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Evaluating the effects of two drying methods on the properties of collagenous proteins: A comparative study

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Abstract
The objective of this research was to assess the impact of distinct drying techniques, specifically freeze drying (FD) and spray drying (SD), on the physicochemical, functional and biological properties of collagenous protein isolate derived from bluefin tuna (CPI). The freeze-dried sample (CPI-FD) displayed superior emulsification contributing to its higher solubility. In contrast, the spray-dried sample (CPI-SD) exhibited the highest foaming capacity and stability, attributed to the smaller particle size generated by the SD process. CPI-FD demonstrated greater gelation capacity, characterized by a lower gelation concentration (LGC), indicating gels prepared with CPI-FD had higher elastic and viscous moduli compared to those from CPI-SD. In summary, diversely dried CPIs showcased intriguing functional, physical, and biological properties, thereby underlining their potential utility in a range of applications, particularly within the food industry as a nutritional ingredient.

Keywords: Collagenous protein isolate, drying methods, freeze-drying, spray-drying, functional properties, biological properties

1. Introduction
The maritime ecosystem, is an untapped source of biologically active substances with significant potential to produce innovative ingredients for the development of products applied in various applications in the food and health sectors. Seafood processing produces a large quantity (50-80%) of inedible by-products, which are discarded or underutilized in many parts of the world [1]. These by-products are veritable sources of new and interesting biomolecules, such as collagen and gelatin, proteins and peptides, chitin, lipids, enzymes and pigments. Those Bioactive substances have been utilized in various biotechnological, nutritional, pharmaceutical, biomedical and other packages consisting of food, cosmetics, drug delivery.
This present study suggests an alternative approach to the transformation of seafood products into by-products, which could be an interesting and sustainable practice to efficiently value this new biomass. Collagen is the most common structural protein in the skin and bones of all animals and accounts for about 30% of the total protein content. It is commonly used in many sectors such as food, pharmacy, cosmetics, tissue engineering and biomedicine [2]. In this context, the head and cartilage of bluefin tuna (*Thunnus thynnus*) were used as by-products of fish processing and were valued by the protein isolation process. As a natural protein, collagenous protein isolate (CPI) has nutritional benefits, such as essential and non-essential amino acids, to improve the quality of different food products.
Several researchers in the characterization of the protein isolate used different drying methods such as spray drying (SD), freeze drying (FD), hot air drying (AD) and drum drying. The present study is, therefore, undertaken to systematically compare the influence of two drying treatments (freeze spray drying and convective drying at 170 °C and -80 °C) on the properties of CPIs.

2. Materials and methods
2.1 Raw material
Thunnus thynnus by-products (Heads, bones and tails) were commercially obtained, under fresh conditions, from a tuna fishing factory "Le Sultan" located in Sfax City, Tunisia.

The sample underwent a succession of steps of washing, cutting, grinding, and then stored in the laboratory at -20 °C until their utilization.

2.1 Preparation of bluefin tuna protein concentrates
The by-products of bluefin tuna were dispersed in distilled water (at a ratio of 1:10 w/v) to eliminate impurities and homogenized with an Ultra-Turrax apparatus (IKAR T18 basic). Subsequently, the homogenate underwent treatment with a 1 M HCl solution to adjust the pH to 5.0 and was incubated with continuous stirring at 50 °C for 18 hours to solubilize the collagenous proteins. After centrifugation at 8000 rpm for 30 minutes, the pH of the protein recovered in the supernatant was adjusted to 7.0 using a 1 M NaOH solution. Finally, the collected supernatant was neutralized to pH 7.0 and then subjected to drying.

2.3 Drying methods
In the freeze-drying process, the protein solution underwent pre-freezing for 24 hours at -80 °C. Following pre-freezing, the frozen samples were subjected to freeze-drying at −50 °C using a Moduloyd Freeze Dryer from Thermo Fisher, USA, for 72 hours, resulting in the production of freeze-dried powder of collagenous protein isolate (CPI-FD).
Conversely, the spray-drying of the CPI solution was conducted using a Mini Spray Dryer B-290 apparatus from BÜCHI Labortechnik AG, Flawil, Switzerland. The inlet air temperature was set at 170 °C, and the outlet air temperature was maintained at 80 °C throughout the spray-drying process. The resulting spray-dried powder of collagenous protein isolate (CPI-SD) was stored at 4 °C for future use.

