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Encapsulation of hydrolyzed protein of yellowfin tuna (*Thunnus albacares*) waste using maltodextrin and gum arabic, and evaluating the physicochemical properties of the resulting microcapsules

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ABSTRACT

The use of protective techniques such as encapsulation is essential to increase the efficiency of hydrolyzed proteins in the food and pharmaceutical industries. In this research, hydrolyzed protein from the viscera of yellowfin tuna (*Thunnus albacares*) was microencapsulated using three types of coatings (maltodextrin, gum arabic and maltodextrin/gum arabic in equal ratio) by the spray drying method, and the characteristics of the produced microcapsules were evaluated. The results showed that microcapsules with maltodextrin wall had the smallest particle size (4.96±0.1 µm), while samples with gum arabic wall showed the highest microencapsulation efficiency (92.76±1.88%) ($p<0.05$). Microencapsulation reduced the water activity, and the lowest amount (0.215±0.01) was observed in microcapsules with maltodextrin wall ($p<0.05$). Also, with microencapsulation, bulk density decreased and its lowest value was observed in microcapsules with gum arabic wall (0.135±0.01 g/ml) ($p<0.05$). The kinematic viscosity of microcapsules with maltodextrin wall and composite wall showed no significant difference at all investigated concentrations ($p>0.05$), and the highest viscosity was recorded in microcapsules with gum arabic wall ($p<0.05$). Microencapsulation of the hydrolyzed protein increased solubility and decreased hygroscopicity ($p<0.05$). The highest levels of these two indices (98.62±1.18% and 30.17±0.95%, respectively) were observed in microcapsules with maltodextrin wall ($p<0.05$). This research showed that microencapsulation of hydrolyzed proteins using the spray drying method and carriers based on maltodextrin and gum arabic improves some of their physicochemical properties, and the effect of the carrier type on these properties is significant.

1- Introduction

Protein hydrolysates are essentially the result of the hydrolysis of proteins present in various tissues using enzymatic and chemical methods. To reduce the production cost of this product, as well as for environmental considerations, meat waste (waste from livestock and poultry slaughterhouses, as well as waste from aquatic processing centers, etc., whose disposal causes environmental pollution and whose optimal use increases productivity) is often used as a substrate. These proteins are used in the production of beverages as stabilizers and flavorings [1], in various foods as antioxidants, antimicrobials, emulsifiers, and foaming agents [2-4], in the manufacture of bacterial culture media as a nitrogen source [5], and in human, livestock, poultry, and aquatic animal feed as a protein supplement with high digestibility. Furthermore, protein powders obtained from hydrolysis contain bioactive peptides. Bioactive peptides consist of short sequences of amino acids that, when consumed by humans, can exert beneficial physiological effects on the body, such as blood pressure modulation, immune system enhancement, anti-inflammatory, anticoagulant, anticancer, antioxidant, and antimicrobial activities, depending on various characteristics like molecular weight, amino acid profile, and degree of hydrolysis [6 and 7]. To exert these properties, bioactive peptides must remain intact and active during digestion and absorption (in the stomach and intestines) before entering the bloodstream [8]. Since these peptides may be denatured and degraded under the acidic and enzymatic conditions of the gastrointestinal tract, the use of protective methods for these peptides seems essential. Similarly, to preserve the preservative (antibacterial and antioxidant) properties of hydrolyzed proteins in foods, appropriate protective techniques must be employed. This is because every food product has specific production (involving high temperatures) and storage conditions, and the bioactive peptides used in the formulation to increase shelf life may undergo undesirable structural changes and lose their efficacy. One of the best methods available today in the food industry to overcome the aforementioned problems is the microencapsulation of hydrolyzed proteins [9 and 10], which is typically carried out using various methods such as freeze-drying [11] and spray drying [12]. Additionally, hydrolyzed proteins have two weaknesses (problems) related to moisture absorption and taste, and according to experts, microencapsulation is the solution [12 and 13]. The moisture absorption rate in hydrolyzed proteins is high, a negative characteristic that leads to physical instability of the product during storage, the occurrence of chemical reactions, microbial spoilage, and ultimately the loss of functional, antioxidant, health-promoting properties, and the nutritional value of hydrolyzed proteins [12]. Hydrolyzed proteins also possess a bitter taste, which creates difficulties in their use in food formulations [13 and 14]. The microencapsulation of hydrolyzed proteins, provided an optimal method and wall material (carrier) are selected, can overcome these weaknesses.

During the microencapsulation process, a microcapsule or nanocapsule is created around the hydrolyzed proteins, protecting the macromolecule from potential changes under adverse conditions. Moreover, the

