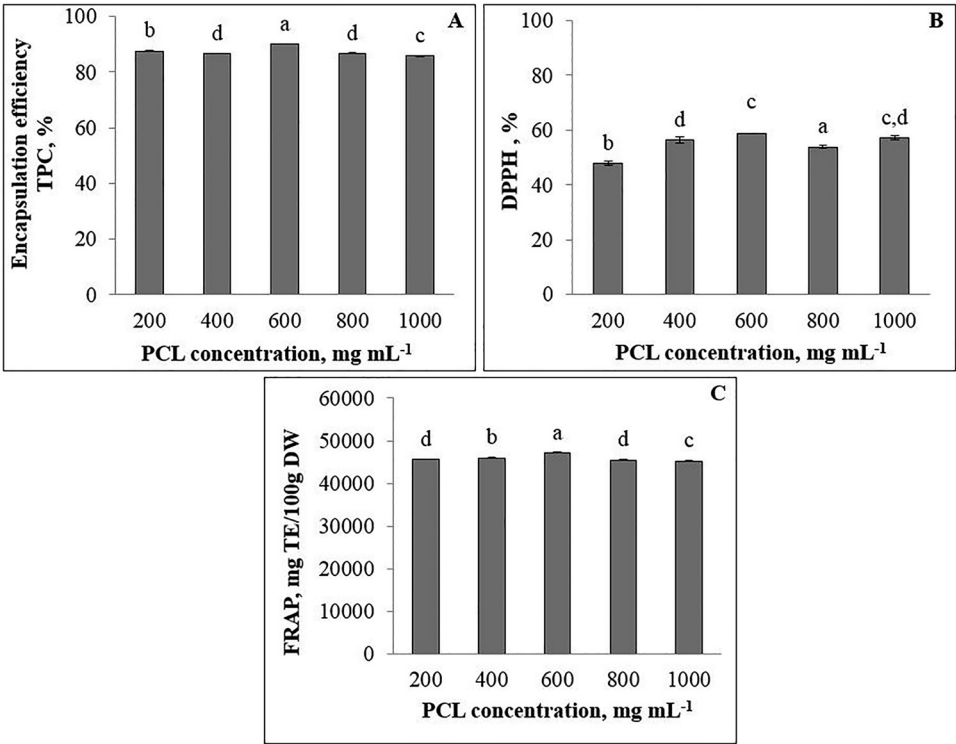


Fig. 2. Effect of PCL concentration on the phenolic compounds' encapsulation efficiency (A) and antioxidant activities DPPH (B) and FRAP (C). a–d: different lowercase letters indicate significant differences at $P < 0.05$

According to Paulo et al. (2021), the polycaprolactone microparticles loaded with propolis extracts exhibited the highest antioxidant activity, reaching a value of 98.3%. This suggests that polycaprolactone is an effective polymer for incorporating and protecting the phenolic compounds present in propolis extracts. Based on these results, it can be concluded that polycaprolactone is the most suitable polymer for encapsulating and preserving the phenolic compounds in propolis extracts, thereby enhancing their antioxidant activity.

3.4. Effect of PVA concentration

The effect of PVA concentration on the phenolic encapsulation efficiency and antioxidant activities of propolis microparticles is shown in Fig. 3. An increase was observed in the rate of phenolic efficiency encapsulation and antioxidants activities (DPPH and FRAP) at the concentration of 2 g mL⁻¹ with values of 88.05 ± 0.08% for the phenolic encapsulation efficiency, 58.63 ± 0.36% for free radical scavenging activity DPPH, and 47,640 mg TE/100 g DW for ferric reducing antioxidant power. Moreover, the encapsulation efficiency of TPC and the antioxidant activity DPPH and FRAP slightly decreased at 1.5 g mL⁻¹ of PVA.



