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ORIGINAL RESEARCH PAPER

PHYSICO-CHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY OF ENCAPSULATED FISH COLLAGEN HYDROLYZATES WITH MALTODEXTRIN

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The aim of the research was to evaluate the effect of the encapsulation process on collagen hydrolysate. Commercial collagen hydrolysate (CH) was spray-dried with maltodextrin (MD) with two different dextrose equivalents (MD12: 10-12 dextrose equivalent (DE) maltodextrin and MD 19: 19-20 DE maltodextrin) and two different core-wall material ratios of 10:90 and 20:80 in pilot scale spray dryer. Four different groups of encapsulated collagen hydrolysate were created such as MD1210 (10% collagen peptide + 90% MD12), MD1220 (20% collagen peptide + 80% MD12), MD1910 (10% collagen peptide + 90% MD19), and MD1920 (20% collagen peptide + 80% MD19). Moisture, water activity, hygroscopicity, solubility and antioxidant activity of collagen peptides significantly ($p<0.01$) decreased with encapsulation ($p=0.01$). The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity analysis results showed that the collagen hydrolysates antioxidant activity was dependent on the dose and the antioxidant activity was significantly decreased by the encapsulation process.

Keywords: antioxidant activity, fish collagen, hydrolysates, maltodextrin, spray drying encapsulation

Introduction

In the past few decades, researchers have focused on bioactive peptides, which are produced by different animal and plant protein sources. Some of them focused on marine products derived from peptides, defined as cobia skin gelatine hydrolysates (Yang *et al*., 2008), Atlantic herring skin (Pampanin *et al*., 2012), Korean Rockfish skin (Kim *et al*., 2011), Grass carp, Nile perch, Nile tilapia skin (Wasswa *et al*., 2008), surimi processing by-products (Wiriyaphan *et al*., 2012; Liu *et al*., 2014), jellyfish collagen hydrolysate (Zhuang *et al*., 2009; Ding *et al*., 2011; Zhuang *et al*., 2012), *Gadus morrhua* skin collagen hydrolysate (Huo and Zhao, 2009),

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Sphyrna lewini muscle (Wang *et al*., 2012; Luo *et al*., 2013), bullfrog skin (Huang *et al*., 2011), sardinelle by-products (Bougatef *et al*., 2008, 2010; Nasri *et al*., 2013), tuna liver (Je *et al*., 2009), Atlantic salmon skin (Gu *et al*., 2011), capelin (Amarowicz and Shahidi, 1997), and silver carp (Dong *et al*., 2008).

Enzymatically hydrolyzed proteins from several sources were reported to have strong antioxidant activities and could be used as antioxidants in food systems. These hydrolyzed proteins have high moisture. Therefore, they could be processed with different methods for preservation. Spray drying is a method used to reduce water activity and formation of capsules. This technique is widely used and its operational costs are very low (Kurozawa *et al*, 2008).

Collagen is a protein which has a special amino acid composition. It is the most important functional building block of intercellular bond and supporting tissues including the most common protein and cartilage in animals and humans (Seifert, 2004). Collagen is an important component of the extracellular matrix. The denatured collagen is called gelatin and is widely used in foods or pharmaceuticals, photographic film, cosmetics, etc. (Felician *et al*., 2018). In order to increase gelatin solubility, partially hydrolysed gelatin products were prepared and named collagen hydrolyzate (Shigemura *et al*., 2011). Enzymatic hydrolysis is used for the production of collagen hydrolyzate from collagenous tissues (bones, hides, fish skin). Proteins with 100 kDa molecular weight were identified at the end of the production of gelatin. Although the same processes are used in the production of collagen hydrolyzates, peptides are formed at the end of the process with a molecular weight of 3-6 kDa. This difference causes the gelling property of the products to be different (Seifert, 2004).

Encapsulation can be used to eliminate the bitterness of protein hydrolysates/ peptides because it coats the bioactive hydrolysates/peptides. The adsorption of bitter peptide to taste receptors could be prevented without altering peptide structure. However, the release mechanism of the encapsulated peptides is still unclear (Mine *et al*., 2010).