capsules formed around the hydrolyzed proteins in this method release their contents at a controlled rate and under specific conditions [11]. One factor that affects all the chemical and physical properties of microencapsulated active compounds is the type of carrier or wall material [12, 15, and 16]. In the microencapsulation process, various carbohydrate-based (maltodextrin, starch, chitosan, dextrin, sucrose, etc.), cellulosic (carboxymethylcellulose, methylcellulose, ethylcellulose, nitrocellulose, etc.), protein-based (casein, whey protein, gelatin, etc.), lipid-based (liposomes, wax, paraffin, beeswax, diglycerides, monoglycerides, etc.), and other compounds are used to coat active compounds [11]. Maltodextrin is a carbohydrate-based coating, a starch derivative, obtained from various starch sources such as potato, corn, and wheat. Due to its high water solubility and lack of distinct color and odor, this substance is one of the most important polysaccharide materials for microencapsulation. Gum arabic is also a polysaccharide that, due to its specific physical and chemical properties, has a wide range of applications in the food industry (emulsifier, stabilizer, thickener, etc.) and has attracted significant attention as a wall material in nanoencapsulation and microencapsulation processes over the past decade. Maltodextrin and gum arabic have characteristics such as reasonable price, edibility, high solubility, and low viscosity, which have led to their widespread use as carriers or wall materials in the microencapsulation of sticky and antioxidant active compounds [11, 12, and 17]. Among the various methods for microencapsulating active compounds (spray drying, extrusion, spray cooling, freeze-drying, etc.), spray drying is the most common method used by researchers. The availability of the required equipment, optimal protection of the active compounds' structure, ideal stability of the final product, and the possibility of large-scale production are among the advantages of this method [12-17].

The yellowfin tuna^1 is one of the high-quality and popular fish for canned food production in the country. During the various product manufacturing processes, a very large volume of viscera from this fish is stored and frozen, eventually being used in the production of products such as fishmeal, silage, blood powder, etc. Another means of efficiently utilizing this waste is the production of hydrolyzed proteins or bioactive peptides [18]. In the present study, first, the viscera of the mentioned fish were hydrolyzed using the microbial enzyme Neutrase, and subsequently, the resulting hydrolysate was microencapsulated with wall materials consisting of maltodextrin, gum arabic, and a combination of both using the spray drying method. Following this, the effect of the wall material type on the physical and chemical properties of the resulting carrier microcapsules was evaluated.

2- Materials and Methods

2-1- Enzymatic Hydrolysis of Waste

To perform enzymatic hydrolysis of the waste (viscera), the microbial enzyme Neutrase (Novozyme, Denmark, 0.8 L) was used after optimizing conditions (temperature 60°C, pH=7.4, enzyme-to-substrate ratio 2% w/w, time 90 minutes). To carry out the hydrolysis process, 100 g of homogenized substrate was poured into 500 mL screw-capped glass containers,

and then 200 mL of phosphate buffer with pH=7.4 was added. Since the waste contains endogenous enzymes and the objective was to examine the effect of the commercial enzyme, before adding the enzyme, the containers with the sample were placed in a water bath (Memmert WNB 29, Germany) at 85°C for 20 minutes to inactivate the endogenous tissue enzymes. After this stage and cooling, the Neutrase enzyme was added to the mixture, which was then placed in a shaking incubator (Cold Shaker Incubator, TM 65, Iran) at 60°C. After 90 minutes of hydrolysis, the sample was placed at 95°C for 15 minutes to inactivate the Neutrase enzyme at this temperature and terminate the reaction. Upon cooling, the sample was centrifuged (D-78532 Tuttlingen, Germany) at 8000 g for 20 minutes at 10°C. The supernatant was then separated, lyophilized using a freeze dryer (Christ, Germany), and stored at -18°C until use [18].

2-2- Degree of Hydrolysis of the Process

After the hydrolysis process concluded, a 20% trichloroacetic acid solution was added at an equal ratio to a portion of the supernatant from centrifugation, and the resulting solution was centrifuged at 6700 g at 4°C for 20 minutes. The nitrogen content in the new supernatant was then measured using the Biuret method [19]. Finally, the degree of hydrolysis was calculated by dividing the nitrogen content in the 10% trichloroacetic acid solution by the total nitrogen of the sample and was reported as a percentage [18]. Bovine serum albumin was used as a standard protein to plot the standard curve and obtain the spectrophotometer equation.

2-3- Sample Preparation for Microencapsulation

To prepare the base solution for the microencapsulation process, the hydrolyzed protein was dissolved in distilled water at a ratio of 40:60 with the wall materials, namely maltodextrin, gum arabic, and a 50:50 combination of both. For this purpose, 1.2 g of hydrolyzed protein and 1.8 g of the various wall materials were fully homogenized and dissolved in 20 mL of distilled water over 30 minutes. Finally, the prepared solutions, effectively considered the feed solutions, were incubated at ambient temperature for 3 hours to allow complete hydration of the core and wall compounds [20].

2-4- Microencapsulation Process by Spray Drying Method

The spray dryer used in this research (Dorsa, Iran) had a cylindrical chamber with a conical lower section, with the cylindrical part measuring 28 cm in diameter and a total chamber height of 80 cm. This spray dryer was equipped with a volumetric peristaltic feed pump with a variable feed flow rate at a pressure of 1 bar to deliver the feed to the nozzle. The spraying process was performed by a pressure atomizer with a 2 cm diameter, operable by an air compressor. Subsequently, inlet air at a temperature of 130°C, directed co-currently with the pre-prepared feeds, was used for drying. For all three treatments, the feed flow rate was set at 3 mL/min, and the outlet air temperature during the process was 75°C. Finally, the powders collected in the glass chamber were kept in a desiccator until reaching a constant temperature to prevent moisture changes. The microencapsulated products were stored in dark glass containers in a dark environment until the time of testing [12 and 20].

