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# By-Products From Jumbo Squid (*Dosidicus gigas*): A New Source of Collagen Bio-Plasticizer?

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## 1. Introduction

The applications of jumbo squid by-products as plasticizer agents have been never received much attention, even though there are several studies in which the properties of the catch and processing discards for productions of films have been reported.

The principal by-products that result from the catch and processing of seafood include viscera, heads, cut-offs, bone, and skin. Therefore, a large and considerable volume of solid waste is obtained, constituting an important source of environmental contaminants unless efforts for their recovery are attained (Arvanitoyannis & Kassaveti, 2008). In the case of squid, one the largest known mollusks, the global capture represents no more than 2% of the total catch. However, by-products from squid processing, which include heads, viscera, backbones or pens, ink, skin, unclaimed fins, mantles, and tentacles, may represent up to 60% of the whole weight. In addition, from all the different anatomical squid components regarded as by-products only the beak and pen are not edible. Thus, since most of the squid is not used, its by-products also pose an environmental issue for this fishery, especially in areas where it is harvested the most. The valuable and profitable components that these by-products contain include among others chitin, chitosan, collagen, and gelatin (Kim & Mendis, 2006; Shahidi, 2006).

Collagen is a fibrous protein responsible for structural sustaining of several animal tissues, being the main protein present in skin, bones, tendons, cartilages, and teeth. In the case of mammals, it accounts for about 20-30% from the total body protein (Quereshi et al., 2010). The term collagen derives from the Greek word *kolla* which means glue and was defined as "that constituent of connective tissue which yield gelatin on boiling" (Oxford University, 1893). Nowadays, although collagen is most of the time referred as to a single item, in fact it is a heterogeneous group of at least 19 different molecules which have a unique triple-helix configuration that forms very strong fibers. Collagen is characterized by its unusual amino acid composition, in which glycine and proline account for about 50% of them. Indeed, each polypeptide called  $\alpha$  chain consists of a repeated sequence of the triplet Gly-X-Y,

where X and Y are often proline and hydroxyproline. Each collagen type varies in the length of the helix and the nature and size of the non-helical portions (Lee, C. R. et al., 2001).

Collagen *per se* is regarded as one of the most useful biomaterials. The excellent biocompatibility and safety due to its biological characteristics, such as biodegradability and weak antigenicity, have made collagen one of the primary resources in medical applications. In addition, other uses include gelatin production, nutritional supplements, sausage casings, and in cosmetic products it claims anti-ageing benefits (Kim & Mendis, 2006; Lee et al., 2001).

Recently, the use of fish collagen in the manufacture of biopolymer films has been reported. Collagens from different species of fish have been extracted using acetic acid, which were used later to produce biodegradable films (Venugopal, 2009). Studies on the production and characterization of films using fish gelatins are quite recent, and all fish gelatins have been observed to exhibit good film-forming properties, yielding transparent, nearly colorless, water soluble, and highly extensible films (Avena-Bustillos et al., 2006; Benjakul et al., 2006; Carvalho et al., 2008; Gomez-Guillen et al., 2007; Zhang et al., 2007).

On the other hand, chitosan is a polysaccharide that is produced by deacetylation of naturally occurring chitin, and it has a great potential for a wide range of applications due to its versatile properties, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection, and recently in gene therapy as well (Venugopal, 2009; Dutta et al., 2009; Shahidi et al., 1999; Shahidi et al., 2002). Nonetheless, pure chitosan, as a film material, does not form films with adequate mechanical properties due to its low percentage of elongation (Butler et al., 1996). For this reason, one of the current trends in designing biodegradable materials for packaging is to combine different biopolymers (Bawa et al., 2003; Bertan et al., 2005; Colla et al., 2006; Le-Tien et al., 2004; Lee et al., 2004; O'Sullivan et al., 2005; Tapia-Blacido et al., 2007; Yu et al., 2006).

Chitin and chitosan belong to a group of natural polymers produced by the shells of crab, shrimp, and lobster. In addition to be nontoxic, chitin and chitosan are inexpensive, biodegradable, and biocompatible. Regarding to film-forming properties, chitosan is more versatile as compared to its precursor chitin. Chitosan has the capacity to form semipermeable coatings which, when used in foods, prolong their shelf life by acting as barriers against air and moisture (Agulló et al., 2004).

Furthermore, since collagen in acid solution exhibited positively charged groups, it has a molecular interaction with chitosan with high potential to produce biocomposites (Liang et al., 2005; Lima et al., 2006; Sionkowska et al., 2006; Wang et al., 2005; Wess et al., 2004), acting as a possible plasticizer agent.

In the first part of this chapter, the most important characteristics of jumbo squid as fishery, as well as the most recent scientific literature dealing with chitosan and collagen films made from seafood by-products, are reviewed. In the second part, thermal, mechanical and morphological properties of chitosan and acid soluble collagen (ASC) produced by casting films are discussed. As-cast films dried in relation to the molecular interaction of ASC by using differential scanning calorimetry (DSC), scanning electron microscope (SEM), and infrared spectroscopy are also discussed in this chapter. Thermal properties by DSC, SEM images, mechanical properties, water vapor barrier properties, and water solubility characteristics of the chitosan/ASC blends are analyzed as a function of ASC content in terms of the individual properties of chitosan and ASC.