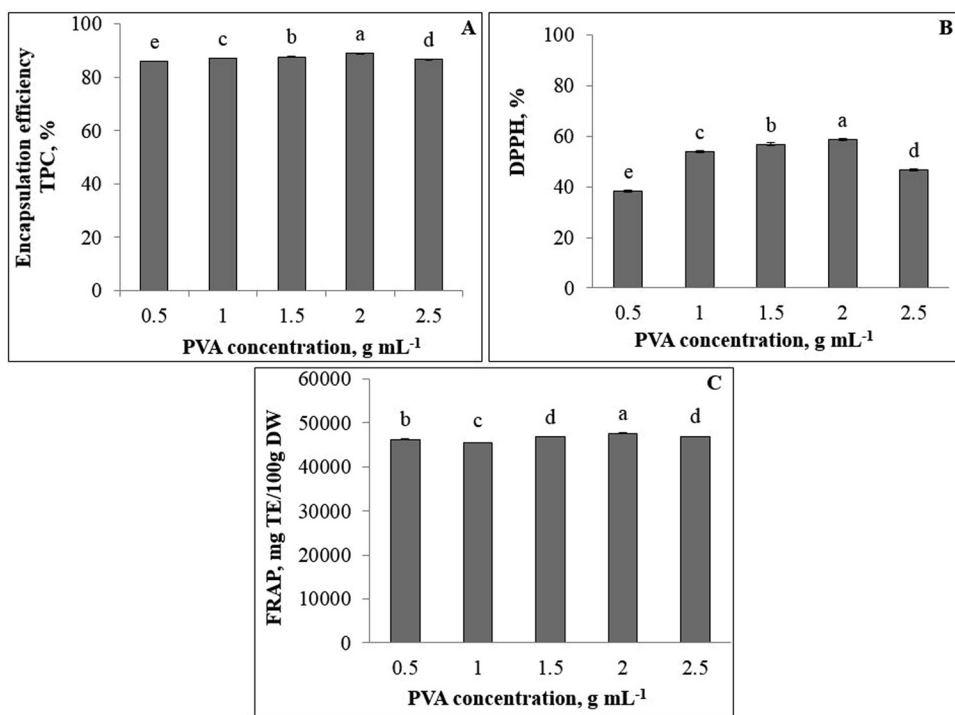


Fig. 3. Effect of the PVA concentration on the phenolic compounds' encapsulation efficiency (A) and antioxidant activities DPPH (B) and FRAP (C). a–e: different lowercase letters indicate significant differences at $P < 0.05$

The obtained results suggest that PVA plays an important role in the formation of emulsions, which can cover larger interfacial areas, create densely packed compliant shell, and improve the textural quality (Speranza et al., 2013).

It was reported that polymerisable emulsifiers increase the emulsion stability by contribution to emulsion polymerisation (Chen et al., 2012). The emulsifiers reduce the surface tension of the continuous phase and minimise the interfacial forces by creating a protective layer around the oil droplets. Moreover, viscosity of the emulsion induces the dispersed resistance in emulsion system (Li et al., 2017). Our findings differ from those reported by Bouriche et al. (2019), who showed that the concentration of polyvinyl alcohol (PVA) did not have a significant effect on the emulsion.

3.5. Effect of stirring speed on the formation of the double emulsion

Figure 4 shows the interactive effect of agitation speed on phenolic encapsulation efficiency and antioxidant activities. The highest phenolic encapsulation rate was recorded with 400 r.p.m. speed with values of $86.98 \pm 0.03\%$ for phenolic efficiency, $53.81 \pm 0.50\%$ for the DPPH free



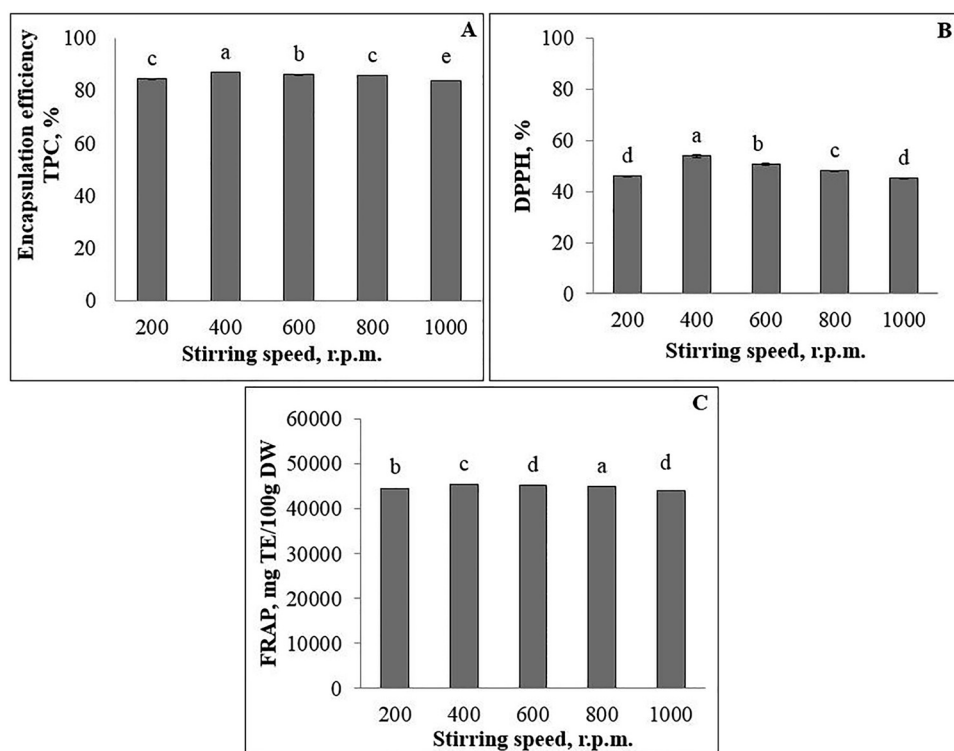


Fig. 4. Effect of stirring speed on the phenolic compounds' encapsulation efficiency (A) and antioxidant activities DPPH (B) and FRAP (C). a–e: different lowercase letters indicate significant differences at $P < 0.05$

