

PROSPECÇÃO TECNOLÓGICA DA RECUPERAÇÃO DE ASTAXANTINA EM RESÍDUOS DE CAMARÃO *LITOPENAEUS VANNAMEI* PELO PROCESSO DE EXTRAÇÃO DE ÓLEO VEGETAL

TECHNOLOGICAL PROSPECTION OF ASTAXANTHIN RECOVERY OF SHRIMP WASTE *LITOPENAEUS VANNAMEI* BY THE VEGETABLE OIL EXTRACTION PROCESS

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Resumo

Os resíduos de camarão descartados durante o processamento de camarão apresentam moléculas bioativas com alto valor agregado. Entre eles, a astaxantina, pigmento natural produzido por microrganismos, algas e plantas amplamente utilizadas nas indústrias de alimentos, cosméticos, nutracêuticos e aquicultura. A extração comercial de astaxantina geralmente envolve o uso de solventes orgânicos. No entanto, alternativas mais sustentáveis foram propostas e uma delas é a extração com óleos vegetais. Assim, a presente prospecção foi utilizada para determinar as técnicas mais utilizadas nos últimos tempos para obter astaxantina a partir de resíduos de camarão. Para isso, foram pesquisados os bancos de patentes do Instituto Nacional de Propriedade Intelectual (INPI), do Escritório Europeu de Patentes (Espacenet) e da Organização Mundial da Propriedade Intelectual (WIPO) e a pesquisa científica foi realizada através do Capes Journal e da Scientific Electronic Library Online (SciELO). A pesquisa retornou aproximadamente 5.000 patentes e 24.000 teses e artigos com o total de palavras-chave pesquisadas. Além disso, a origem da astaxantina e suas áreas de uso também foram analisadas. Os resultados mostraram que as técnicas de extração de astaxantina dos resíduos de camarão estão nos estágios iniciais do desenvolvimento tecnológico. A astaxantina natural vem atraindo mais atenção do que o processo sintético, encontrando ampla aplicação nos setores alimentício e farmacêutico.

Palavras-chave: camarão; recuperação de astaxantina; *litopenaeus vannamei*; prospecção.

Abstract

Shrimp residues discarded during the shrimp processing process present bioactive molecules with high added value. Among them, astaxanthin, a natural pigment produced by microorganisms, algae

and plants widely used in the food, cosmetics, nutraceutical and aquaculture industries. Commercial extraction of astaxanthin usually involves the use of organic solvents. However, more sustainable alternatives have been proposed and one of them is the extraction with vegetable oils. Thus, the present prospection was used to determine the techniques most used in recent times to obtain astaxanthin from shrimp residues. For this purpose, the National Intellectual Property Institute (INPI), the European Patent Office (Espacenet), and the World Intellectual Property Organization (WIPO) patent banks were searched and the scientific research was done through the Capes Journal and the Scientific Electronic Library Online (SciELO). The search returned approximately 5,000 patents and 24,000 theses and articles with the total of searched keywords. In addition, the astaxanthin origin and its areas of use were also analyzed. The results showed that the techniques of extracting astaxanthin from shrimp residues are in the early stages of technological development. Natural astaxanthin has been attracting more attention than the synthetic process, finding wide application in the food and pharmaceutical sectors.

Key-words: shrimp; astaxanthin recovery; *litopenaeus vannamei*; prospection.

1. Introduction

One of the major bottlenecks in industrial production processes is the generation of by-products and waste during these processes, and in shrimp farming this reality is also present (CORRÊA *et al.*, 2012).

According to the United Nations Food and Agriculture Organization (FAO), in 2015, world production was approximately 8.4 million tons of sea shrimp, and Brazilian production was estimated at 105 thousand tons and the state of Sergipe 2.8 thousand tons of shrimp (CHAN *et al.*, 2014; IBGE, 2018).

During the shrimp processing steps, the cephalothorax and head are discarded resulting in the main residues (MEZZOMO *et al.*, 2013). Depending on the species, size and peeling procedure, about 40 to 50% of the weight is discarded (RAZI PARJIKOLAEI *et al.*, 2017).

