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Crustacean chymotrypsin: estate of art

Quimotripsina en crustáceos: estado del arte

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ABSTRACT

Chymotrypsin in crustaceans is an enzyme whose importance has not been fully recognized over time, despite being a fundamental component in the digestion of proteins in their food. Enzymes are basic catalytic components of cellular metabolism; of great diversity classified according to the function they exert (hydrolysis, oxidation-reduction, synthesis, isomerization, among others). Chymotrypsin belongs to hydrolyses, which catalyzes the breaking of peptide bonds adjacent to the carboxyl groups of the aromatic amino acids tryptophan, tyrosine and phenylalanine. Since the 1980s there have been few studies related to this enzyme in crustaceans, concentrating a third of these (13 studies) in the last five years. Therefore, the paper aims to present a review about the existing information on these enzymes in crustaceans from a general perspective, helping to identify areas of opportunity to expand the knowledge of their function and properties in these invertebrates, as well such as the ecological, ethological, management, feeding and nutrition implications of aquaculture.

Keywords: Enzymatic activity, digestive physiology, protease, crustaceans.

RESUMEN

La quimotripsina en crustáceos es una enzima cuya importancia no ha sido del todo reconocida a lo largo del tiempo, a pesar de ser un componente fundamental en la digestión de las proteínas de sus alimentos. Las enzimas son componentes catalíticos básicos del metabolismo celular, de gran diversidad clasificadas acorde a la función que ejercen (hidrolisis, oxido-reducción, síntesis, isomerización, entre otros). La quimotripsina pertenece a las hidrolasas, que cataliza la ruptura de enlaces peptídicos adyacentes a los grupos carboxilo de los aminoácidos aromáticos triptófano, tirosina y fenilalanina. Desde la década de 1980 se han realizado pocos estudios relacionados con esta enzima en crustáceos, concentrando un tercio de estos (13 estudios) en los últimos cinco años. Por lo anterior, el presente artículo tiene como objetivo el presentar una revisión acerca de la información existente de estas enzimas en crustáceos desde una perspectiva general, contribuyendo a identificar las áreas de oportunidad para ampliar el conocimiento de su función y propiedades en estos invertebrados, así como las implicaciones ecológicas, etológicas, de manejo, alimentación y nutrición acuícola.

Palabras clave: actividad enzimática, hidrólisis, fisiología digestiva, proteasa, crustáceos.

INTRODUCTION

Chymotrypsin is an enzyme that belongs to the hydrolases, which break covalent bonds by incorporating water between peptide bonds. It has a serine residue in the active site, this being the reason that classifies it as a serine endoprotease (Di Cera, 2009; Navarrete del Toro & García Carreño, 2019). This enzyme catalyzes the hydrolysis of peptide bonds adjacent to the carboxyl groups of the aromatic amino acids tryptophan, tyrosine and phenylalanine ((Zwilling & Neurath, 1981; Zhou *et al*., 2011), although it can also hydrolyze peptide bonds on the side of other large hydrophobic residues, such as methionine and leucine (Zwilling & Neurath, 1981; Zhou *et al*., 2011; Navarrete del Toro & García Carreño, 2019) to reduce the size of polypeptide chains and allow the action of exoproteases (McDonald, 1985; Barrett, 1994).

Studies on this biological catalyst have focused mainly on fish, mainly to seek alternatives for obtaining enzymes from waste materials generated by the fishing industry (Zhou *et al*., 2011). However, studies in crustaceans are limited when compared to those reported in fish and terrestrial organisms (Balti *et al*., 2012; Navarrete del Toro & García-Carreño, 2019), only approximately 36 investigations between 1980 to date that point to this enzyme and almost a third of them were conducted in the last five years (13 studies), this may be due to the fact that studies have been channeled and focused on trypsin, since this can represent up to 60% of the digestive proteolytic activity (Cruz-Suarez, 1996; Muhlia-Almazán *et al*., 2008).

