Solid lipid nanoparticles loaded with Jackfruit (*Artocarpus heterophyllus* Lam) seed-derived bioactive peptides: Characterization and antioxidant activity

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ABSTRACT

Objective: Characterize and evaluate the antioxidant activity of solid lipid nanoparticles loaded with bioactive peptides derived from the cotyledon of jackfruit.

Design/methodology/approach: The peptides were obtained from seed cotyledon by ultrasound-assited sequencial enzymatic hydrolysis. Four peptide fractions (PF) of ≥ 10 kDa, ≤ 10 kDa, ≥5 kDa, and ≤5 kDa, and their antioxidant activities were evaluated by using the DPPH radical scavenging method. A double emulsion method encapsulated PF≥ 5 kDa and ≤ 5 kDa into solid lipid nanoparticles (SLN).

Results: The UA-AF 2 treatment resulted in higher HD (40.39±2.82%). Besides, the effect of ultrasound improved the antioxidant activity on the DPPH. The SLN had a particle size (Ps) mean of 235 nm, polydispersity index < of 0.4, and negative zeta potential (ζ) > −30 mV and the SLNs were considered stables. The antioxidant activities of the peptide fractions in SLN decreased by 67.65±3.19% and 52.21±1.15%, associated with higher encapsulation efficiencies of peptide fraction in SLNs, ≥ 5 kDa (84.43±7.44 %) and ≤ 5 kDa (81.68±8.31%).

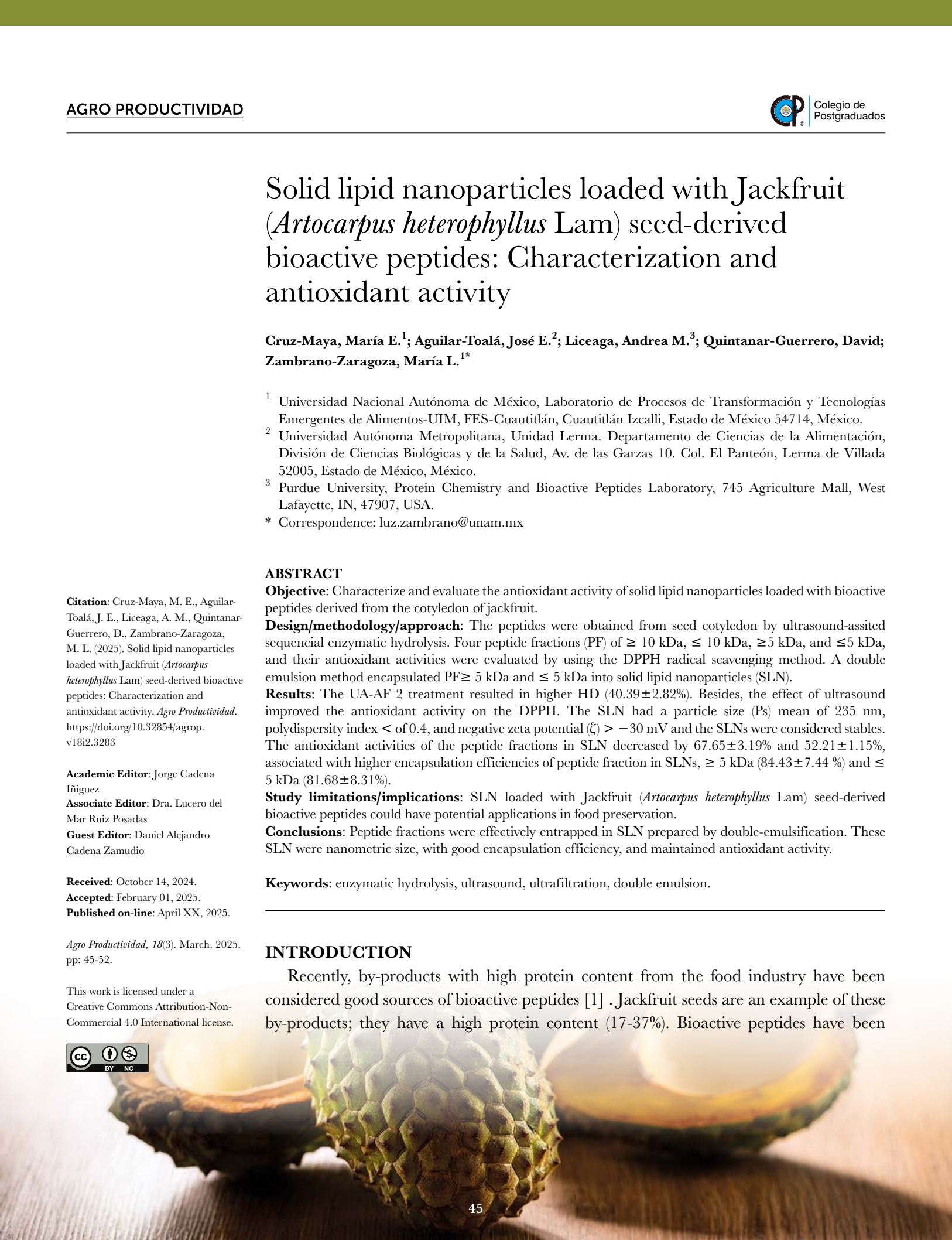
Study limitations/implications: SLN loaded with Jackfruit (*Artocarpus heterophyllus* Lam) seed-derived bioactive peptides could have potential applications in food preservation.