2-5- Mean Particle Size, Polydispersity Index, Zeta Potential, and Microencapsulation Efficiency

The mean particle size and polydispersity index were measured by diluting the samples 10-fold with phosphate-buffered saline (PBS) using the Dynamic Light Scattering method with a Zetasizer instrument (Nanosizer, 3000, manufactured in England, Malvern Company, 90° angle, and a special cell with a 0.01 m path length) [10]. The Zetasizer instrument was also used to measure the zeta potential of the microcapsule surfaces. For this method, the microcapsules were diluted 10-fold using 50 mM phosphate buffer (pH=7.4). Zeta potential evaluation was performed at a 173° angle and a helium-neon laser wavelength of 633 nm (at a temperature of 25°C) [10]. The microencapsulation efficiency of the process was calculated using the method of Liu et al. (2015) and the following formula [21]:

Microencapsulation Efficiency = Total Protein / (Total Protein - Unloaded Protein)

2-6- Measurement of Moisture and Water Activity

To measure moisture, a container designated for the sample was first dried in an oven at 105°C for half an hour and cooled in a desiccator to room temperature. The container was weighed using a precision balance (0.0001 g accuracy), then 1 g of sample was added, and it was weighed again. Next, the container was transferred to an oven at 105°C and kept under these conditions until a constant weight was reached (approximately 3 hours). The moisture content was calculated from the weight difference of the container and sample before and after drying, relative to the sample weight, and reported as a percentage. A water activity meter (Decagon Devices, USA) was used at ambient temperature to measure the water activity of the samples [22].

2-7- Bulk Density

To measure the bulk density of the hydrolyzed protein and the carrier microcapsules, a 10 mL graduated cylinder was first weighed, a specific volume of the cylinder was filled with the treatments, and the weighing operation was repeated. Finally, the value of this index was calculated as the ratio of the sample weight to the sample volume and reported in grams per milliliter [23].

2-8- Kinematic Viscosity

The kinematic viscosity of 1, 2, 3, 4, and 5% solutions of the hydrolyzed protein and carrier microcapsules was calculated as a function of concentration using a capillary viscometer (Cannon-Fenske Viscometer, Schott Gerate, Germany) size 100. The viscometer temperature was maintained constant at 10°C using a water bath. The transit time of the sample through the viscometer was measured, and the kinematic viscosity of the samples was calculated according to the following formula and reported in centistokes. In this formula, C_0 is equivalent to 0.01536 and B is equivalent to $2.86*10^{-6}$ per °C [24].

Viscosity (centistokes) = Flow time * C_0 [1 - B (Test temperature - Filling temperature)]

2-9- Solubility

To measure the solubility of the treatments, 1 g of the samples was added to 100 mL of distilled water under stirring with a magnetic stirrer (at 700 rpm) over 4 minutes. In the next step, this solution was centrifuged (D-78532 Tuttlingen, Germany) at 3000 g for 4 minutes. After centrifugation, a specific volume of the

supernatant was separated, transferred to a Petri dish, and dried in an oven (Fan Azma Gostar, BM 55, Iran) at 105°C for 8 hours. Finally, the weight of the dried substance relative to the initial powder was used to determine the solubility limit and reported as a percentage [25].

2-10- Hygroscopicity

To investigate the hygroscopicity of the treatments, 1 g of each treatment was stored in a desiccator containing a saturated sodium chloride solution with a relative humidity of 75% for one week. The hygroscopicity of the treatments was measured and reported as the percentage of weight gain (grams of water absorbed per 100 g of powder) [26].

2-11- Statistical Analysis

The present study was conducted in a completely randomized design, and SPSS statistical software (version 22) was used to analyze the data. Data were analyzed using one-way ANOVA^2, and the evaluation of the significant difference between means was performed using Duncan's test (at a 95% confidence level). EXCEL software was used to draw the tables and figures.

3- Results

3-1- Mean Particle Size, Zeta Potential, Polydispersity Index, and Microencapsulation Efficiency

Table 1 shows some physical characteristics of the microcapsules carrying hydrolyzed protein (degree of hydrolysis 15.63±0.49%) with different wall materials. As observed in this table, the mean particle size in the three treatments varied significantly, ranging from 4.96±0.1 to 7.84±0.12 µm ($p<0.05$). Furthermore, the minimum mean particle size corresponds to the microcapsules with a maltodextrin wall ($p<0.05$). Subsequently, it was determined that the produced microcapsules possess variable, differing negative zeta potentials, from -23.39±1.1 to -11.63±0.9 mV ($p<0.05$). According to Table 1, the type of coating or wall material significantly affected the polydispersity index, and the lowest value for this index was recorded for microcapsules with a maltodextrin wall (0.329±0.07) ($p<0.05$). The microencapsulation process efficiency was also influenced by the type of wall material, and the highest level of this index corresponded to microcapsules with a gum arabic wall (92.76±1.88%) ($p<0.05$).

Table 1. Physical properties of microcapsules carrying hydrolyzed protein

Treatments	Mean particle size (µm)	Zeta potential (MV)	Particle distribution index	Encapsulation efficiency (%)
FPH encapsulated using MD	4.96±0.1^{c}	-23.39±1.1^{c}	0.329±0.07^{c}	74.85±0.52^{c}
FPH encapsulated using GA	7.84±0.12^{a}	-11.63±0.9^{a}	0.695±0.02^{a}	92.76±1.88^{a}
FPH encapsulated using MD+GA	6.17±0.05^{b}	-17.87±1^{b}	0.538±0.06^{b}	81.23±0.14^{b}

- FPH: Fish Protein Hydrolysate, MD: Maltodextrin, GA: Gum Arabic
- Different letters in each column indicate significant difference between the data ($p<0.05$).