Early research on the detection of this enzyme did not consider it relevant (Lee *et al*., 1984; Glass & Stark, 1994); however, this thinking has been changing as the presence of chymotrypsin has been found in several studies on crustaceans (Tsai *et al*., 1986; Tsai *et al*., 1991; Von Elert *et al*., 2004; Navarrete del Toro *et al*., 2015; Torres Ochoa, 2020) (Table 1).

Enzymatic characterization in crustaceans

In most of the research carried out in crustaceans, chymotrypsin is not specifically evaluated, but is part of a battery of digestive enzyme study protocols. Likewise, most of the characterizations have been made in decapod crustaceans such as the tiger shrimp *Penaeus monodon*, the Malaysian shrimp *Macrobrachium rosenbergii*, the white shrimp *Penaeus vannamei*, the common Caribbean lobster, *Panulirus argus*, the red lobster *Panulirus interruptus* and the brown shrimp *Penaeus californiensis* (Tsai *et al*., 1986; Tsai *et al*., 1991; Hernández-Cortes *et al*., 1997; Perera *et al*., 2008; Bibo-Verdugo *et al*., 2015; Navarrete del Toro *et al*., 2015; Torres-Ochoa, 2020); as they are exploited species or with potential to be exploited in aquaculture cultures (Espinosa-Chaurand *et al*., 2019; Torres-Ochoa *et al*., 2020).

Table 1. Studies in crustaceans dealing with the presence of chymotrypsin, period 1980-2020

	Species	Common name	Characterization	Study
1.	*Artemesia longinaris*	Long-faced shrimp	No	Fernández-Gimenez *et al.*, 2002.
2.	*Artemia salina*	Artemia	Yes	Serrano, 2015
3.	*Caridina cantonensis*	Bee shrimp, glass shrimp	No	Kattakdad *et al.*, 2018
4.	*Daphnia magna*	Water flea, daphnia	Yes	VonElert *et al.*, 2004
5.	*Homarus americanus*	American lobster	No	Brockerhoff *et al.*, 1970
6.	*Lithodes santollla*	Patagonian spider crab	No	Bañuelos-Vargas *et al.*, 2018
7.	*Macrobrachium amazonicum*	River shrimp, Amazonian shrimp	No	Da Silva *et al.*, 2014
8.	*Macrobrachium australiense*	River shrimp, shrimp, prawn	No	Bonorino & Anderson, 2009
9.	*Macrobrachium carcinus*	Acamaya	No	Manriquez-Santos *et al.*, 2018