radical scavenging activity, and 45,480 mg TE/100 g DW for the ferric reducing antioxidant power. Above 400 r.p.m., the encapsulation efficiency decreased with the increase of stirring speed.

The stirring speed is an important factor for the optimisation of encapsulation efficiency; with the increase of the stirring speed both encapsulation efficiency and phenolic and antioxidant activities significantly decreased. These results are similar with the result reported by Calligaris et al. (2016). The mechanical forces, turbulence, and elongation stress, break down the droplets to the smaller ones (Li et al., 2017). This phenomenon may explain the observed decrease in encapsulation efficiency and antioxidant activity at higher stirring speed.

3.6. Correlation analysis

Correlation analysis was carried out to explore the relationships between phenolic encapsulation efficiency and the antioxidant activities (DPPH and FRAP) measured in encapsulated propolis extracts (Fig. 5).

The results showed a highly significant correlation between phenolic compounds encapsulation efficiency and antioxidant activities of propolis extract. This finding was in agreement



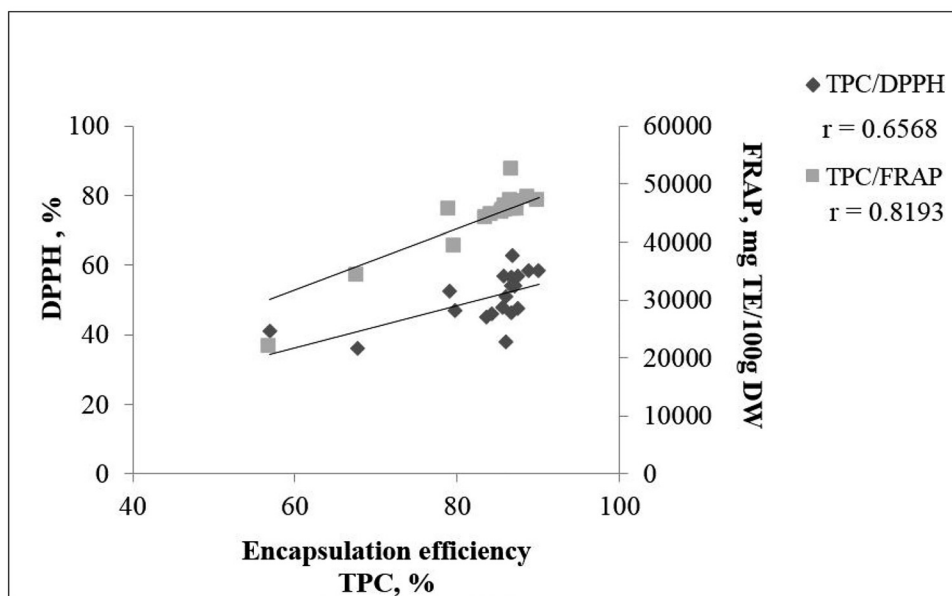


Fig. 5. Correlation between phenolic compounds' encapsulation efficiency and antioxidant activities (DPPH and FRAP)

with the results reported by [Da Rosa et al. \(2014\)](#) in their study on the encapsulation of phenolic compounds from blackberries.

4. CONCLUSIONS

In this work, propolis was microencapsulated through a method of double emulsion solvent evaporation, using polycaprolactone as polymer. The encapsulation parameters investigated exhibited significant effects ($P < 0.05$) on the phenolic encapsulation efficiency and the antioxidant activities tested. The results show that the optimum conditions of encapsulation were: 30 mg mL^{-1} for sample concentration, 600 mg mL^{-1} for PCL concentration, 2 g mL^{-1} for PVA concentration, and 400 r.p.m. for stirring speed with values of $86.98 \pm 0.03\%$ for the phenolic encapsulation efficiency, $53.81 \pm 0.50\%$ for free radical scavenging activity, and $45,480 \text{ mg TE/100 g DV}$ for the ferric reducing antioxidant power. This finding underscores the potential of propolis-loaded microparticles as a means to enhance the stability and bioavailability of propolis in various applications.

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