Casein (Rocha *et al*., 2009), chicken meat protein (Kurozawa *et al*., 2009, 2011) and shark skin protein (Rodríguez-Díaz *et al.,* 2014) were investigated for protein hydrolysate encapsulation with maltodextrin by using spray drying.

Encapsulation technology was reported to prevent undesirable flavour and bitterness (Rocha *et al*., 2009; Kurozawa *et al*., 2011) and to reduce moisture, water activity (a_w), hygroscopicity and water solubility of hydrolysates (Rocha *et al*., 2009; Rodríguez-Díaz *et al*., 2014). Antioxidant activity of encapsulated hydrolysates is influenced by the presence of maltodextrin (Rodríguez-Díaz *et al*., 2014).

Bioactive peptides have numerous functions in human health. One of these is the collagen hydrolysate (CH), which has an antioxidative effect (Song and Li, 2017). The encapsulation of bioactive peptides can provide an alternative application to overcome problems associated with direct administration in food (Rao *et al*., 2016).

In addition, some protein hydrolysates were found to be very hygroscopic and reactive (Favaro-Trindade *et al*., 2010).

The aim of this research was to evaluate the influence of encapsulation on collagen hydrolysate by maltodextrin with two different dextrose equivalents and two different cores, which are core-wall material ratios (10:90, 20:80). Some physicochemical and morphological properties and antioxidant capacity of non-encapsulated materials and encapsulated materials were also analyzed.

Materials and Methods

Materials

Maltodextrin Maldex 120 and 190 were purchased from Tereos Syral (Aalst, Belgium). The fish collagen hydrolysate (Peptan F 2000 HD) was obtained from Rousseoult Angouléme S.A.S. (Rue de Saint-Michel an Angouléme, Angouléme, France). Fish-hydrolyzed collagen characteristics are presented in Table 1. Analytical grade chemicals and standards were used for analysis (Sigma or Merck) unless otherwise stated.

Table 1. Fish hydrolyzed collagen data

Tests	Units	Specifications	Values
Physico-Chemical Limits			
Typical average molecular weight (Mw)	Da	2000	Compliant
Protein content	%	≥ 90	Compliant
Loss on drying (105 °C, 17 h)	%	≤ 10	7.2
Bulk density	g/cm^3	0.40 - 0.55	0.50
Residue on ignition (550 °C)	%	≤ 2	0.88
Particul size < 425 µm (40 mesh)	%	≥ 95	≥ 95
Particul size < 75 µm (200 mesh)	%	≤ 15	≤ 15
Sulfites (as SO_2)	ppm	≤ 10	≤ 10
Peroxides (H_2O_2)	ppm	≤ 10	≤ 10
Physical & Chemical Properties			
pH			5.0-6.5 (in water)
Physical form		Powder	
Solubility		Cold soluble in water	
Water solubility		Completely soluble	

Dispersion preparation

Dispersions were prepared with 30% solids (w/w). Solids consisted of maltodextrin (MD12 or MD19) and fish collagen hydrolysate at wall material/core ratios of 90:10 and 80:20 respectively. Powders were dissolved in distilled water and then the samples were homogenized by using a homogenizer (Ultra Turrax T25, Janke

and Kunkel GmbH & Co. KG, Staufen, Germany) at 18000 rpm for 2 min at room temperature (Rocha *et al*., 2009).

Microencapsulation by spray drying

A laboratory-scale spray dryer (Buchi-B290, Flawil, Switzerland), with a chamber diameter of 16.5 cm, a chamber length of 60 cm and a standard nozzle with 0.7 mm was used in microencapsulation experiments. The inlet air temperature and outlet air temperature were maintained at 140 ± 1 °C and 80 ± 0.5 °C, respectively. Microcapsule powders were collected from the bottom of the dryer's cyclone. Powders were stored in an amber bottle in a cool and dry place (Rocha *et al*., 2009).

Moisture content and a_w

Powder moisture, which is contained in encapsulated products, was determined by a moisture analyzer (Ohaus, MB45, USA).

The water activity (a_w) values were analyzed in accordance with the method of Rödel *et al*. (1975). Water activity meter was used to calculate the encapsulated products a_w (LabTouch -aw, Novasina AG, Neuheimstrasse Lachen, Switzerland). Approximately 5g of homogeneous sample was put in a disposable cup, completely covering the bottom of the cup, and filling not more than half of it. The water activity value was directly measured by a hygrometer with an accuracy of ±0.003.