3-2- Moisture and Water Activity of Hydrolyzed Protein and Carrier Microcapsules

Table 2 shows the moisture content and water activity of the hydrolyzed protein and its carrier microcapsules. According to this table, the microencapsulation process significantly increased the moisture content of the microcapsules (relative to the hydrolyzed protein) ($p<0.05$). Among the microcapsules, the minimum moisture content was for the protein microencapsulated with a maltodextrin wall (3.36±0.07%), and the maximum moisture content was recorded for the protein microencapsulated with a gum arabic wall (5.11±0.15%) ($p<0.05$). Microcapsules with a composite maltodextrin/gum arabic wall, with a moisture content of 4.28±0.11%, were placed intermediate. As observed in Table 2, in contrast to moisture, the microencapsulation process significantly decreased the water activity of the microcapsules ($p<0.05$), and among the microcapsules, the highest water activity was related to the microcapsules empasulated with gum arabic (0.389±0.02) ($p<0.05$). Also, the water activity in the microcapsules with a maltodextrin wall (0.215±0.01) was minimal ($p<0.05$).

Table 2. Moisture and water activity of FPH and carrier microcapsules

Treatments	Moisture (%)	Water activity
FPH	2.14±0.05^{d}	0.472±0.03^{a}
FPH encapsulated using MD	3.36±0.07^{c}	0.215±0.01^{d}
FPH encapsulated using GA	5.11±0.15^{a}	0.389±0.02^{b}
FPH encapsulated using MD+GA	4.28±0.11^{b}	0.299±0.01^{c}

- FPH: Fish Protein Hydrolysate, MD: Maltodextrin, GA: Gum Arabic
- Different letters in each column indicate significant difference between the data ($p<0.05$).

3-3- Bulk Density of Hydrolyzed Protein and Carrier Microcapsules

Figure 1 shows the bulk density of the hydrolyzed protein and carrier microcapsules with different coatings. As seen in this figure, the microencapsulation process significantly decreased the bulk density of the carrier microcapsules compared to the hydrolyzed protein (0.311±0.03 g/mL) ($p<0.05$). According to Figure 1, the value of this index was minimal in microcapsules with a gum arabic wall (0.135±0.01 g/mL) and maximal in those with a maltodextrin wall (0.212±0.02 g/mL) ($p<0.05$).

Figure 1. Bulk density of FPH and carrier microcapsules

FPH: Fish Protein Hydrolysate, MD: FPH encapsulated using maltodextrin, GA: FPH encapsulated using gum Arabic, MD+ GA: FPH encapsulated using maltodextrin and gum Arabic

Different letters indicate significant difference between the data ($p<0.05$).

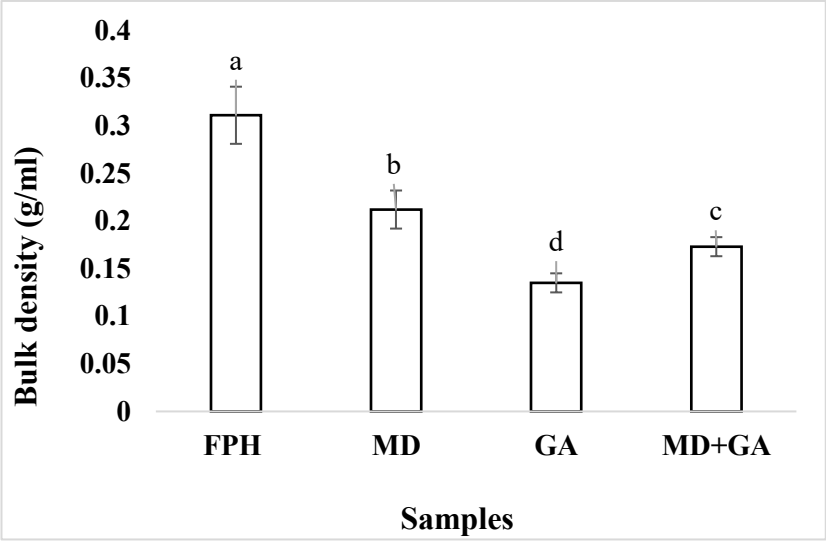

Figure 1. Bulk density of FPH and carrier microcapsules

FPH: Fish Protein Hydrolysate, MD: FPH encapsulated using maltodextrin, GA: FPH encapsulated using gum Arabic, MD+ GA: FPH encapsulated using maltodextrin and gum Arabic

Different letters indicate significant difference between the data ($p<0.05$).

3-4- Kinematic Viscosity of Hydrolyzed Protein and Carrier Microcapsules

Figure 2 presents the kinematic viscosity of the hydrolyzed protein and carrier microcapsules with different coatings at concentrations of 1, 2, 3, 4, and 5%. According to this figure, the viscosity values of all three carrier microcapsules (at all concentrations) are significantly higher than those of the hydrolyzed protein ($p<0.05$). Furthermore, the values of this index for carrier microcapsules with a maltodextrin coating and carrier microcapsules with a composite maltodextrin and gum arabic wall (at all concentrations) showed no significant difference ($p>0.05$). As seen in Figure 2, the highest kinematic viscosity among treatments at all investigated concentrations corresponds to the carrier microcapsules with a gum arabic wall ($p<0.05$). Also, with increasing concentration, the kinematic viscosity of all samples showed an increasing trend. The maximum kinematic viscosity values for the hydrolyzed protein and carrier microcapsules with maltodextrin, gum arabic, and composite walls were 2.11±0.02, 2.74±0.05, 3.41±0.09, and 2.76±0.08 centistokes, respectively.