Conclusions: Peptide fractions were effectively entrapped in SLN prepared by double-emulsification. These SLN were nanometric size, with good encapsulation efficiency, and maintained antioxidant activity.

Keywords: enzymatic hydrolysis, ultrasound, ultrafiltration, double emulsion.

INTRODUCTION

Recently, by-products with high protein content from the food industry have been considered good sources of bioactive peptides [1] . Jackfruit seeds are an example of these by-products; they have a high protein content (17-37%). Bioactive peptides have been



identified from jackfruit seeds, including the antioxidant peptides JFS-2 and glutathione, the hormonal peptide leptin, and the antiviral α-peptide from jacalin [2,3].

Sequential hydrolysis has been used to extract bioactive peptides, resulting in a higher degree of hydrolysis and yielding bioactive peptides, thereby enhancing the peptide profile bioactivity. Additionally, ultrasound-assisted hydrolysis has been considered a promising method for improving hydrolysis and enhancing peptide bioactivity, owing to its positive effects on enzyme activity, protein structure, and enzyme-substrate interactions [4].

Encapsulation ensures the peptides' functionality by delaying their degradation, preventing undesired interactions with components of the food matrix, and increasing consumer acceptance by masking unpleasant tastes [5]. In this context, solid lipid nanoparticles (SLN) represent one of the main strategies used to encapsulate hydrophilic or lipophilic peptides, offering significant advantages [6]. Thus, the aim of this study was to characterize and evaluate the antioxidant activity of solid lipid nanoparticles loaded with bioactive peptides derived from the cotyledon of jackfruit.

MATERIALS AND METHODS

Materials

The ripened Jackfruits (*Artocarpus heterophyllus* Lam.) were collected in El Llano, San Blas, Nayarit, Mexico. The peptidases Alcalase® from *Bacillus licheniformis*, Subtilis A (specific activity ≥2.4 U/g), Flavourzyme® from *Aspergillus oryzae* (specific activity ≥500 U/g), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and Folin-Ciocalteu were purchased from Sigma-Aldrich Chemical® S. A. de C. V. (State of Mexico, Mexico). The surfactants Tween 80, soy lecithin, cacao butter, and beeswax were provided by Drogueria Cosmopolita S. A. de C. V. (CDMX, Mexico).

Obtention and hydrolysis of jackfruit cotyledon

Jackfruit seed parts were manually separated, and the cotyledons were washed and dipped in 0.05% (w/v) sodium metabisulfite solution for 24 h. The cotyledons were ground (Nutribullet® food processor), dried (Sunix® dehydrator), and milled (KRUPS® mill) into a powder. The hydrolysis of jackfruit cotyledons (5% w/v) in two sequential stages of enzymatic hydrolysis at 55 °C/90 min by enzyme [7]. Initially, Alcalase® (8%) was used, followed by Flavourzyme® (6%) considering enzyme/protein relation. The enzymes were then inactivated at 80-85 °C/10 min.

Two treatments with ultrasound were used to assist the hydrolysis process. An ultrasound equipment UP200HT with a sonotrode of 14 mm diameter at 50 W and 50 kHz (Helshier, Warthestrasse, Germany) was used. The treatment conditions were UA-AF1 (3 pulses/60s-on/60 s-off) and UA-AF2 (5 pulses/40s-on/20s-off). After hydrolysis (65-and 125 min), each treatment's aqueous extract was recovered to determine the protein content and hydrolysis degree (HD).

Hydrolysis Degree (HD)

The hydrolysate samples were centrifuged at 3,000 g/10 min and separated from the supernatant. The protein content was measured using the modified Lowry assay, and

the HD was calculated by dividing the protein content by the protein content of seed flour [1,8].