2.4 Chemical property analysis
The determination of dry matter and ash contents in both freeze-dried (CPI-FD) and spray-dried (CPI-SD) powders adhered to the AOAC standard method [3]. Lipids were quantified gravimetrically following Soxhlet extraction of desiccated samples with hexane. The total protein content (N% × 6.25) of the protein isolate was assessed using the Kjeldahl method. All analyses were conducted in triplicate. The expressions of dry matter, protein, ash, and fat contents were standardized on a dry weight basis.

2.5 Physical characterization of the dried powders
2.5.1 Analysis of amino acid composition
The determination of the amino acid composition was performed according to ISO 13903:01-2015. The determination of total amino acids was performed after acid hydrolysis of the peptide bonds of CPIs for 24 h at 110 °C. Amino acid content was determined by chromatographic analysis using an HPLC system (Infinity 1260, Agilent, USA) equipped with a DAD detector (λ = 338 nm; λ = 208 nm). Amino acid separation was performed on a Zorbax eclipse AAA column at a temperature of 40 °C. A binary gradient mobile phase was used: Valve (A): monobasic sodium phosphate solution NaH_2PO_4 (40 mM) adjusted to pH 7.8 and Valve (B): acetonitrile/methanol/water (45/45/10) (v/v/v) mixture.

2.5.2 Surface morphology analysis
The surface morphology of dried powders was examined using a Scanning Electron Microscope (SEM, Hitachi S4800) operating at an accelerating voltage of 2.0 kV and an absolute pressure of 60 Pa.

2.5.3 Particle size distribution
The particles size was determined using a laser scattering particles size distribution analyzer Litesizer 500 (Anton Paar, GmbH, France) at 25 °C in triplicate.

2.6 Functional properties of the dried powders
2.6.1 Protein solubility Index
The solubility index (SI) was determined for various concentrations and across a broad pH range, following the methodology outlined by Binsi *et al.* [4] with slight modifications. In brief, 100 mg of dried powders were dispersed in a centrifuge tube by adding 10 ml of distilled water at room temperature, followed by homogenization. The pH of the dispersion was then adjusted to specific values using 6 M HCl or 6 M NaOH solutions as required. Subsequently, the protein solutions were agitated for 15 minutes and centrifuged at 8000 g for 10 minutes at 4 °C. The protein content of the supernatants was determined using the Lowry method.

2.6.2 The emulsifying properties
The emulsifying activity index (EAI) and emulsifying stability index (ESI) were determined using the method described by Pearce & Kinsella, [5]. A volume of 30 ml of protein solutions (0.5-4%) was mixed with 10 mL of corn oil and then homogenized with an Ultra-Turrax apparatus for 5 min at 25 °C. Aliquots (50 µL) from the emulsions were extracted from the emulsion base at 0- and 10-minutes post-homogenization and meticulously blended with 950 µL of a 0.1% sodium dodecyl sulfate solution (SDS). Subsequently, the absorbance was promptly measured at 500 nm, both immediately (A0) and after 10 minutes (A10) following emulsion formation.

2.6.3 Foaming properties of protein concentrates
Foam Capacity (FC) and Foam Stability (FS) were measured according to a slightly modified version of the method of Fekria *et al.* [6]. Briefly, 20 ml of protein solution (0.5-4%) was mixed with distilled water and whipped to incorporate the air for 3 min using an Ultra-Turrax at room temperature. Then, the volume was recorded before and after whipping. Foam Capacity (FC) and foam stability (FS) were calculated, respectively at 0 min and 1h.

2.6.4 Least gelation concentration (LGC)
The LGC was determined by the method of Sathe *et al.* [7]. Test tubes containing suspensions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20% (w/v) were heated for 1 h in boiling water. After cooling, the tubes were placed at 4 °C for 12 hours and then inverted. The LGC is the smallest concentration from which the contents of the inverted tube do not flow.

2.7 Biological capacities
2.7.1 Determination of *in vitro* antioxidant activities
Four different antioxidant assays, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and metal chelating activity were used. The DPPH radical scavenging activity was determined according to the method of Bersuder *et al.* [8]. The FARP was assessed based on the method described by Prieto *et al.* [9]. The Chelation capacity was measured according to Shantha & Decker, [10].