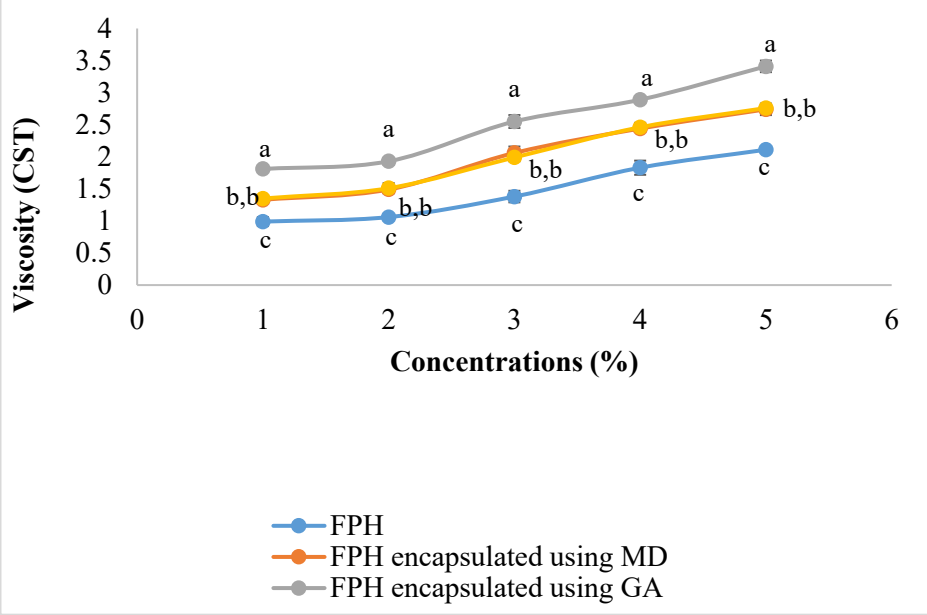

Figure 2. Viscosity of fish protein hydrolysate and carrier microcapsules

Different letters indicate significant difference between the data ($p<0.05$).

3-5- Solubility and Hygroscopicity of Hydrolyzed Protein and Carrier Microcapsules

Table 3 presents the solubility and hygroscopicity of the hydrolyzed protein and carrier microcapsules with different wall materials. According to this table, microencapsulation of the hydrolyzed protein significantly increased its solubility, and this index in all three carrier microcapsules ($p<0.05$) was higher than that of the hydrolyzed protein (85.36±1.57%) ($p<0.05$). As seen in Table 3, the highest and lowest solubility were related to microcapsules with a maltodextrin wall (98.62±1.18%) and microcapsules with a gum arabic wall (90.12±1.92%), respectively ($p<0.05$). According to this table, the hygroscopicity of all three carrier microcapsules ($p<0.05$) was significantly lower than that of the hydrolyzed protein, and the lowest level of this index was recorded for the carrier microcapsules with a composite maltodextrin and gum arabic wall (20.96±1.79%) ($p<0.05$).

Table 3. Solubility and hygroscopicity of FPH and carrier microcapsules

Treatments	Solubility (%)	Hygroscopicity (%)
FPH	85.36±1.57^{d}	41.29±1.23^{a}
FPH encapsulated using MD	98.62±1.18^{a}	30.17±0.95^{b}
FPH encapsulated using GA	90.12±1.92^{c}	24.58±1.01^{c}

FPH encapsulated using MD+GA	94.86±0.83[b]	20.96±1.79[d]

- FPH: Fish Protein Hydrolysate, MD: Maltodextrin, GA: Gum Arabic
- Different letters in each column indicate significant difference between the data ($p<0.05$).

4- Discussion

One of the most important physical properties of microencapsulated materials (microcapsules) is the mean particle size. This index is a primary characteristic and influences their stability [27]. Moreover, mean particle size significantly affects the microencapsulation process efficiency and the rate of core release from the wall material [28]. Factors influencing this index include the type of microencapsulation method, the microencapsulated active compound, the coating or wall material type, the core-to-wall ratio, etc. [29 and 30]. In the present study, the mean particle size was influenced by the type of wall material (maltodextrin, gum arabic, and their combination), and the resulting capsules ranged in size from 4.96 to 7.84 µm. In a study where the physical properties of microcapsules carrying hydrolyzed casein with similar coatings were evaluated, the mean particle size varied depending on the wall material type and was in the range of 5.16-6.31 µm [12]. Also, in that research, similar to the present study, microcapsules produced with a gum arabic wall had a larger mean particle size, which was attributed to the greater viscosity increase in feeds produced with gum arabic compared to maltodextrin, subsequently leading to the formation of larger droplets [22]. In research where casein hydrolysate was microencapsulated using soy isolate via the spray-drying method, the mean particle size of the produced microcapsules ranged from 9.18 to 11.32 [31].