However, shrimp residues present in their constitution bioactive compounds such as mineral salts (15-35%), proteins (18-40%), chitin (14-30%) and lipids, as well as carotenoids that can be used in other segments of industry (AMBIGAIPALAN; SHAHIDI, 2017). Thus, the study and development of techniques for extracting the molecules present in the shrimp residues are relevant due to the high added value of these molecules and the reduced environmental impact generated by the residues (MEZZOMO *et al.*, 2013; RAZI PARJIKOLAEI *et al.*, 2017).

Carotenoids represent one of the most important groups of natural pigments, generating the colors red, orange and yellow (CARDOSO *et al.*, 2017; LERFALL, 2016). The major carotenoid found in shrimps and their waste is astaxanthin, red coloring pigment, much used in aquaculture for pigmentation of salmonid meat, shrimp and lobster shells. In addition, it has been gaining

prominence in the food, cosmetic and nutraceutical industries (AMADO *et al.*, 2015; AMBATI *et al.*, 2014).

With a wide range of applications, the global carotenoid market reached \$ 1.5 billion in 2017, with expectations of reaching \$ 2.0 billion by 2022, and an annual growth rate of 5.7% (MUSSAGY *et al.*, 2019). The major market is Europe, representing 42% of the world total, followed by North America (25%) and Asia (20%) of the total (LIMA *et al.*, 2019).

Astaxanthin, due to its powerful antioxidant activity, is in second position among the carotenoid markets. The global astaxanthin market is valued at US \$ 288.7 million in 2017, and is estimated a value of US \$ 426.9 million dollars in 2022, making the most of astaxanthin carotenoid world market (MUSSAGY *et al.*, 2019). Astaxanthin production is dominated by chemical synthesis through organic solvents representing 95% of the global market and its value ranges from \$ 2500-7000 per kilo (PANIS; CARREON, 2016).

Astaxanthin production through shrimp residues using vegetable oils is a relatively new area of study. Its early stage makes the amount of information on the subject reduced. Thus, technological prospecting studies addressing the topic are needed to understand the big picture and assess their development

Technological prospecting can be defined as a systematic way of outlining scientific and technological developments that can generate future impacts on society by adding value to the information obtained in the present (MAYERHOFF, 2009; TEIXEIRA, 2013).

Thus, the present work aims to search existing information in scientific articles, theses and patents on the recovery of astaxanthin present in shrimp residues using vegetable oils to obtain data and further analysis of current behavior and future scenario of research in this area.

2. Theories

2.1. Carotenoids and Astaxanthin

Carotenoids is the term used to define the main natural pigments found in the animal and plant kingdoms (MEZZOMO; FERREIRA, 2016), they are isoprenoid metabolites synthesized throughout the photosynthetic organism (such as plants, algae), some non-photosynthetic (such as bacteria, fungi), and with some animal exceptions do not produce carotenoids, obtaining them through their food (MUSSAGY *et al.*, 2019; RODRIGUEZ-CONCEPCION *et al.*, 2018).

In photosynthetic organisms, carotenoids are essential for capturing light and energy transfer during photosynthesis also has a photoprotective function by neutralizing free radicals and prevented the cell from oxidative damage. In non-producing organisms, carotenoids are employed

as necessary precursors for vitamin A production. In addition, some research has placed carotenoids as substances with high potential to prevent some cancers and cardiovascular disease (ADADI *et al.*, 2018; MUSSAGY *et al.*, 2019; RODRIGUEZ-CONCEPCION *et al.*, 2018).

Carotenoids are fat soluble and can be divided into 2 subclasses based on their structures. Thus, they are classified as carotenes and xanthophylls (NGAMWONGLUMLERT; DEVAHASTIN, 2018). Carotenes are composed of carbon and hydrogen structures, while xanthophylls are carotenoids obtained from the oxidation of carotenes. Therefore, they have carbon, hydrogen and oxygen in their structures (MARTINS; FERREIRA, 2017).