2.7.2 Antibacterial activity: The assessment of

antibacterial activity in the solutions of dried powder was conducted using the disc diffusion method. Four Gram-negative bacteria (Salmonella enterica ATCC 43972, Pseudomonas aeruginosa ATCC 15442, Enterobacter sp., Escherichia coli ATCC 4698) and four Gram-positive strains (Staphylococcus aureus ATCC 25923, Micrococcus luteus ATCC 4698, Listeria monocytogenes ATCC 13932, Bacillus cereus ATCC 11778) were employed in the assay.

2.8 Statistical analysis
Statistical analyses were performed using SPSS version 20.0 professional edition (SPSS, Inc., Chicago, IL, USA) through ANOVA analysis. Significance was considered at $p< 0.05$.

3. Results and Discussion
3.1 Effect of drying method on chemical composition
The proximal compositions of CPI-SD and CPI-FD are listed in Table 1. In terms of protein, ash and lipid contents, no significant difference was found between the samples, which means that spray and freeze drying has comparable drying efficiency under the experimental conditions. Joshi *et al*. [11], also reported that the drying methods only change the physical characteristics and not the chemical composition of the powders. On the other hand, CPI-SD and CPI-FD were characterized by high protein contents in the range of 77.49% and 76.64% (g/100 g dry matter), respectively. According to Table 1, the yield of the obtained CPIs differs depending on the drying process. The yield of CPI-SD was higher (11.00%, g/100 g wet weight) than that of CPI-FD (8.10%, g/100 g wet weight) ($p<0.05$). These results are consistent with those reported by Wan Omar et Sarbon [12], who explained that the frozen sample will be directly transformed into powder by lyophilization and this may cause some of the sample stick on the freeze drier wall chamber. This is because the freeze-dried sample was too fine with light weight as compared to sample spray dried.
Zeta potential (ζ) is a parameter that provides information about the surface charge of protein particles and electrostatic interactions. The Zeta potential values of CPIs were shown in Table 1. At pH 7.0, the overall charge of the CPIs particles was negative. The surface charge value of CPI-SD was lower in absolute value (3.58 mV) compared to that observed for CPI-FD (9.27 mV).

Table 1: Proximate composition (%, in dry matter) and yield (%, in wet matter) of the dried CPI powders

	CPI-SD	CPI-FD
Protein (%) **	77.49±0.35^b	76.64±0.55^a
Ash (%) **	20.76±0.42^a	22.29±0.52^b
Lipids (%) **	1.2±0.04^b	1.05±0.08^a
Drying yield (%) *	11.00	8.10
Size of particles	3.85±0.04^a	33.39±0.70^b
Zeta Potential (ξ)	-3.58±0.02^b	-9.27±0.03^a

*: g dry matter per 100 g wet matter, **: g dry matter per 100g dry matter. All the data are expressed as mean ± SD and are the mean of three replicates.

3.2 Effect of drying method on physical characterization
3.2.1 Amino acid composition analysis
The amino acid profile of CPIs was determined, and the results are presented in Table 2. Both CPIs were found to be rich in Gly (11.82 and 11.67 g/100 g protein), Pro (7.26 and 7.47 g/100 g protein), Hyp (5.45 and 7.07 g/100 g protein), Ala (6.03 and 6.54 g/100 g protein), Glx (5.81 and 7.97 g/100g protein) and Arg (6.14 and 6.78 g/100 g protein) for CPI-SD and CPI-FD, respectively. However, CPI-SD and CPI-FD have low levels of Trp (0.02 and 0.08 g/100 g protein), Tyr (0.12 and 0.38 g/100 g protein), Ile (0.48 and 0.88 g/100 g protein) and Met (0.86 and 0.89 g/100g protein), respectively. In contrast, no detectable values were observed for Cys. Overall, the amino acid profile observed in the current study is in agreement with that obtained for collagen reported by Carvalho *et al*. [13], who showed that the structure of collagen is stabilized by the presence of a glycine residue every three residues, a high content of proline (Pro) and hydroxyproline (Hyp).