The polydispersity index indicates the degree of uniformity or non-uniformity of colloidal systems. High values of this index suggest the presence of large and non-uniform particles in the colloidal system [10]. In the present study, the polydispersity index in the produced microcapsules, influenced by the coating type, varied and ranged from 0.329 to 0.695, a range that suggests approximate uniformity of the microcapsules. In the research by Mosquera et al. (2014), the polydispersity index for liposomes carrying peptides derived from fish collagen was recorded as 0.25 [32]. The polydispersity index value in the study by Baygan et al. (2022) on the microencapsulation of Ziziphora clinopodioides essential oil using a maltodextrin and gum arabic coating was reported as 0.681 [33]. In research where the pigment astaxanthin was microencapsulated with a composite coating of maltodextrin and sodium caseinate and the physical properties of nanocapsules were evaluated, the polydispersity index was reported as 0.423 [28].

One of the most important physical characteristics of microencapsulated particles is the zeta potential, which is measured to determine the electrical state

of colloidal systems. Generally, the presence of charged surface-active compounds such as ionic surfactants, polysaccharides, and proteins in emulsion systems creates different electrical charges (positive and negative) on the droplet surface. Zeta potential actually indicates the magnitude of this electrical charge and the electrostatic interactions between suspended particles. The higher the zeta potential value of the microencapsulated particles, the greater the repulsive force between the particles and the lower their tendency to aggregate, whereby the emulsion droplets repel each other. As a result of these interactions, the emulsion system becomes stable [10 and 34]. A decrease in zeta potential below critical values disrupts the electrical double layer around the particles and causes particle aggregation (flocculation). In general, particles with a zeta potential above +30 or below -30 mV are at the highest level of stability [35]. Depending on the wall material type, the microcapsules produced in the present study possessed varying zeta potentials in the range of -11.63 to -23.39 mV, which, according to the above information, are favorable values for the stability of emulsion systems. The negative zeta potential of the microcapsules can be attributed to the anionic nature of gum arabic [36].

Microencapsulation efficiency is one of the most important stability indicators for encapsulated compounds, as it demonstrates the ability of microcapsules (or nanocapsules) to prevent the leakage of the inner core. The process efficiency in the present study was significantly influenced by the wall material type, varying from 74.85 to 92.76%. In a study where peptides derived from the enzymatic hydrolysis of trout skin gelatin were encapsulated using nanoliposomes and chitosan concentrations of 0.2 to 1%, the microencapsulation efficiency of the treatments ranged from 46.1 to 80.2% [37]. The microencapsulation efficiency for the production of nanocapsules carrying the pigment phycocyanin extracted from the microalgae Spirulina using a composite coating of maltodextrin-sodium caseinate was reported as 73.41% [11]. In a study using maltodextrin, gum arabic, and inulin for the microencapsulation of cardamom essential oil, the microencapsulation efficiency varied from 23 to 80% depending on the type and percentage of the wall material [38]. In that study, the highest microencapsulation efficiency belonged to a wall composition of gum arabic, maltodextrin, and inulin at ratios of 8:14:78, respectively, while the lowest level of this index was for the treatment of gum arabic, maltodextrin, and inulin at ratios of 36:50:14, respectively [38].

One of the most important causes of spoilage in food, agricultural products, and animal products is the free water present within them.

Moisture in food consists of two distinct parts: a portion that is bound to various materials, known as bound or absorbed water, and another portion that exists in a free state, which is unbound and is precisely the fraction related to water activity. Water activity is a highly important characteristic of foods, possessing extraordinary significance from the perspectives of maintaining food safety, shelf life, taste, nutritional value, and ultimately economic aspects. This index is defined as the ratio of the vapor

pressure of the product's water to the vapor pressure of pure water after reaching moisture equilibrium at the same temperature. At low water activity (less than 0.6), the growth and reproduction of most microorganisms are often halted due to the lack of synthesis of essential functional enzymes and proteins [39]. Therefore, controlling water activity is a very effective method to prevent the proliferation of spoilage and pathogenic species in food. In the present study, the moisture content and water activity of the microcapsules ranged from 3.36 to 5.11% and 0.215 to 0.389, respectively. Considering the points mentioned above, it can be claimed that the products produced in the current research possess standard microbial stability. It should be noted that these two indices in the produced microcapsules were significantly influenced by the type, composition, and nature of the wall material. In a study where mountain tea extract was microencapsulated with maltodextrin and gum arabic walls, microcapsules with a maltodextrin wall had the lowest, and those with a gum arabic wall had the highest moisture content [40], a finding consistent with the results of the present study. The reason for this result in that research was reported as the higher water-holding capacity of hydrocolloids (gum arabic) compared to starch derivatives (maltodextrin) [40]. Similarly, in research where hydrolyzed casein was microencapsulated using gum arabic, maltodextrin, and a composite wall of the two (spray drying method), the minimum moisture and water activity (3.19±0.21 and 0.273±0.01, respectively) were reported in carrier microcapsules with a maltodextrin wall, and the maximum for these indices (4.27±0.14 and 0.305±0.01, respectively) was in carrier microcapsules with a gum arabic wall [12], a finding also consistent with the present study. In the current research, the microencapsulation process increased the moisture content of the powders, which aligns with the results of Fávaro-Trindade et al. (2010) and Akbarbaglu et al. (2020) [12 and 14].