Carotenoids are widely recognized for their antioxidant capacity. The mechanisms involved in its antioxidant activity include its role as an electron donor, oxygen suppressor and the ability to sequester free radicals due to the double conjugate bonds in its structure, in addition to the hydroxyl groups (MARIA *et al.*, 2015; MARTINS; FERREIRA, 2017).

Thus, they are widely used in the pharmaceutical, cosmetic and nutraceutical industries where they have effects on the prevention of degenerative diseases and reduce the risk of carcinogenic and coronary diseases. In addition, they protect the skin against oxidative damage, aging and stabilize and improve cosmetic products. Another use is given by the food and beverage industry where they are used as colorants (EGGERSDORFER; WYSS, 2018; MARTINS; FERREIRA, 2017).

Astaxanthin (3,3'-dihydroxy- β , β -carotene-4,4'-dione) is a xanthophyll found in crustaceans (such as shrimp), algae (such as *Haematococcus pluvialis*), fish (such as salmon). Its structure is composed of a long hydrocarbon chain with double conjugated bonds. At the ends of the chain are two benzene rings containing in their constitution a hydroxyl and ketone group (AMBATI *et al.*, 2014).

Thus, astaxanthin has lipophilic and hydrophilic characteristics. These dual properties aid in the binding of astaxanthin to the cell membrane, and generate better antioxidant activity compared to other carotenoids. In addition, astaxanthin also has antimicrobial properties (AMBATI *et al.*, 2014; WEERATUNGE; PERERA, 2016). These properties, especially the high antioxidant activity, make astaxanthin used in many industrial processes, such as cosmetics, pharmaceuticals, foods and functional foods (SHAH *et al.*, 2016; WEERATUNGE; PERERA, 2016).

The cosmetic industry uses astaxanthin in the formulation of creams and sunscreens to protect the skin from damage due to its effectiveness in protecting against ultraviolet light, sunburn and skin aging (EGGERSDORFER; WYSS, 2018). The pharmaceutical and functional food industry are using it in their formulations to reduce the chance of having certain diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases, among others (AMBATI *et al.*,

2014). While in aquaculture, astaxanthin is used in pigmentation of fish meat (salmon and trout), shrimp and lobster shells. And in the food industry, astaxanthin is used as a colorant (PRAMEELA *et al.*, 2017).

The production of astaxanthin commonly happens through three routes, chemical synthesis, biotechnological production and extraction in crustaceans. In the crustacean extraction residues, the most used technique is the extraction through organic solvents. However, this technique is expensive, inflexible and some important compounds can undergo changes in their structure and consequently the loss of functionality (LIM *et al.*, 2018; RAZI PARJIKOLAEI *et al.*, 2015). Thus, alternative extraction methods have been researched for the use of waste without the use of organic solvents.

2.2. Solid-liquid extraction

Solid-liquid extraction is a separation process involving the mass transfer of a solute from the solid matrix to a solvent. It is an old separation process with reports of its use 3500 years a.C. for obtaining drugs and perfumes (BART; PILZ, 2011). Interest in the technique is due to its wide use in various industrial processes, such as polymer engineering, food engineering, bioengineering and the growing demand for natural products (QIN *et al.*, 2018).

Common to all solid-liquid extraction methods is the intimate contact between liquid and solid. This contact can be divided into 2 categories, immersion and percolation. Immersion is the configuration where the solid is dipped in the liquid, usually agitation occurs. Percolation occurs when the solvent passes through a fixed bed of the solid (BART; PILZ, 2011).

The extraction of astaxanthin from shrimp residues by vegetable oils was proposed in 1975 by Cheng and Meyers. The procedure consisted of immersing the shrimp residue in soybean oil followed by mixing, heating and centrifuging to recover the pigmented oil (PRAMEELA *et al.*, 2017).

3. Methodology

The methodology employed is the search for information in the form of theses, scientific articles, and patents in a free access database on the recovery of astaxanthin from shrimp *Litopenaeus vannamei* residues through soybean oil extraction.

The scientific articles and theses were searched in the Periódicos Capes and the Scientific Electronic Library Online (SciELO). Patents were consulted in the databases of the Instituto Nacional

de Propriedade Intelectual (INPI), the European Patent Office (Espacenet), and the World Intellectual Property Organization (WIPO).