Hygroscopicity

The method of Rodríguez-Díaz *et al*. (2014) was used for determining the hygroscopicity. One g of sample was placed at 25 °C in a container with NaCl saturated solution, and samples were weighed after seven days. Results were expressed as g moisture/100 g powder.

Dissolution time

The method of El-Tinay and Ismail (1985) with some modifications was used for the determination of the dissolution time of the powders. In short, 2 g of powder was added to 50 ml distilled water at 26 °C. The mixture was stirred with a magnetic stirrer (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) at 900 rpm. The dissolution time was recorded after the complete dissolution of the material.

Scanning electron microscopy (SEM)

The microstructure of the spray dried microcapsule powder was observed under SEM (Leo 1430 VP, Leo Electron Microscopy Ltd., Cambridge, UK) coupled with Energy Dispersive X-ray (SEM/EDX) Spectroscopy. The powder was placed on the SEM stub using a double-sided adhesive tape. The microcapsule collagens, coated with a thin layer of gold were immediately analyzed by SEM, operated with an accelerating voltage of 15–20 kV.

DPPH radical scavenging activity

Scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical of aqueous solutions with different concentrations (0.5%, 1%, 2%, 3%, and 4%) of powders was determined by using the method described in the study of Tang *et al*. (2013),

with some modifications. An aliquot sample of 2 ml was added to 2 ml of 0.1 mM DPPH in ethanol (96%) prepared on a daily basis. The mixture was shaken vigorously for 30 min and then centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was then measured using a spectrophotometer (OPTIZEN™ POP, Mecasys Co., Daejeon, Korea) at a wavelength of 518 nm. The antioxidant activity of each sample was expressed in percentage inhibition of free radicals.

Statistical analysis

All experiments were performed in two replicates. One-way analysis of variance (ANOVA) was applied on all the variables, using the SPSS 17 statistical package for Windows (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ($p<0.05$) was used to determine the differences between treatment means.

Results and Discussion

Moisture and water activity

The moisture content of the encapsulated collagen hydrolysate samples was found to be significantly different from collagen hydrolysate and maltodextrins ($p<0.01$). The moisture content of powders was found between 4.15±0.36% and 8.42±0.39% on dry basis (Table 2). There was no significant difference found between the moisture content of MD1210, MD1910 and MD1920 encapsulated hydrolyzates but their moisture content was significantly lower than the MD1220 group.

Rocha *et al*. (2009) reported that casein hydrolyzates had significantly higher moisture content than the maltodextrin encapsulated hydrolysates. Also, Kurozawa *et al*. (2009) concluded in their research that the spray dried chicken meat protein hydrolysate with maltodextrin in different concentrations significantly reduced the moisture content of free hydrolysate. According to Rodríguez-Díaz *et al*. (2014), a higher solid content of feed solution resulted in a lower moisture content of encapsulated blue sharkskin protein hydrolysate. The water ratio of the solution fed to the spray dryer was found to affect the moisture content of the encapsulated powder. When the solid content of the solution increased, the water content of the powder decreased. Hence, the moisture content of the encapsulated powder is important (Goula and Adamopoulos, 2007). In our study, the moisture content of collagen hydrolysate significantly decreased in accordance to the increase of the concentration of the maltodextrins.

The water activity of powders ranged between 0.11 ± 0.007 and 0.36 ± 0.006. Because of the moisture content, water activity of collagen hydrolysates was significantly higher than the two different maltodextrins and four different encapsulated collagen hydrolysates ($p<0.01$). Encapsulated collagen hydrolysates with MD12 had significantly lower a_w than the one encapsulated with MD19, and a_w of the encapsulated materials were significantly different from each other. The lowest a_w was registered for the MD1210 group because MD12 had the lowest moisture content and during encapsulation only 10% collagen hydrolysate was used. Our results were in agreement with other scientific reports. Encapsulation of casein (Rocha *et al*., 2009) and bovine liver sarcoplasmic protein hydrolysates (di

Bernardini *et al*., 2011) with maltodextrin was found to lower the a_w of free hydrolysates. Due to the lower moisture content, and lower a_w the studied samples could be considered microbiologically stable products.