Table 2: Composition of amino acids (g/100 g Protein) of the dried CPIs powders

Amino acids	CPI-SD	CPI-FD
Essential amino acids		
Isoleucine	0.48	0.88
Leucine	1.79	2.37
Lysine	3.38	4.50
Methionine	0.86	0.89
Phenylalanine	1.44	1.80
Threonine	1.81	2.18
Tryptophan	0.02	0.08
Valine	1.79	2.32
EAA	11.57	15.02
Non-essential amino acids		
Histidine	1.98	2.03
Tyrosine	0.12	0.38
Arginine	6.14	6.78
Asx	3.02	4.06
Alanine	6.03	6.54
Glycine	11.82	11.67
Glx	5.81	7.97
Hydroxyproline	5.45	7.07
Proline	7.26	7.47
Serine	2.51	2.83
Cysteine	ND	ND
NEAA	50.14	56.8
THAA	30.61	33.14

The aspartic and glutamic acids contents include, respectively, asparagine and glutamine, Asx = Asp + Asn; Glx = Glu + Gln. EAA: Essential amino acid; NEAA: Non-essential amino acid; THAA: Total hydrophobic amino acids; ND: Non detected

The results revealed that the total amino acid compositions were equivalent (100%) with some variations in the total amount of essential and non-essential AAs for both CPIs. Therefore, the CPIs showed a richness in amino acids, which could serve as promising sources of useful nutrients and bioactive peptides.

3.2.2 Surface morphology analysis
The SEM micrographs of CPI dried powders are shown in Fig. 1. The morphology of the CPI-SD was an asymmetric spherical structure with a smooth surface (Fig. 1-a and c). Some particles also appear to be folded or wrinkled. This structure was mainly formed by protein powders dissolved in water, and the asymmetric spherical structure could be due to the inequal distribution of protein and water [14]. In contrast, CPI-FD has a rough plate-like surface and an amorphous shape rather than individual particles. This was probably due to the fact that freeze-drying tends to produce aggregation after a long processing time by increasing the interaction of protein molecules (Fig. 1-b and d). In

addition, the ice sublimation phase in freeze-drying has a significant impact on the shape and volume of the powders due to the formation of large pores.

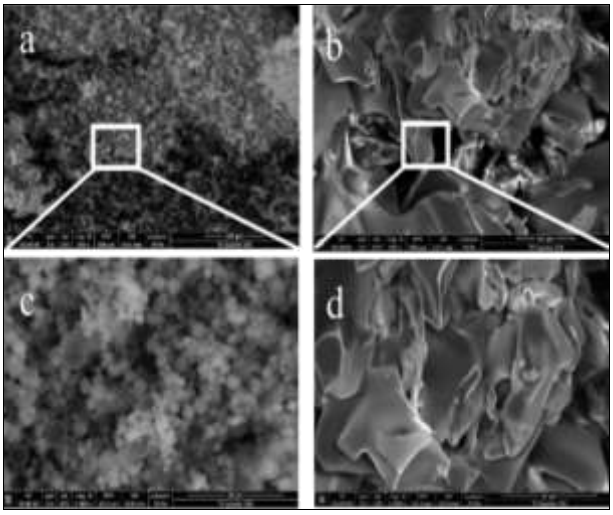


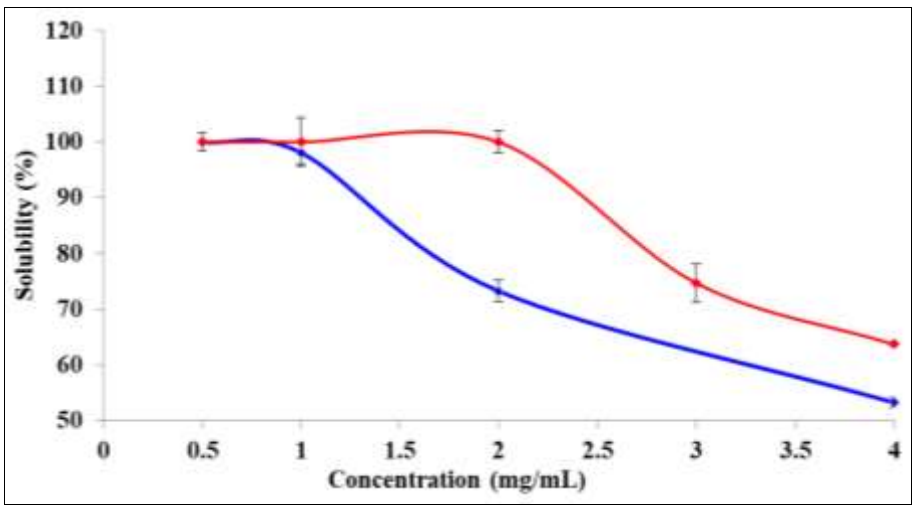
Fig 1: Microscopic morphology of the CPI obtained by two drying methods. SD (a: × 250, c: × 2000), FD (b: × 250, d: × 2000)