The bulk density of micro and nanocapsules depends heavily on the inter-particle attractive forces, particle size, number of contact points between them, particle shape and size distribution, moisture content, chemical composition, amount of air trapped between particles, and the nature of the coating or wall material [41]. This index indicates the behavior of the product in a dry mix and is important in the packaging of hydrolyzed protein powder and its carrier microcapsules [42]. In the present study, the value of this index in microcapsules, depending on the coating type, varied from 0.135 to 0.212 g/mL, with the minimum bulk density recorded in microcapsules with a gum arabic wall. This finding is consistent with the research by Akbarbaglu et al. (2020), and the reason was stated to be the lower viscosity of gum arabic compared to maltodextrin [12]. Increased viscosity leads to the formation of larger droplets sprayed into the dryer chamber and ultimately the formation of larger particles. Moreover, the higher ability and capacity of gum arabic to form a film around the particles and trap a larger volume of air within the particles is another contributing factor to this result [43]. In the conducted research, the bulk density of the hydrolyzed protein (with a degree of hydrolysis of 15.63±0.49%) was measured as 0.311

g/mL. In the study by Shokrpour Roodbari et al. (2015), the bulk density of hydrolyzed proteins obtained from the meat of the white cheek shark^3 using the Alcalase enzyme at three degrees of hydrolysis (1.91, 2.25, and 2.53%) was reported as 0.1547, 0.19966, and 0.254 g/mL, respectively [44]. In another study where the meat of the same shark was hydrolyzed with Alcalase for 120 minutes and the supernatant was then spray-dried, a bulk density of 0.11346 g/mL was reported for the hydrolyzed protein [42]. In the research by Sathivel et al. (2009), the bulk density of spray-dried catfish hydrolyzed protein was reported as 0.340 g/mL [45]. The bulk density of hydrolyzed proteins depends on various factors, including the degree of hydrolysis, the type of enzyme or hydrolysis method, the drying method, molecular weight, etc., among which the degree of hydrolysis and drying method are of particular importance. Typically, as the degree of hydrolysis increases, resulting in a decrease in molecular weight, the bulk density of hydrolyzed proteins increases. This is likely due to the creation of a powdery substance with smaller particle size and thus less porosity in solutions with smaller molecular weights [44]. Additionally, freeze-drying the supernatant from the hydrolysis reaction typically yields a powder with high porosity and low bulk density [41].

Kinematic viscosity is a very important characteristic of food products, significantly affecting the texture of liquids (in the beverage industry) and processes such as pumping, extrusion, and drying [46]. In this study, the viscosity of the hydrolyzed protein solution was very low, and this index increased substantially in solutions containing microcapsules compared to the protein solution, but overall did not exceed a value of 3.41±0.09 centistokes (carrier microcapsules with a gum arabic wall at 5% concentration). This low viscosity of the produced products enables their use in the formulation of products requiring functional ingredients with weak viscosity and gel properties, such as yogurt and ready-to-eat soups [12]. It should be noted that in the present study, the kinematic viscosity of the hydrolyzed protein and its carrier microcapsules was influenced by concentration and increased with increasing concentration. This finding aligns with the research by Shokrpour Roodbari et al. (2015) and Alinejad et al. (2016). However, besides concentration, the degree of hydrolysis is another influential factor on the kinematic viscosity of hydrolyzed proteins, which was not investigated in this study. Researchers believe that the kinematic viscosity of proteins with a lower degree of hydrolysis is higher due to the larger peptide chains and the greater likelihood of entanglement and interaction among peptides, resulting in a stronger matrix. As the degree of protein hydrolysis increases, the length and size of the peptide chains decrease, and viscosity also decreases [44].

The most important functional property of hydrolyzed proteins, which affects all their functional properties, is solubility [47]. Hydrophobic and ionic interactions are the main factors influencing protein solubility. Hydrophobic interaction promotes protein-protein association, thereby reducing solubility. In contrast, ionic interaction promotes protein-water association and increases protein

solubility. Enzymatic hydrolysis of proteins, up to a certain point, progressively breaks peptide bonds, forms smaller peptides, and consequently increases solubility. Additionally, the ionization and carboxyl groups of amino acids increase the hydrophilicity of hydrolyzed proteins [47 and 48]. The solubility of hydrolyzed proteins varies depending on the enzyme used, degree of hydrolysis, ratio of hydrophilic to hydrophobic amino acids, and pH. The enzyme type can influence this index through the degree of hydrolysis and the production of peptides with different ratios of hydrophilic and hydrophobic amino acids. pH, by causing changes in the number of charged groups on the protein surface, also significantly affects water absorption characteristics and subsequently solubility [47 and 49]. When hydrolyzed proteins are microencapsulated with different coatings, in addition to the factors mentioned above, other factors such as surface characteristics, particle microstructure, microencapsulation method, drying conditions (temperature used, inlet air flow rate, atomizer pressure and speed), feed type (solid concentration), wall material type, etc., also influence solubility [12]. In the present study, microencapsulating the hydrolyzed protein with three types of coatings significantly increased solubility, and the maximum value for this index corresponded to carrier microcapsules with a maltodextrin wall. Nadeem et al. (2011), Wang and Zhou (2015), and Akbarbaglu et al. (2020), who microencapsulated active compounds using gum arabic and maltodextrin via the spray-drying method, reported, similar to the present study, higher solubility for microcapsules with a maltodextrin wall compared to those with a gum arabic wall [12, 40, and 50].