Table 2. Moisture, water activity, hygroscopicity and water solubility results of powders.

Treatment	Moisture (%)	Water activity	Hygroscopicity (g/100 g powder)	Dissolution time (sec)
MD12	$4.15^{e} \pm 0.36$	$0.11^{g} \pm 0.007$	$12.13^{c} \pm 0.04$	$47.08^{f} \pm 0.71$
MD19	$4.71^{d} \pm 0.17$	$0.19^{f} \pm 0.003$	$12.66^{b} \pm 0.07$	$69.28^{cd} \pm 6.49$
CH	$8.42^{a} \pm 0.39$	$0.36^{a} \pm 0.006$	$15.01^{a} \pm 0.17$	$90.44^{a} \pm 8.95$
MD1210	$6.19^{c} \pm 0.34$	$0.23^{e} \pm 0.006$	$9.86^{f} \pm 0.29$	$82.56^{ab} \pm 0.01$
MD1220	$6.88^{b} \pm 0.24$	$0.28^{d} \pm 0.010$	$9.61^{g} \pm 0.12$	$76.85^{bc} \pm 8.03$
MD1910	$5.89^{c} \pm 0.36$	$0.30^{c} \pm 0.005$	$10.73^{d} \pm 0.04$	$57.07^{e} \pm 2.37$
MD1920	$6.35^{c} \pm 0.33$	$0.35^{b} \pm 0.004$	$10.48^{e} \pm 0.22$	$63.80^{de} \pm 7.11$

Values represent the mean ± standard deviation.

Mean values followed by different superscripts within the same column indicate a statistically significant difference between the mean values ($p<0.01$).

MD12: Maltodextrin DE10-12, MD19:Maltodextrin DE19-20, CH: Free collagen hydrolysate, MD1210: Collagen hydrolysate encapsulated with MD12 (%10 CH- %90 MD12), MD1220: Collagen hydrolysate encapsulated with MD12(%20 CH- %80 MD12), MD1910:Collagen hydrolysate encapsulated with MD19 (%10 CH- %90 MD19), MD1920: Collagen hydrolysate encapsulated with MD19 (%20 CH- %80 MD19).

Hygroscopicity

Encapsulated collagen hydrolysates presented lower hygroscopicity values in comparison to the free collagen hydrolysate and maltodextrins. The results given in Table 2 showed that collagen hydrolysates had the highest hygroscopicity. Encapsulation of collagen hydrolysates with MD 19 resulted in a significantly higher hygroscopicity than collagen hydrolysates encapsulated with MD 12. Results showed that for all studied powders, hygroscopicity was significantly different from each other.

Rodríguez-Díaz *et al.* (2014) concluded that hygroscopicity of protein hydrolysate was affected by the maltodextrin concentration. Maltodextrins were used as carrier agent in the present study and the obtained results were in agreement to those presented by Kurozawa *et al*. (2009) and Rodríguez-Díaz *et al*. (2014) for blue sharkskin hydrolysate, chicken meat protein hydrolysate and mussel meat hydrolysate, respectively. Rocha *et al*. (2009) concluded that encapsulated casein hydrolysates hygroscopicity was significantly lower than that of the free hydrolysate. Our results are similar to their conclusion.

Dissolution

The solubility of powders in distilled water was significantly different among the treatment groups ($p<0.01$), as can be observed from Table 2. Collagen hydrolysate solubility was significantly higher than other powders except MD1210 group.

According to Rocha *et al.* (2009), the solubility time of free casein hydrolysates was higher than the encapsulated hydrolysates with maltodextrin (10-12 DE and 19-20 DE). Our results showed similar solubility characteristics.

Morphology of encapsulated collagen hydrolysate

Scanning electron micrographics of free collagen hydrolysate and encapsulated collagen hydrolysates are showed in Figure 1.