The particle size data further corroborate the SEM results (Table 1). According to the laser particle size analyzer, the highest particle size was attributed to the freeze-dried CPI (33.39 µm) compared to that of spray-dried CPI (3.85 µm). These results are in agreement with those reported by Joshi *et al*. [11], who showed that the particle size of spray-dried lentil proteins was smaller than those freeze-dried. In the freeze-drying process, sample had a larger particle size due to the low temperature used and the absence of forces to break the frozen liquid into droplets during the saturation process, resulting in an agglomeration, thereby, significant increase in particle size [15]. While, spray-drying process generate small particle size which allows larger surface area to reflect more light compared to freeze-drying [16]. The main advantage of the spray drying technique is the ability to control the particle size in terms of shape and morphology. These results suggest that the adopted drying method can lead to changes in the particle size distribution of the samples.

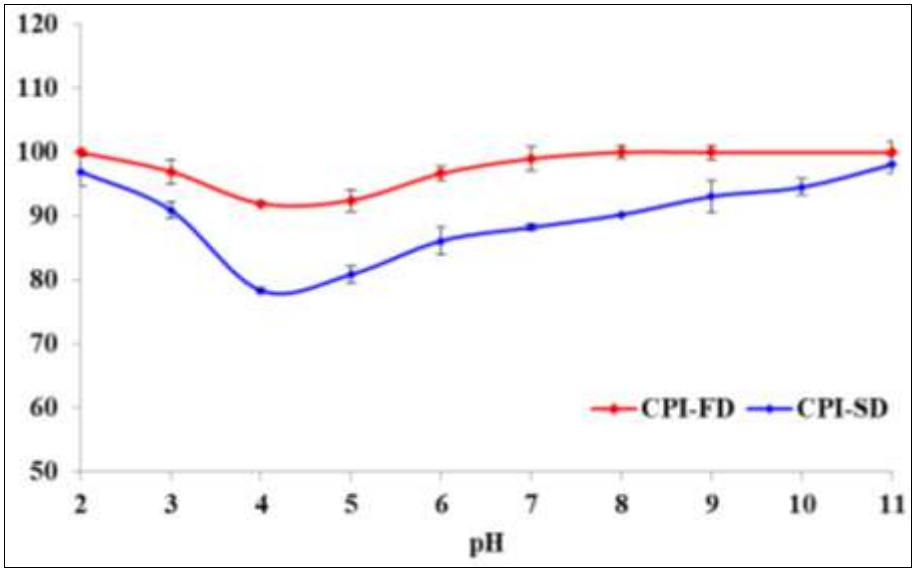
3.3 Effect of drying method on functional properties

3.3.1 Solubility

Solubility is the most reliable criterion to evaluate the behavior of powder in aqueous solution. The effect of concentration and pH on the solubility of CPIs was shown in Fig. 2. The results, presented in Fig. 2A, showed that the solubility decreased with increasing CPIs concentration, as at concentrations of 0.5 and 1.0%, the protein isolates are completely soluble in water (100%), however, at a concentration of 4%, the solubility of CPI-SD and CPI-FD decreased to 53.27% and 63.90%, respectively. At a high concentration (4%), the proteins will be highly aggregated. In the case of aggregation, protein molecules bind together, often due to an irreversibly altered conformation, and form high molecular weight insoluble forms [17]. The effect of pH on protein solubility was also studied (Fig. 2B). The minimum solubility of CPIs was observed at pH 4.0. This low solubility could be explained by the fact that this pH was probably close to the isoelectric pH of the studied CPIs. Indeed, at pH close to the isoelectric point, the net charge of the protein was zero, which reduced the repulsion of protein molecules by electrostatic forces and promoted their self-aggregation by hydrophobic forces, thus contributing to the precipitation of proteins. While, at basic or acidic pH values, the number of positive or negative charges accumulated on proteins was significant favoring the replacement of protein-protein interactions between molecules by protein-water interactions when the protein was dissolved in water. It is also interesting to note that protein solubility depends on the drying method adopted (Fig. 2). The solubility of CPI-FD was higher than that of CPI-SD. At pHs ranging from 7.0 to 12.0, CPI-FD exhibited complete solubility. In contrast, 88% solubility of CPI-SD was observed at pH 7.0. The variation in solubility of the CPIs may be related to the drying conditions used. These results are consistent with those presented by Liu *et al*. [18], who reported that spray-dried peanut protein concentrates have lower solubility than freeze-dried proteins due to the high drying temperature.