Obtention of peptide fractions from hydrolysates

The hydrolysates from ultrasound and control treatments were sequentially separated and filtered through 10 kDa and 5 kDa molecular weight cut-off (MWCO) ultrafiltration membranes using two centrifugal filters (Visvaspin® 2, Sigma Aldrich, UK) under centrifugation at 2408 RCF/10 min (centrifuge Frontier™ 5707, Ohaus, Germany). The resulting peptide fractions were named PF ≥10 kDa, ≤ 10 kDa, ≥ 5 kDa, and ≤ 5 kDa.

DPPH Radical scavenging

The DPPH radical scavenging activities of the peptide fractions and SLNs were determined [8]. The reaction mixture's absorbance was measured at 515 nm against a standard curve of Trolox using a UV-Vis spectrophotometer. The DPPH radical scavenging activity was expressed in μM Trolox/L.

Preparation of solid lipid nanoparticles (SNL)

The SNL loaded with PF > 5 kDa and < 5 kDa were prepared using the double emulsification method assisted with ultrasound, as illustrated in Figure 1.

Characterization and encapsulation efficiency (EE)

The particle size (Ps) and polydispersity index (PDI) were determined by dynamic light scattering, and the zeta potential (ζ) by electrophoretic motion, using a Zetasizer Nano® ZS90 instrument (Malvern Instruments Ltd, Worcestershire, U.K.). The EE was determined by the indirect method after centrifugation at 16278 RCF/10 min

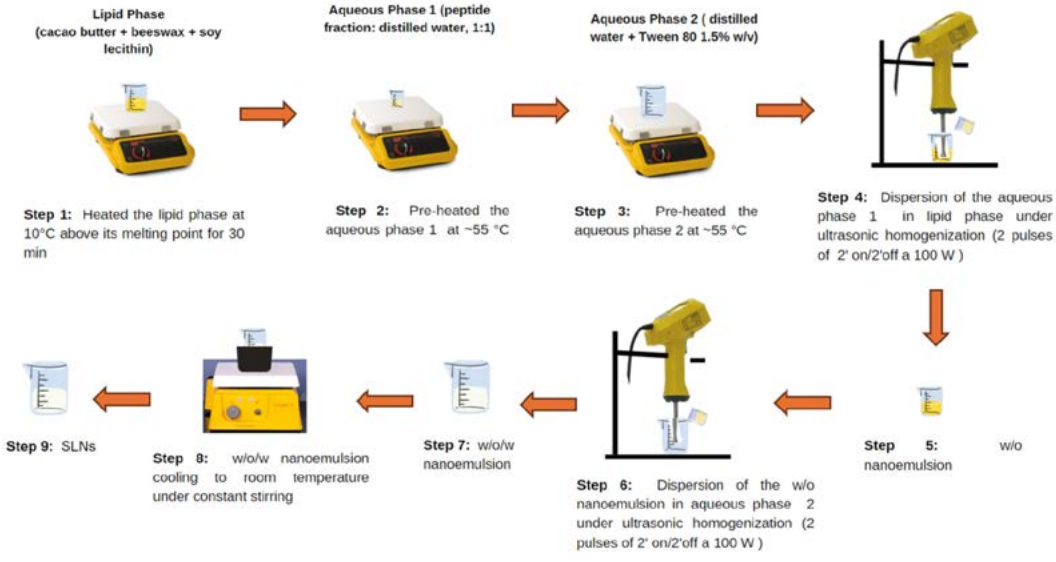


Figure 1. Double emulsification-ultrasound assisted in preparing SLN encapsulating bioactive peptides from jackfruit cotyledon.

(HERMLE Z323K, Germany). Protein content was determined using the Lowry assay, and the difference between protein content in the supernatant and peptides aggregated was reported as EE (%).

SLN stability

The backscattering profile was obtained using diffuse reflectance in a Turbiscan MA200 (Formulation, Toulouse, France), employing a detection wavelength of 55 nm throughout 24 h at 25 °C.

Statistical analysis

Statistical analysis was performed using Minitab® 19 Statistical Software, ANOVA, and Tukey test to mean differentiation, with significance set at $P<0.05$.

RESULTS AND DISCUSSION

Hydrolysis Degree (HD)

Table 1 shows the final HD; the most significant increase in final HD was observed with UA-AF2. The longer the ultrasound exposure time, the higher the HD [9]. However, this holds only if the ultrasound time is not excessive.