10.	*Macrobrachium rosenbergii*	Malaysian shrimp	No	Tsai *et al.*, 1986
11.	*Macrobrachium tenellum*	Shrimp, pigua	No	Espinosa-Chaurand *et al.*, 2017; Montoya, 2018; Espinosa-Chaurand *et al.*, 2019.
12.	*Metacarcinus edwardsii*	Marmola crab	No	Bañuelos-Vargas *et al.*, 2018
13.	*Metapenaeus bennetae*	Shrimp	No	Bonorino & Anderson, 2009
14.	*Metapenaeus monoceros*	Spotted shrimp	No	Tsai *et al.*, 1986
15.	*Penaeus californiensis*	Brown shrimp	Yes	Navarrete del Toro *et al.*, 2015; Torres-Ochoa, 2020
16.	*Penaeus chinensis*	Meaty shrimp	No	Shi*et al.*, 2008; Xue *et al.*, 2013
17.	*Penaeus esculentus*	Brown tiger shrimp	No	Bonorino & Anderson, 2009
18.	*Penaeus indicus*	Indian shrimp	Yes	Omondi, 2005
19.	*Penaeus japonicus*	Kuruma shrimp	No	Tsai *et al.*, 1986
20.	*Penaeus monodon*	Tiger shrimp	Yes	Tsai *et al.*, 1986; Tsai *et al.*, 1991; Jiang *et al.*, 1991
21.	*Penaeus notialis*	Southern pink shrimp	No	Fernández *et al.*, 1997
22.	*Penaeus paulensis*	Sao Paulo shrimp	Yes	Souza *et al.*, 2009
23.	*Penaeus penicillatus*	Red tail shrimp	No	Tsai *et al.*, 1991
24.	*Penaeus plebejus*	Shrimp	No	Bonorino & Anderson, 2009
25.	*Penaeus schmitti*	Southern white shrimp, white caribbean shrimp	No	Lemos *et al.*, 2002
26.	*Penaeus stylirostris*	Blue shrimp	Yes	Navarrete del Toro *et al.*, 2011
27.	*Penaeus subtilis*	Southern brown shrimp	Yes	Buarque *et al.*, 2010
28.	*Penaeus vannamei*	White shrimp, Pacific white shrimp	Yes	Van Wormhoudt *et al.*, 1992; Hernández-Cortes *et al.*, 1997; Navarrete del Toro *et al.*, 2011
29.	*Palaemon serratus*	Serrated shrimp	Yes	Trellu & Ceccaldi, 1980
30.	*Panulirus argus*	Common Caribbean lobster	Yes	Perera *et al.*, 2008
31.	*Panulirus homarus*	Spiny lobster	No	Gora *et al.*, 2018
32.	*Panulirus interruptus*	Red lobster	Yes	Bibo-Verdugo *et al.*, 2015
33.	*Pleoticus muelleri*	Argentine shrimp	No	Fernández-Gimenez *et al.*, 2001
34.	*Portunus pellagicus*	Blue crab	No	Bonorino & Anderson, 2009
35.	*Scylla paramamosain*	Mud crab	No	DuyKhoa *et al.*, 2019
36.	*Scylla serrata*	Mangrove crab	No	Bonorino & Anderson, 2009; Serrano, 2015