a)



b)

Fig 2: Effect of concentration (A) and pH (B) on the solubility of the CPIs

3.3.2 Emulsifying Activity and Emulsion Stability

The emulsifying capacity, represented by EAI and ESI, depends on the nature of the protein solution, the protein concentration, the pH, the solubility of the proteins. As observed in Table 3, the change in pH greatly affected the EAI and ESI values. In general, the EAI and ESI values were lowest at the pH 2.0 and it increased at pH 11.0. This could be probably explained by the fact that when the pH is close to the isoelectric point (pH=5), electrostatic repulsion among the molecules is the lowest, resulting in protein aggregation, and thereby, lower solubility and emulsifying properties. Whereas, when the pH value is higher than the isoelectric point, the protein molecules had a net negative charge which greatly enhanced protein-water interaction and resulted higher solubility, therefore, the emulsifying capacity was increased. As observed in the Table 3, CPI-FD presented the highest EAI and ESI followed by CPI-SD, indicating the best emulsion stability. This is further supported by Gong *et al.* [19], who observed that the EAI of freeze-dried peanut protein isolate (PPI) was higher than the spray-dried PPI, when the pH is higher than 8.0. Regarding CPI-SD, the low solubility and the damaged surface hydrophobic groups tend to generate coalescence and reduce the protein emulsification. Thus, drying methods certainly affect the emulsifying properties of the CPIs by generating different structural configurations.

Table 3: Effects of different drying methods on the functional properties of the dried CPIs

	CPI-SD		CPI-FD	
	pH 2.0	**pH 11.0**	**pH 2.0**	**pH 11.0**
Emulsifying activity index (m^2/ g)	5.26±0.24^a	21.06±0.77^a	12.48±0.24^b	25.13±0.68^b
Emulsifying stability index (min)	11.00±0.07^a	23.36±1.62^a	40.34±0.17^b	52.22±0.01^b
Foaming activity (%)	125.0±0.17^b	150.0±0.02^b	112.5±0.04^a	137.5±0.2^a
Foam stability (%)	100.0±0.07^b	125.0±0.12^b	100.00±0.21^a	118.7±1.01^a

Results are the means of three determinations ± standard deviation. $^{a-b}$ Different letters in the same column indicate a significant difference ($p< 0.05$)

3.3.3 Foam Capacity and Foam Stability

Foamability is an important factor in the application of food and cosmetic to obtain a final product with a better consistency and sensory quality. The foaming capacity (FC) and the foaming stability (FS) at pH 2.0 and pH 11.0 are shown in Table 3. The data presented indicated that there were no significant differences in foam capacities and stabilities between CPI-SD and CPI-FD. Indeed, the FC and FS of both CPIs increase significantly at a high pH (11.0) relative to pH 2.0. These observations are in agreement with the previous results of solubility and emulsifying properties, which confirmed that the highest CP values were observed at alkaline pH. The CPI-SD had good foaming and foam stability (Table 3). This can be attributed to the shape of the protein structure in the SD process, which could easily adsorb to the newly created air-water interface with the reduction of surface tension during bubbling. This result is consistent with that of Lin *et al.* [20], which demonstrated higher foaming properties in the phosphorylated krill spray-dried protein. In addition, the smaller particle size of CPI-SD could be adsorbed more rapidly during whipping or bulling, generating a slightly higher foam capacity than that of CPI-FD. Similarly, CPI-FD has a high foaming capacity and foam stability. These results could be explained by high solubility and hydrophobicity, which are already presented in the previous sections.

3.3.4 Least gelation concentration

The determination of the least gelation concentration (LGC) is a simple but effective method often used for studying the gelling capacity of proteins [21]. LGC is defined as the lowest protein concentration at which gel remained in the inverted tube was used as index of gelation capacity. Proteins with lower LGC have greater gelling capacity. The LGC of the CPI-FD and CPI-SD was about 6% and 20% (w/v), respectively. This result corroborates with the

solubility profile of both CPIs, as the samples with high solubility (CPI-FD) require a lower concentration of protein to make good gels. Thus, this finding infers the superior gelling capacity of CPI-FD compared to CPI-SD. Regarding the higher LGC of CPI-SD, may be assigned to the globular nature of its proteins, which is consistent with the SEM micrographs. According to Wei *et al.* [22] a considerably higher protein concentration is usually required for the gelation of globular proteins.