DPPH radical scavenging of the peptide fractions

Figure 2 shows the DPPH radical scavenging activities. The results demonstrated that ultrasound increased the DPPH activities of protein extract (66.34±14.47 %) and PF ≥ 5 kDa (5.31 ± 1.68 %) compared to the control. The higher DPPH radical scavenging activities of PF ≤ 10 kDa and PF ≥ 10 kDa observed can be attributed solely to enzyme specificity. In this case, Alcalase®, primarily an endopeptidase, exhibits broad specificity cleaving at Glu, Met, Leu, Tyr, Lys, Trp, and Gln. In contrast, Flavourzyme®, an exopeptidase, cleaves only the peptide bond between Leu-Pro and Pro-Pro [10]. This enhancement is attributed to the sonication effects, which promote hydrolysis, releasing peptides with diverse amino acid compositions that enhance antioxidant activity [11].

SLN characterization

Table 2 presents Ps, PDI, and ζ of SLN, both empty and loaded with PF with molecular weights ≥ 5 kDa and ≤ 5 kDa. These SLNs exhibited Ps ranging between 200-600 nm and PDI values ≤ 0.4. So, a PDI ≤ 0.5 indicated homogeneity in the Ps distribution, while a PDI ≥ 0.7 suggests particle aggregation [12]. The ultrasound conditions employed

Table 1. Effects of Ultrasound Treatment in the HD.

	UA-AF 1	UA-AF 2	Control
Total ultrasound time (s)	720	800	0
H.D. (%)	36.32±1.64 [a]	40.39±2.82 [a]	30.77±0.70 [b]
Increment (%)	18.12±7.20	31.18±6.46	---

Mean values with different letters in each row are statistically different ($P<0.05$).

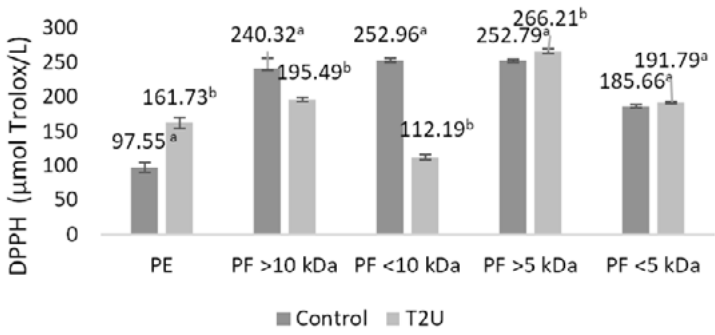


Figure 2. PF's antioxidant activity from DPPH assay. the mean values with different letters were statistically different (P<0.05).

Table 2. Particle sizes and polydispersity indexes of SLNS.

Parameter	NLS empty	NLS PF > 5kD	NLS PF < 5kD
Ps (nm)	218.7±4.980 [a]	228.8±2.501 [b]	235.2±3.993 [b]
PDI	0.258±0.037 [a]	0.336±0.055 [a]	0.412±0.094 [a]
ζ(mV)	−30.7±0.458 [a]	−34.8±0.100 [b]	−36.0±0.651 [c]

Mean values with different letters in each row are statistically different (P<0.05).

during SLN preparation with loaded PF contributed to the production of nanoparticles with smaller Ps and narrow distribution. The shear stress and shock waves generated by acoustic cavitation during the ultrasound reduced Ps [13].

The zeta potential (ζ) of empty SLN and those loaded with PF was around −30 mV, indicating electrostatic stables. However, there was a significant difference in the ζ between SLN loaded with PF ≥ 5kDa and those loaded with PF ≤ 5kDa. The differences are attributed to specific peptides loaded with exposed negatively charged amino acid residues on the nanoparticle surface, thereby increasing the negative potential of the SLN [14]. Establishing that the double nanoemulsion approach enables the production of a system for encapsulating and protecting bioactive peptides at the nanometric size.

Encapsulation efficiency (EE)

The SLN loaded with PF ≥ 5 kDa exhibited a higher EE (84.43±7.44%) compared to SLN ≤ 5 kDa (81.68±8.31) and demonstrated a statistically significant difference (P<0.05). The double emulsion facilitated the entrapment of hydrophilic peptides by incorporating the peptides into the inner aqueous phase of the W/O/W structure [14]. The combination of cacao butter (4.5%) and beeswax (0.5%) results in a less ordered lipid crystal structure with ample space for peptide embedding in the lipid matrix due to their different lipid composition and properties [15].