In enzymatic characterization studies for the identification of chymotrypsin in crustaceans, parameters of optimum temperature, thermo stability, optimum pH, pH stability, isoelectric point and ion effect have been evaluated, finding that the ranges of activity can vary depending on the species between 30 to 60 °C in the optimum, between 0°C and 75 °C for its thermo stability, between 7 and 10 in the optimum pH and pH stability between 3 and 12 points (Table 2). In the particular case of isoelectric point

analysis in chymotrypsin, studies mention the detection of its isoforms Navarrete del Toro *et al*., 2015), for example, Hernández-Cortes *et al*. (1997) identified a single isoelectric point in *P. vannamei*, as did Navarrete del Toro *et al*. (2015) in *P. californiensis,* which determines the presence of a single form of chymotrypsin; while, Tsai *et al*. (1991) reported the presence of two isoelectric points in *P. monodon* with values of 3.0 and 3.2, indicating two isoforms. In most of the species studied, two isoforms of the enzyme have been found (Navarrete del Toro *et al*., 2011); with exceptions such as *Panulirus interruptus*, which presented five isoforms (Celis, 2003).

Table 2. Results of chymotrypsin characterization in different crustacean species, period 1980-2020

	Species	Optimal pH	Optimal temp	pH stability	Thermo-stability	Study
1.	*Artemia salina*	7.5	30°C	6.0-8.5	0-55°C	Serrano, 2015
2.	*Daphnia magna*	7	--	3.0-12.0	--	VonElert *et al*., 2004
3.	*Penaeus californiensis*	10	50°C	3.0-10.0	30-60°C	Navarrete del Toro *et al*., 2015; Torres-Ochoa, 2020
4.	*Penaeus indicus*	8	--	--	--	Omondi, 2005
5.	*Penaeus japonicus*	7	--	5.0-9.0	--	Tsai *et al*., 1986
6.	*Penaeus. monodon*	7	40°C	4.0-10.0	25-70°C	Tsai *et al*., 1986
7.	*Penaeus. paulensis*	8	55°C	---	25-75°C	Souza *et al*., 2009
8.	*Penaeus stilyrostris*	7	60°C	4.0-11.0	10-70°C	Navarrete del Toro *et al*., 2011
9.	*Penaeus subtilis*	8	55°C	---	25-65°C	Buarque *et al*., 2010
10.	*Penaeus vannamei*	8	60°C	4.0-11.0	10-70°C	Navarrete del Toro *et al*., 2011
11.	*Palaemon serratus*	---	30°C	---	14-30°C	Trellu & Ceccaldi, 1980
12.	*Panulirus argus*	7.5	50°C	2.0-12.0	30-60°C	Perera *et al*., 2008
13.	*Panulirus interruptus*	8	55°C	3.0-12.0	25-65°C	Bibo-Verdugo *et al*., 2015
14.	*Scylla serrata*	8	30°C	6.5-8.5	0-45°C	Serrano, 2015

Determination techniques

The techniques used for the determination of chymotrypsin-like activity in crustaceans are supported by different tools. It is possible to carry it out by using specific synthetic substrates, where techniques described by Hummel (1959), Erlanger & Edel (1964) and Del Mar *et al*. (1979) are used; on the other hand, enzymatic inhibitors can be used, such as those employed by Tsai *et al*. (1986), Vega-Villasante *et al*. (1995) and Navarrete del Toro *et al*. (2015).

It is possible that the simplest way to determine the presence of chymotrypsin in organisms is by using specific substrates for the enzyme, such as labenzoyl-tyrosine ethyl-ester (BTEE) proposed in the research of Hummel (1959); or 2-Nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC), mentioned by Erlanger & Edel (1964); or Succinyl-Ala-Ala-Pro-Phe-P-nitroanilide (SAAPNA or SAAPFNA) which was proposed

by Del Mar *et al*. (1979). The principle by which the above techniques work is the detection of changes in the interaction between the substrate and the enzyme, which promote the release of the dye molecule that can be read by colorimetry in spectrophotometry (Hummel, 1959; Erlanger & Edel, 1964; Del Mar *et al*., 1979).

With Erlanger & Edel (1964) method, it has been reported that no chymotrypsin-like activity was detected with *P. vannamei* and *P. setiferus* (Lee *et al*., 1984); while when using BTEE (Hummel, 1959) as substrate, contradictory results have been reported since in species such as *H. gammarus* no chymotrypsin enzymatic activity was found (Glass & Stark, 1994), but in *P. bennetae*, *P. plebejus*, *M. austreliense* and *S. serrata* activity was detected Bonorino & Anderson, 2009). SAAPNA use as a substrate (Del Mar *et al*., 1979) is one of the most widely used to date for its detection, since it is a very sensitive substrate to chymotrypsin activity (Tsai *et al*., 1986), showing its activity in all studies where it has been used, such as the investigations carried out in *P. vannamei* (Le Moullac *et al*., 1996; Hernández-Cortes *et al*., 1997), *P. muelleri* (Fernández-Gimenez *et al*., 2001), D. magna (Von Elert *et al*., 2004), *P. interruptus* (Celis-Guerrero *et al*., 2004), *P. subtilis* (Souza *et al*., 2009) and *P. californiensis* (Navarrete del Toro *et al*., 2015; Torres-Ochoa, 2020).