The words, terms or expressions used in the search were astaxanthin, shrimp residues, *Litopenaeus vannamei*, astaxanthin extraction, astaxanthin and shrimp waste; astaxanthin, shrimp waste and vegetable oils. In addition, we searched for the term astaxanthin together with the terms food, aquaculture, cosmetics, pharmaceutical and nutraceutical, the main areas of use of astaxanthin today, in order to identify the main areas of interest in carotenoid application. We have also searched for the term astaxanthin in association with the natural and synthetic terms to determine which type of astaxanthin has been attracting the most interest.

All terms and words were searched in Portuguese for the national database and in English for those where the most pertinent was English terms. Finally, the results of the design refer to the period between 2013 and 2019.

4. Discussion and Results

The data obtained for technological prospection were processed and are presented in Tables 1 and 2. Table 1 presents patent data for each keyword searched in the three previously chosen databases. The largest volume of patents has been found in WIPO's databases, as expected, as WIPO is a United Nations entity and houses patent information from around the world.

Table 1 - Number of patents per keyword in each database analyzed between 2013 and 2019

Keywords	INPI	Espacenet	WIPO	Total
Astaxanthin (Asx)	8	663	3998	4669
Shrimp Waste	3	25	40	68
<i>Litopenaeus Vannamei</i>	1	115	145	261
Astaxanthin Extraction	2	17	86	105
Asx e Shrimp Waste	0	4	20	24
Asx, Shrimp Waste e Vegetable Oils	0	0	2	2
Total	14	924	4410	5348

Source: Own Authorship (2019)

Looking at the more specific search term, “astaxanthin, shrimp residues and vegetable oils”, only two patents were found. Belonging both to WIPO. These patents addressed the production of animal feed from the production of shrimp waste astaxanthin.

In relation to the term “astaxanthin and shrimp residues” they include astaxanthin extractions employing the residues. One of the patents simultaneously produces astaxanthin and

chitin by means of *Bacillus subtilis* and *Gluconobacter* sp. Another patent for astaxanthin is obtained through solid-liquid and liquid-liquid extractions based on shrimp residues.

Regarding the national patent database, the INPI, the number of patents on the subject in the latter is reduced. Analyzing the patents found with the term “astaxanthin” in this database, the main focus is the use of algae to produce astaxanthin, being the main algae used *Haematococcus pluvialis*. The subject corresponds to 50% of the term patents. The other patents address the production of food and feed with astaxanthin in its constitution (2), a method of preparing a non-therapeutic astaxanthin diester and the production of carotenoids from carotene hydroxylase.

With the term “shrimp residues” patents were found for chitin production, polysaccharide production, such as xanthan gum, by fermenting these residues and obtaining animal feed. Finally, the term “astaxanthin extraction” found two patents, which dealt with extracting the astaxanthin obtained in microalgae through solvents. It is noteworthy that the two patents found with this term were also encompassed by the term astaxanthin.

Table 2 presents the number of articles and theses according to the keywords searched, and most of the documents are in the Capes platform. The largest amount of data found in Periódicos Capes is due to the fact that the platform covers works from a larger number of countries, while Scielo accesses works from only 16 countries, the vast majority of South American countries.

Table 2 - Number of articles and theses found for each keyword or term between 2013 and 2019

Keywords	Periódicos Capes	Scielo	Total
Astaxanthin (Asx)	5614	11	5625
Shrimp Waste	7178	8	7186
<i>Litopenaeus Vannamei</i>	7543	27	7570
Astaxanthin Extraction	2696	3	2699
Asx e Shrimp Waste	305	1	306
Asx, Shrimp Waste e Vegetable Oils	1	1	2
Total	23337	51	23388

Source: Own Authorship (2019)

The articles and theses found in Scielo with the terms “astaxanthin extraction” employ biotechnological processes, organic solvents and vegetable oils. In addition, the article dealing with the extraction of astaxanthin by vegetable oil is the same found in the more specific terms.