One of the challenges of using hydrolyzed proteins in food formulations, as well as in edible films [51], is their high level of hygroscopicity. This index significantly affects the storability, stability, and shelf life of powders dried by various methods under adverse and unfavorable packaging and storage conditions [12]. In this study, after microencapsulating the hydrolyzed protein, the degree of hygroscopicity significantly decreased. The value of this index, under the influence of the wall material type, differed among the three types of microcapsules, and its minimum was recorded for microcapsules with a composite maltodextrin and gum arabic wall (20.96±1.79%), a finding consistent with the research by Akbarbaglu et al. (2020) [12]. The reason for this phenomenon is attributed to the high capability of gum arabic to form a film with low and negligible hygroscopicity around the particles [22]. In a study evaluating the effect of microencapsulation of whey protein hydrolysates with a maltodextrin coating and a composite coating of maltodextrin and beta-cyclodextrin on the physicochemical properties of the hydrolysate, a significant reduction in the hygroscopicity index in the microcapsules was reported, to the extent that this index decreased from 64.31% in the hydrolysate to 43.09% and 36.92% in the microcapsules, respectively [13]. Kurozawa et al. (2009) microencapsulated chicken meat hydrolyzed protein using maltodextrin and gum arabic. The findings of that research indicated a significant decrease in the hygroscopicity levels of

the microcapsules (15.9% and 21.2%, respectively) compared to the hydrolyzed protein (40.9%), consistent with the results of the present study [52].

5- Conclusion

According to the results of the present study, the microencapsulation of hydrolyzed proteins utilizing the spray-drying method and carriers based on maltodextrin and gum arabic leads to significant improvement and enhancement in a wide range of their physicochemical properties, such as moisture, water activity, hygroscopicity, solubility, density, and viscosity. It should be noted that these mentioned properties are significantly influenced by the nature of the coating or carrier (wall material type). In other words, it cannot be claimed that microcapsules with a specific coating demonstrate the most desirable or undesirable performance across all physical and chemical properties. Therefore, selecting an optimal and suitable coating is crucial and depends on the ultimate goal of the carrier microcapsules' application in various food formulations. This informed choice guarantees the achievement of desired characteristics and optimal performance of the microcapsules in the final product.

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Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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انکپسولاسیون پروتئین هیدرولیزشده ضایعات ماهی تن زردباله (*Thunnus albacares*) با استفاده از مالتودکسترین و صمغ عربی و ارزیابی خواص فیزیکوشیمیایی میکروکپسول‌های حاصل

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چکیده

استفاده از تکنیک‌های حفاظتی مانند انکپسولاسیون برای افزایش کارایی پروتئین‌های هیدرولیزشده در صنایع غذایی و دارویی، ضروری است. در این تحقیق، پروتئین هیدرولیز شده حاصل از امعاء و احشاء ماهی تن زرد باله (*Thunnus albacares*) توسط سه نوع پوشش (مالتودکسترین، صمغ عربی و مالتودکسترین/صمغ عربی با نسبت برابر) با استفاده از روش خشک‌کردن پاششی ریزپوشانی شد و خصوصیات میکروکپسول‌های حامل مورد ارزیابی قرار گرفت. نتایج نشان داد که میکروکپسول‌های با دیواره مالتودکسترین کوچک‌ترین اندازه ذرات را داشتند (۹۶/۱ ۴/۰±µm)، در حالی که نمونه‌های با دیواره صمغ عربی بالاترین بازده ریزپوشانی (۹۲/۷۶±۱/۸۸٪) را نشان دادند (p<۰/۰۵). با انجام میکروکپسولاسیون، فعالیت آبی کاهش یافت و کمترین مقدار آن (۰/۲۱۵±۰/۰۱) در میکروکپسول‌های دارای دیواره مالتودکسترین مشاهده شد (p<۰/۰۵). همچنین با میکروکپسولاسیون، دانسیته توده‌ای کاهش یافت و کمترین مقدار آن در میکروکپسول‌های دارای دیواره صمغ عربی (۱۳۵/۰۱±۰/۰g/ml) مشاهده شد (p<۰/۰۵). میزان ویسکوزیته سینماتیک میکروکپسول‌های دارای دیواره مالتودکسترین و دیواره ترکیبی در تمامی غلظت‌های مورد بررسی فاقد اختلاف معنی‌دار بود (p>۰/۰۵) و بیشترین میزان ویسکوزیته در میکروکپسول-های دارای دیواره صمغ عربی ثبت شد (p<۰/۰۵). میکروکپسولاسیون پروتئین هیدرولیزشده موجب افزایش انحلال‌پذیری و کاهش جاذب‌الرطوبه‌بودن گردید (p<۰/۰۵). بیشترین میزان این دو شاخص (به ترتیب ۹۸/۱±۶۲/۱۸٪ و ۳۰/۱۷±۰/۹۵٪) در میکروکپسول‌های دارای دیواره مالتودکسترین مشاهده شدند (p<۰/۰۵). این تحقیق نشان داد که ریزپوشانی پروتئین‌های هیدرولیزشده با استفاده از روش خشک‌کردن پاششی و حامل‌های مبتنی بر مالتودکسترین و صمغ عربی، موجب بهبود برخی از خصوصیات فیزیکوشیمیایی آن‌ها می‌شود و اثر نوع حامل بر این خواص معنی‌دار است.