3.4 Biological potential

3.4.1 Antioxidant activities

The antioxidant results showed that the antioxidant activity of CPIs increases with concentration (Fig. 3). In order to reveal the free radical scavenging activity in the different dried protein isolates, the DPPH test as a function of the concentration of CPIs (1-4 mg/ml) was performed (Fig. 3A). Data revealed that both CPI-FD and CPI-SD have good antiradical activities with a better activity detected for CPI-FD (79% at a concentration of 4 mg/mL), compared to that of CPI-SD (56% at a concentration of 4 mg/mL). It is interesting to note that CPIs were active, endowed with antiradical power, which can react with free radicals to convert them into more stable products. TAs seen in the Fig. 3B, the capacity pf reducing power was correlated with increasing concentration of both CPIs. The highest value was attributed to CPI-FD with a value of 1.22 (OD 700nm) at 4 mg/ml. Also, CPI-SD followed the same trend as CPI-FD but with a low efficiency reaching 0.75 (OD700 nm). Thus, CPI-FD showed the highest capacity of an antioxidant to donate an electron or hydrogen. The same trend was observed for the total antioxidant capacity and the results obtained are expressed as µmol/ml equivalent of α-tocopherol (Fig. 3C). CPI-FD displayed the highest value reaching 180.51 µmol/ml at 4mg/ml. Nevertheless, findings reported in Fig. 3C, revealed that spray-drying method had no significant increase in the total antioxidant activity about 54.15 µmol/ml at 4 mg/ml. Current findings indicate that increased total antioxidant capacity may have a protective effect against oxidative stress. With regard to chelating power, the results recorded in Fig. 3D highlight the effectiveness of the antioxidants present in these two CPIs in chelating iron ions. Data showed a dose-dependent chelation potency for Fe^{2+}. From a dose of 0.5 mg/ml, CPI-FD and CPI-SD displayed the highest chelating activity reaching 100% with a similar efficiency than EDTA. Thus, these isolates could be considered as potential chelating agents in various biotechnological applications.