This article uses palm oil to extract astaxanthin from the residues of pink shrimp (*Farfantepenaeus subtilis*). In addition, the article evaluates the influence of shrimp residue drying on extraction and seeks to obtain the kinetic parameters of extraction.

It can be observed through Tables 1 and 2 that the more specific the keywords are, the fewer the patents, articles and theses on the subject.

In addition, when compared, Tables 1 and 2 show that obtaining astaxanthin from shrimp residues through vegetable oil extraction is in the initial research phase and needs to be optimized for industrial use.

Looking at Tables 3 and 4 below, it is possible to see the trend in recent years of searching for more natural products, meeting consumer demand and seeking to reduce cost and/or maximize profits through new processes and products. However, it is interesting to consider that the synthetic astaxanthin production processes that dominate the carotenoid market are well established, thus generating fewer patents. In addition, those databases with the largest collection obtained the largest number of articles found on the search terms.

Table 3 - Comparison between natural and synthetic astaxanthin patents between 2013 and 2019

Keywords	INPI	Espacenet	WIPO	Total
Synthetic Astaxanthin	0	6	95	101
Natural Astaxanthin	0	75	131	206
Total	0	81	226	307

Source: Own Authorship (2019)

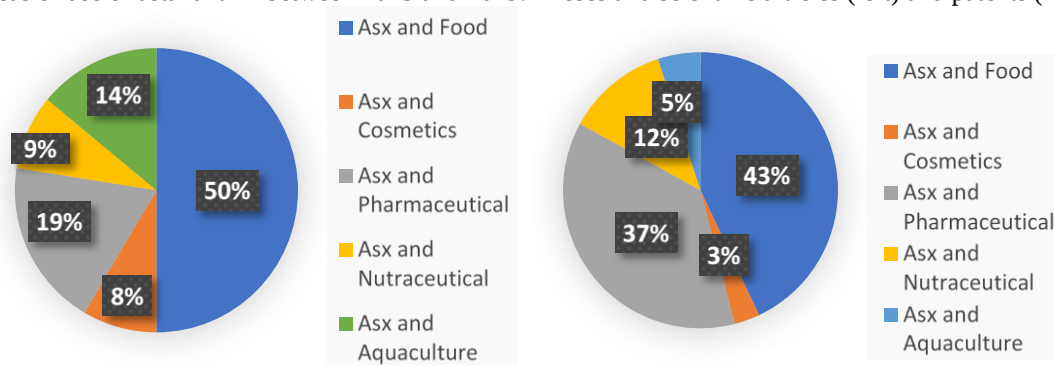
Table 4 - Comparison between articles and theses on natural and synthetic astaxanthin during the period between 2013 and 2019.

Keywords	Periódicos Capes	SciELO	Total
Synthetic Astaxanthin	175	1	176
Natural Astaxanthin	309	2	311
Total	484	5	489

Source: Own Authorship (2019)

Figure 1 presents the main research areas for the use of astaxanthin. In patents, theses and scientific articles, astaxanthin's main area of employment is food. The pharmaceutical area has a significant number of patents, and aquaculture, there are a large number of studies or theses in the area, denoting a growing interest of greater application of carotenoid in the area.

Figure 1 - Areas of use of astaxanthin between 2013 and 2019. Theses and scientific articles (left) and patents (right).



Source: Own Authorship (2019)

5. Discussion and Results

Through searching the databases for patents, theses and articles it can be concluded that most patents are found in international databases.

This fact is due to the great involvement between industry and foreign universities in the search and development for new technologies. In addition, international platforms span a large number of countries.

The theses and scientific articles were found mostly by the national platform Periódicos Capes that gathers various documents from around the world in their databases.

Natural astaxanthin in recent years has attracted more interest than synthetic astaxanthin. This can be seen from the greater number of patents, theses and articles addressing naturally obtained astaxanthin, meeting society's desire for healthier products and raw materials. The major application of astaxanthin is in the food and beverage area as a colorant.

Regarding the recovery of astaxanthin from shrimp residues of *L. vannamei* species by extraction of vegetable oils is still little explored, requiring further studies to be applied industrially. Thus, the number of patents, theses and articles on the subject is small, despite a growing interest on the subject.

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