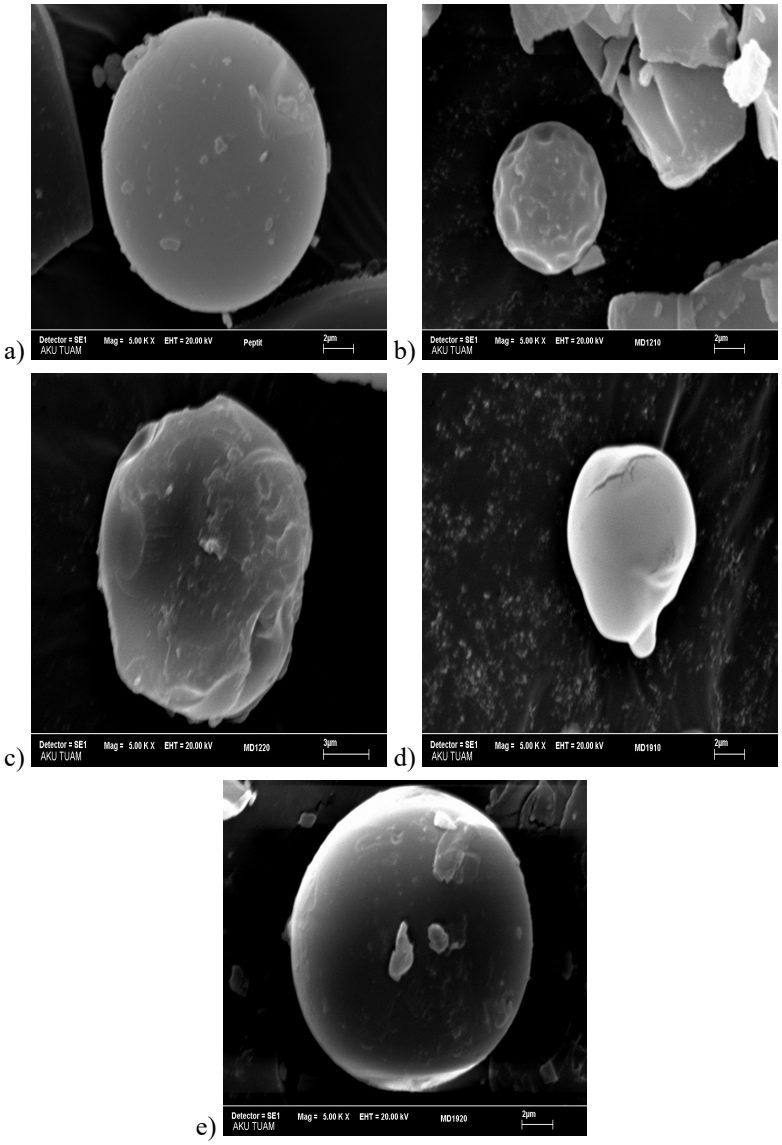

Figure 1. Scanning electron micrographs of free collagen hydrolysate and encapsulated collagen hydrolysates. a: CH (Mag. a; x5000), b: MD1210 (Mag. b; x5000), c: MD1220 (Mag. c; x5000), d: MD1910 (Mag. d; x5000), e: MD1920 (Mag. e; x5000)

It can be observed that microspheres presented different and various shapes such as smooth-spherical, shrunk-spherical and amorphous. Results were similar to those of Kurozawa *et al*. (2009) and Rodríguez-Díaz *et al*. (2014).

As can be seen in Figure 1a the pure collagen hydrolysate showed a spherical shape and smooth particular structure. Some amorphous structures were observed, most probably because of the maltodextrins presence. When collagen hydrolysate was encapsulated with the MD12 molecules the powder showed the spherical shapes but the surfaces were not smooth. This behavior can be explained by the evaporation of moisture during spray drying, causing some shrinkage which resulted in shrunk particles. Kurozawa *et al.* (2009) concluded that the formation of dents caused adverse effects on the flow characteristics of the encapsulated material. Favaro-Trindade *et al.* (2010) concluded that the occurrence of the concavities on the surface of the capsules could be associated with the rapid evaporation of the liquid droplets during the spray drying process.

Antioxidant activity

The antioxidant activity results of encapsulated collagen hydrolysates are given in Table 3. Antioxidant activity of collagen hydrolysate was significantly higher than the other powders and the lowest activity was seen in maltodextrin powders ($p<0.01$). Encapsulation of collagen hydrolysates with maltodextrin reduced the antioxidant activity. There was no significant differences found in the antioxidant activity of encapsulated collagen hydrolysates.