Just as it is desired to observe the activity of chymotrypsin, its inhibition is also studied, which is done at the same time as its characterization or presence through specific substrates, and which are intended to make evident the total or partial inhibition of enzymatic hydrolysis in the presence of these substances (García-Carreño, 1992). The most widely used enzymatic inhibitors for chymotrypsin are chymostatin (Tsai *et al*., 1986); tosyl-phenylalanine chloromethyl ketone (TPCK) (Tsai *et al*., 1986; Hernández-Cortes *et al*., 1997; Omondi, 2005; Navarrete del Toro *et al*., 2015; Manriquez-Santos *et al*., 2018); carbobenzoxy-phenylalanine chloromethyl ketone (ZPCK) (Lemos *et al*., 2000); phenylmethylsulfonyl fluoride (PMSF) (Tsai *et al*., 1986; Hernández-Cortes *et al*., 1997; Omondi, 2005; Bibo-Verdugo *et al*., 2015; Manriquez-Santos *et al*., 2018); soybean-derived trypsin inhibitor (SBTI) (Tsai *et al*., 1986; Manriquez-Santos *et al*., 2018); and Z-L-allaniglycyl-L-phenylalanine-chloroketone (ZAGPCK) (Tsai *et al*., 1986; Chen *et al*., 1991).

Knowing the structure, function, physicochemical characteristics of activity thresholds, reaction substrates, as well as inhibition, can help us to adequately characterize this enzyme, which leads us to think about the formulation of functional foods for the species of interest, possible resources with which these organisms feed, or even its potential as a biomolecule in biotechnological processes for the treatment and use of different substrates. Thus, from basic physiological knowledge to industrial application, understanding this type of enzyme will provide an advance in its study and will denote avenues of research and application.

Function and action of chymotrypsin

Although chymotrypsin-type enzyme activity has been recorded since the 1980s with the studies of Galgani *et al*. (1984), it has always been considered as an enzyme with low catalytic activity in crustaceans (García-Carreño *et al*., 1994; Cruz-Suarez, 1996); its presence in these organisms has even been questioned, as in the research of Lee *et al*. (1984), where they specified that chymotrypsin-type enzyme activity was nonexistent in *P. monodon* juveniles, similar to what was reported in other crustacean species such as *H. americanus* (Brockerhoff *et al*., 1970), *Lithodesa esquispinus* and *Paralithodescam tschaticus* (Galgani & Nagayama, 1987), where the same conclusion is reached. Possibly because the activity of this enzyme was not detected in the first investigations, there was a decrease in interest in studies related to it (García-Carreño *et al*., 1994; Von Elert *et al*., 2004; Omondi, 2005), which caused a delay in the body of information on this enzyme, compared to trypsin (Muhlia-Almazán *et al*., 2008). Due to the above, an information gap has been generated on mechanisms of action of chemotrypsin in digestion processes in crustaceans (Bibo-Verdugo *et al*., 2015; Navarrete del Toro & García Carreño, 2019).

This lack of information becomes more evident when comparing the available information of chymotrypsin with respect to that of trypsin; which has been the most studied digestive enzyme in crustaceans and it is considered responsible for approximately 60% of the digestive proteolytic activity in these organisms (Vega-Villasante *et al*., 1995; Albuquerque-Cavalcanti *et al*., 2001; Carrillo-Farnés *et al*., 2007; Muhlia- Almazan *et al*., 2008). The importance given to trypsin is mainly due to the fact that it was the first protease detected and characterized in crustaceans (Muhlia-Almazán *et al*., 2008; Sainz-Hernandez & Cordova-Murueta, 2009). For its detection, the techniques of Erlanger *et al*. (1961), which uses benzoyl-DL-arginine 4-nitroanilide chlorohydrate (BAPNA); and Hummel (1959), where tosyl-arginine-methylester hydrochloride (TAME) is used, are commonly used. Both methods were developed for the detection of trypsin in other organisms; however, their use has been ratified for the detection of chemotrypsin in crustaceans (Brockerhoff *et al*., 1970; Sainz *et al*., 2004; Von Elert *et al*., 2004; Omondi, 2005; Navarrete del Toro *et al*., 2011).