Antioxidant activity of the peptides in the SLN

Figure 3 illustrates the DPPH radical scavenging activities. The DPPH radical scavenging activities were lower in SLN with PF ≥ 5kDa (67.65±3.19%) compared with

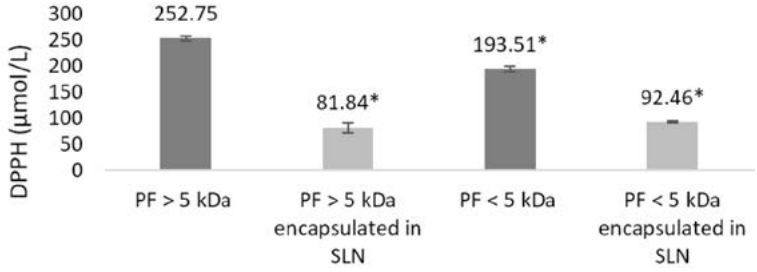


Figure 3. Antioxidant activity of the P.F. > 5 kDa and P.F. < 5 kDa with and without encapsulation in the SLN.

PF ≤ 5 kDa (52.21±1.15%). This behavior is attributed to a significant portion of the PF being entrapped within the SLN matrix, reducing the number of amino acid residues available to donate a proton to the DPPH radical, consequently decreasing the DPPH radical scavenging activities. Thus, encapsulating peptides from the cotyledon of jackfruit seeds in the SLN contributed to preserving their antioxidant activity.

SLN stability

Figure 4 (a and b) shows the backscattering (B.S.) profiles of the loaded SLNs. The figure depicts a slight increase in the B.S. intensity in the middle of the cell. However, the stability of SLN remained unaffected by this instability phenomenon, as indicated by the B.S. intensity variation in the middle (18-25 mm length), being less than 10% [16]. Following these results, SLNs loaded with PF ≥ 5 kDa and ≤ 5 kDa were deemed stable since they showed no significant variation in B.S. intensity. The stability of the SLNs can be attributed to higher ζ (>±30 mV), smaller Ps, and low PDI. Increased ζ results in higher electrostatic repulsion between nanoparticles, thus reducing instability phenomena [17]. Meanwhile, the hydrophilic surfactant Tween 80® stabilized the O/W_2 nanoemulsion by absorbing in the interface oil-water with the hydrophobic tail (carbon chain of oleic acid) directed towards the lipidic phase and the hydrophilic groups (hydroxyl moiety groups) oriented towards the aqueous phase [18].

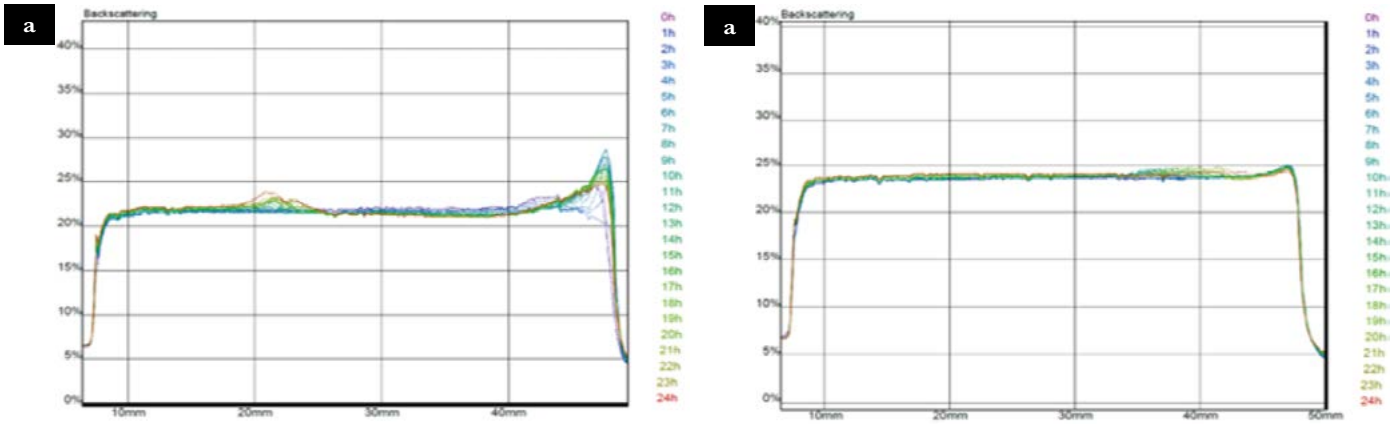


Figure 4. Backscattering profiles of the SLNs loaded with P.F. > 5 kDa (a) and P.F. < 5kDa (b).

CONCLUSIONS

Sequential enzymatic hydrolysis using Alcalase® and Flavourzyme® increased the degree of hydrolysis, thereby enhancing the antioxidant activity of the protein extract and protein fractions. The combination of the double emulsion method with ultrasound assistance, along with the selective use of lipids (beeswax and cacao butter) and surfactants (soy lecithin and Tween 80®), facilitated the encapsulation of antioxidant peptides into SLNs with higher encapsulation efficiency and good stability, as evidenced by low backscattering.

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