Table 3. Antioxidant activity (%) of aquatic solutions of maltodextrins, collagen hydrolysate and encapsulated collagen hydrolysates

Treatments	Concentrations				
	% 0.5	% 1	% 2	% 3	% 4
MD12	12.98 ± 1.29^{bcAB}	10.81 ± 2.35^{cdB}	14.88 ± 1.05^{cdA}	13.36 ± 2.30^{cAB}	14.84 ± 2.37^{dA}
MD19	8.87 ± 0.45^{cC}	9.65 ± 0.42^{dC}	11.83 ± 0.63^{dB}	13.97 ± 1.22^{cA}	12.99 ± 1.01^{dAB}
CH	26.94 ± 3.35^{aC}	36.54 ± 5.70^{aC}	53.02 ± 8.14^{aB}	68.65 ± 15.78^{aA}	78.36 ± 12.29^{aA}
MD1210	11.89 ± 0.09^{bcC}	12.75 ± 1.90^{cdC}	20.86 ± 3.85^{bcB}	27.62 ± 3.36^{bA}	29.47 ± 3.73^{cA}
MD1220	12.45 ± 1.64^{bcC}	15.51 ± 4.38^{bcC}	22.45 ± 4.96^{bB}	29.56 ± 3.36^{bA}	33.59 ± 6.47^{bcA}
MD1910	15.11 ± 4.81^{bC}	18.82 ± 3.59^{bBC}	24.30 ± 4.73^{bAB}	24.67 ± 4.97^{bAB}	$30.,51\pm7.70^{bcA}$
MD1920	14.58 ± 2.83^{bC}	18.33 ± 2.64^{bBC}	24.92 ± 6.14^{bB}	33.75 ± 4.37^{bA}	40.27 ± 7.01^{bA}

Values represent the mean ± standard deviation.

[a-c:] Mean values followed by different superscripts within the same column indicate a statistically significant difference between the mean values ($p<0.01$).

[A-C:] Mean values followed by different superscripts within the same row indicate a statistically significant difference between the mean values ($p<0.01$).

MD12: Maltodextrin DE12, MD19: Maltodextrin DE19, CH: Free collagen hydrolysate, MD1210: Collagen hydrolysate encapsulated with MD12 (%10 CH- %90 MD12), MD1220: Collagen hydrolysate encapsulated with MD12(%20 CH- %80 MD12), MD1910:Collagen hydrolysate encapsulated with MD19 (%10 CH- %90 MD19), MD1920: Collagen hydrolysate encapsulated with MD19 (%20 CH- %80 MD19).

The values presented in Table 3 show that the antioxidant activity of encapsulated hydrolysates increased with the increase of solution concentration, but no significant differences were found. The highest antioxidant activity of collagen hydrolysate was observed at 4% aqueous solution (78.36±12.29) and there was no significant difference found with 3% concentration (68.65±15.78). According to Kurozawa *et al*. (2011) the antioxidant activity of spray dried chicken meat hydrolysate was in the range of 38.7% to 59.4%. In addition to this, they reported that inlet air temperature (from 120 to 200 °C) of spray dryer positively affected antioxidant activity.

Rodríguez-Díaz *et al.* (2014) suggested that the antioxidant capacity of blue sharkskin protein hydrolysates was influenced by the presence of maltodextrin in the samples. This effect was explained by the reducing power of dextrose and by-products produced during starch hydrolysis. They found that increases in maltodextrin concentration resulted in higher antioxidant capacity, but inlet air temperature did not significantly affect antioxidant capacity because of the temperature range employed (156–190 °C).

Conclusions

Collagen hydrolysate and maltodextrin powders characteristics were significantly influenced by the encapsulation process. The encapsulation process lowered the moisture content, a_w, hygroscopicity and solubility of collagen hydrolysates by maltodextrin coating. Antioxidant activity of maltodextrin powders significantly increased and activity of free collagen hydrolysate was decreased by the encapsulation process. In this research concentration of the collagen hydrolysate in the spray dryer fed solution was selected at low concentrations. Some researchers showed that feed solution concentration could be increased, in order to improve the antioxidant activity. More studies are needed to find the encapsulation efficiency of protein hydrolysates in model systems and in food systems.

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