It was not until the development of other substrates, such as SAAPNA, that it was possible to detect the presence of the hydrolytic activity of chymotrypsin (García-Carreño *et al*., 1994). Despite this, research on this enzyme in crustaceans continued to focus on the detection and analysis of trypsin, leaving aside the analysis of chymotrypsin, which was considered a complementary enzyme with low activity (Muhlia-Almazán *et al*., 2008; Sainz-Hernández & Córdova-Murueta, 2009).

The probable reason why the enzyme does not hydrolyze the BTEE substrate is the one exposed by Tsai *et al*. (1986), where it is mentioned that although BTEE presents the amino acids on which chymotrypsin acts, it lacks other amino acids that interact in a

secondary way with the enzyme so that it can carry out its function, this is also mentioned by Van Wormhoudt *et al*. (1992), when they report that crustacean chymotrypsin is more reactive to natural substrates than to synthetic ones, due to the length of the polypeptide chains alone.

This has led to consider trypsin as the protease responsible for the highest percentage of protein hydrolysis (Carrillo-Farnés *et al*., 2007; Muhlia-Almazán *et al*., 2008). However, subsequent studies consider that the enzyme with the highest hydrolysis capacity is chymotrypsin (Tsai *et al*., 1986; Buarque *et al*., 2010; Navarrete del Toro *et al*., 2011; Gora *et al*., 2018), and this enzyme has not been given greater importance. For this reason, information gaps are observed and new questions arise around the activity of this enzyme, because the lack of information forces researchers to use enzymatic activity models of other groups of organisms (Navarrete del Toro & García-Carreño, 2019), thus establishing an area of opportunity to generate new and basic knowledge that affects the studies of the digestive physiology of this group of organisms so important as a source of food for humans.

Among the new aspects of study on chymotrypsin is its function within the collagenolytic activity (Navarrete del Toro *et al*., 2015), which involves review from classification to applications, and currently through more precise equipment, generated knowledge and adaptation of techniques make it feasible to reach specific results.

The collagenolytic function of chymotrypsin offers it a similarity with the enzyme brachyurin (Hernández-Cortes *et al*., 1997; Navarrete del Toro *et al*., 2015); in this sense Rudenskaya (2003) proposes an approach for the separation of chymotrypsin and brachyurin into two distinct groups of enzymes, since when analyzing the structure of the chain of amino acids that integrate brachyurins it has been observed that there are differences with chymotrypsin in the polypeptide chains, mainly in the amino acids in charge of anchoring the enzyme to the polypeptide chain to be hydrolyzed. Contrary to this, Navarrete del Toro & García-Carreño (2019) state that brachyurin classification is incorrect and should not be considered as a group of enzymes distinct from chymotrypsin, since in crustaceans the collagenolytic activity of chymotrypsin is a generality. Due to these different positions and the lack of more precise information in this regard, it is suggested that these groups should be called digestive enzymes with chymotrypsin-like activity (Navarrete del Toro & García-Carreño, 2019).

Chymotrypsin in crustaceans

Although there are few studies of chymotrypsin in economically important crustacean species, this is repeated in other groups of aquatic organisms such as fish (Lauff & Hofer, 1984; Rungruangsk-Torrissen *et al*., 2006; Castillo-Yañez *et al*., 2009; Hadj Ali *et al*., 2010). In this group, chymotrypsin is composed of a single polypeptide chain (Lauff & Hofer, 1984; Zhou *et al*., 2011), as in crustaceans (Hernández-Cortes *et al*., 1997;

Navarrete del Toro *et al*., 2015), and studies have mainly focused on the comparison of chymotrypsin activity among organisms of this group, using standards of the enzyme derived from ruminants (Tsukada & Blow, 1985). According to these studies, it has been observed that chymotrypsin has been shown to have higher hydrolytic activity than its mammalian simile (Lauff & Hofer, 1984; Celis, 2003; Rungruangsk-Torrissen *et al*., 2006; Zhou *et al*., 2011; Navarrete del Toro *et al*., 2015)