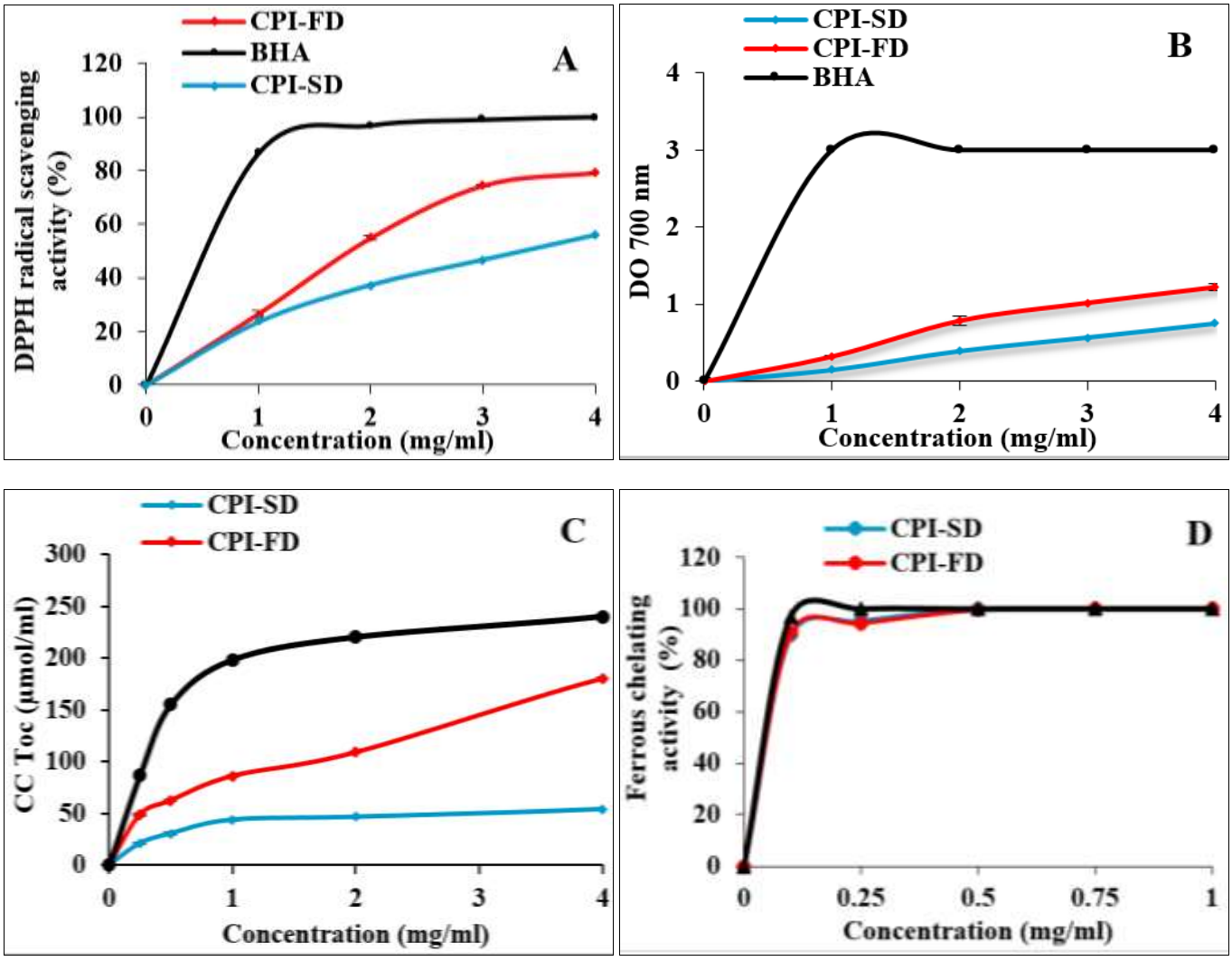


Fig 3: Antioxidant activities of the CPI obtained by two drying methods: DPPH radical scavenging (A), Reducing power (B), Total antioxidant activity (C), and Ferrous ion-chelating activity (D)

3.4.2 Antibacterial potential

The antibacterial activity of CPI obtained by freeze and spray drying at 70 mg/ml was examined against both Gram-negative and Gram-positive. Both CPI-FD and CPI-SD showed similar antibacterial activity against these bacterial strains. The results obtained (Table 4), expressed as the diameter of the inhibition zones, showed that the CPIs were endowed with antibacterial activity, almost similar, against the tested strains.

Table 4: Sensitivity of the microbial strains towards the dried CPIs powders

		Diameter of inhibition zone (mm)	
	Bacteria	**CPI-SD**	**CPI-FD**
Gram -	*S. enterica*	+++	+++
	P. aeruginosa	+++	+++
	Enterobacter sp.	--	--
	E. coli	+++	+++
Gram +	*S. aureus*	++	++
	M. luteus	--	--
	L. Monocytogenes	+	+
	B. cereus	--	--

The results, reported in Table 4, revealed that Gram-negative bacteria were significantly more inhibited than Gram-positive strains, especially for *S. enterica*, *P. aeruginosa*, *E. coli*, probably due to the difference of bacterial membrane structures. On the other hand, the weakest effect was obtained against Gram-positive strains. Indeed, CPI-FD and CPI-SD were ineffective against *E. sp*, *M. luteus* and *B. cereus*.

4. Conclusion

The findings of this study highlight the notable distinctions in chemical and physical structures, morphology and biological properties between spray-dried and freeze-dried collagenous protein isolates (CPIs). The choice of drying method, whether it be spray-drying or freeze-drying, exerts a substantial influence on both the yield and quality of CPI, with freeze-dried CPI yielding the highest at 11% as opposed to 8.10% for spray-dried CPI. It is noteworthy that the microstructure is notably affected by the drying method employed. Specifically, CPI-SD is characterized by spherical or oval-shaped particles, whereas CPI-FD exhibits a plate-shaped morphological structure. Importantly, there were no statistically significant differences observed in the amino acid composition, implying that the drying methods did not induce alterations in the physical properties of the proteins. Regarding functional properties, it was observed that freeze-drying presented enhanced emulsification and foaming properties. In terms of biological activities, both CPIs demonstrated intriguing antioxidant and antibacterial potential. Consequently, these differently dried CPIs present themselves as suitable candidates for various industrial applications, with particular relevance to the food industry.

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