As mentioned above, in commercially important crustaceans, research on the study of chymotrypsin, since 2000, has focused mainly on species such as *P. chinensis* (Shi *et al*., 2008), *P. subtilis* (Buarque *et al*., 2010), *P. vannamei* (Navarrete del Toro *et al*., 2011), as well as studies have also been carried out on this enzyme in species that present potential to be exploited in culture conditions, such as *P. indicus* (Omondi, 2005), *P. californiensis* (Navarrete del Toro *et al*., 2015; Torres-Ochoa, 2020), and *M. tenellum* (Espinosa-Chaurand *et al*., 2017); this because it is of great importance to have information on enzymatic dynamics and digestive physiology of organisms (Gamboa-Delgado *et al*., 2003; Simon, 2009) to influence and use their physiological potential within the formulation of balanced feed (Carrillo-Farnés *et al*., 2007; Simon, 2009; Buarque *et al*., 2010) and consequently increase the yield in commercial crops. This digestive physiological potential in crustaceans is evidenced by the results of chymotrypsin activity during protein digestion, since through its activity smaller polypeptides are released on which other enzymes act to obtain the different amino acids present (Tsai *et al*., 1991; Navarrete del Toro & García-Carreño, 2019).

Analyses of chymotrypsin activity between wild organisms and organisms in culture systems have been carried out, such as the one performed by Da Silva *et al*. (2014) in *M. amazonicum*, where they report that the enzymatic activity of chymotrypsin, and in general of proteases, is higher in cultured organisms; contrary to this, in *P. californiensis*, Torres-Ochoa (2020) observed that the activity of this enzyme was lower in cultured organisms than in wild organisms. These discrepancies could be due to two particular situations, the type of food or substrate and the time of consumption or time between feedings (sporadic starvation). It has been mentioned that chymotrypsin activity has been observed better from more specific substrates, such as SAAPNA, and in different magnitude according to the type of ingredient or diet evaluated, which in cultured organisms is supervised and guaranteed, promoting stability in the dynamics and digestive cycle of these organisms, contrary to what may occur with a wild organism that consumes whatever it has available, when it has it available and generally only one or two different ingredients, which would lead to the possible explanation where the feeding and food status of the wild samples in these studies is totally unknown.

In the starvation case, there is the possibility that wild organisms present a higher reserve of zymogens, which can only be activated in the presence of the substrate, so they may be prepared for the processing of these substrates, while fed organisms present lower values as they are constantly using enzymes for digestive processes

(Gora *et al*., 2018; Torres-Ochoa, 2020). Gora *et al*. (2018) conducted a study with *P. homarus*, where they evaluated the differences in enzyme activity under starvation and dieting schemes, reporting that fed organisms presented lower enzyme activity than those in starvation. This is possible due to the fact that wild organisms may spend periods of starvation because they do not have food and when they find it, they must ingest it quickly to avoid competition with other animals. Therefore, it is necessary that they have a high reserve of zymogens, which when activated are able to quickly hydrolyze food to make the most of the available nutrients.

CONCLUSIONS

So far, chymotrypsin in crustaceans is considered as an enzyme with no or low activity within the digestive enzymatic dynamics in these species; however, its study is of utmost importance to understand the bioavailability of amino acids, especially tryptophan, tyrosine and phenylalanine. As well as to understand the mechanism of hydrolysis of large residues involving the presence of essential amino acids for these species such as methionine and leucine. Due to the advance of techniques for the assessment of enzymatic activity and the use of synthetic substrates such as SAAPNA, it has been possible on the one hand to detect the presence of this enzyme in crustaceans and, on the other hand, to understand in more detail the digestive physiology of these species. This will serve to define nutritional guidelines in which it is possible to optimize and efficiently take advantage of the ingredients in functional foods, establish feeding correlations and study the behavior in both wild and farmed organisms, and will allow exploring new biotechnological uses of this enzyme and its possible application in the hydrolysis of proteins.

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Errata Erratum

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