## 2. Characteristics of jumbo squid as fishery

Squid or calamari are cephalopods which comprises a group around 300 species, being the jumbo squid or *Dosidicus gigas* one of them (Figure 1). Jumbo squid is a member of the flying squid family, Ommastrephidae (Nesis, 1985), and are known to eject themselves out of the water to avoid predators. Jumbo squid are the largest known mollusks and the most abundant of the nektonic squid. They can reach up to 2 m in length and weigh up to 45 kg. This specie is characterized by its large, tough, thick-walled mantle and long tentacles. These organisms are aggressive predators. Jumbo squid earned the nickname of "red devils" because of their red hue when hooked, which they use to camouflage from predators in deep waters where most animals cannot see the red color. This coloration is due, like other cephalopods, to the presence of chromatophores. Also, squid possess the ability to squirt ink as a defense mechanism (Nigmatullin et al., 2001).

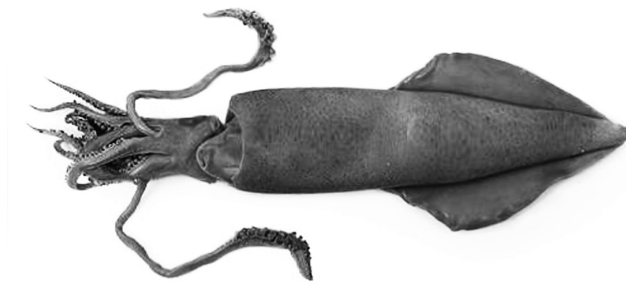


Fig. 1. Jumbo squid (*Dosidicus gigas*).

Jumbo squid is an endemic species to the Eastern Pacific, ranging from northern California to southern Chile and to 140 degrees W at the equator. Exploratory commercial fishing for *Dosidicus gigas* began in the 1970s off the Pacific coast of America. The catches of this fishery increased from 14 tons per year in 1974 to over 250,000 tons in 2005. Since then, it has become an extremely important fisheries resource in the Gulf of California, Costa Rica Dome and Peru (Marakadi et al., 2005).

The commercial fishery of jumbo squid consists of a multinational jigging fleet, which fish at night using powerful lights to attract squid (Waluda et al., 2004). The caught of this organism depends of the season and the region. In the Gulf of California for example, this organism enter to the Gulf from the Pacific in January, to reach their northernmost limit by April, and to remain in the central Gulf from May through August; the highest aggregations of specimens are found along the western (Baja California) coast. From September squids appear to migrate onward the eastward to the Mexican mainland coast and then southwards, to the Gulf back into the Pacific (Ehrhardt et al., 1983). Whereas, in Peruvian waters the highest squid concentrations occur along the coast of northern Peru, from Puerto Pizarro to Chimbote, with low to medium squid concentrations off Pisco and Atico. The highest catches occur during autumn, winter, and spring, since squid tend to be dispersed in summer (Taipe et al., 2001).

Although the growth of this fishery has been spectacular, great contrasts have characterized it. Of the total catch, a major portion remains unused or minimally used. In Mexico for instance, no more than 11% of the resource is used for human consumption, regardless of its low price and high nutritional value (De la Cruz et al., 2007). Moreover, only the mantle

(42%) is usually used, which is later primarily marketed fresh, frozen or pre-cooked (Luna-Raya et al., 2006). Some of the by-products produced after filleting, like fins and heads, are utilized but huge amounts are wasted. Fortunately, a number of studies have reported that this waste is an excellent raw material to obtain important by-products with high commercial value, such as collagen (Gómez-Guillen et al., 2002; Kim et al., 2005, Shahidi, 2006; Torres-Arreola et al., 2008; Gimenez et al., 2009).

### 3. Collagen from jumbo squid by-products

Collagen is the main fibrous component of the connective tissue and the single most abundant protein in all organisms, since it represents up to 30% of the total protein in vertebrates and about 1-12% in aquatic organisms (Brinckmann, 2005). In general, collagen fibrils in the muscle of fish, form a delicate network structure with varying in the different connective tissues and is responsible for the integrity of the fillets (Shahidi, 2006). Moreover, the distribution of collagen may reflect the swimming behavior of the species (Sikorski et al., 1994). In several species of fish the weakening of the connective tissues may lead to serious quality deterioration that manifests itself by disintegration of the fillets. Also, thermal changes in collagen contributes to the desirable texture of the meat, however when heating is conducting under not controlled conditions, this may lead to serious losses due to the reduction in the breaking strength of the tissues (Sikorski et al., 1986).

The name collagen is in fact a generic term for a genetically distinct family of molecules that share a unique basic structure: three polypeptide chains coiled together to form a triple helix. About 19 different types of collagen molecules have been isolated and these not only varies in their molecular assembly, but also in their size, function, and tissue distribution (Table 1) (Exposito et al., 2002; Brinckmann, 2005).

Type	Chain composition	Subfamily	Tissue distribution
I	$[(\alpha 1(I))_2\alpha 2(I)]$	Fibrillar	Skin, tendon, bone, ligament, vessel
II	$[(\alpha 1(II))_3]$	Fibrillar	Hyaline cartilage, vitreous
III	$[(\alpha 1(III))_3]$	Fibrillar	Skin, vessel, intestine, uterus
IV	$[(\alpha 1(IV))_2\alpha 2(IV)]$	Network	Basement membranes
V	$[\alpha 1(V)\alpha 2(V)(3(V))]$	Fibrillar	Bone, skin, cornea, placenta
VI	$[\alpha 1(VI)\alpha 2(VI)\alpha 3(VI)]$	Network	Interstitial tissue
VII	$[(\alpha 1(VII))_3]$	Anchoring fibrils	Epithelial tissue
VIII	$[\alpha 1(VIII)\alpha 2(VIII)]$	Network	Endothelial tissue, descemet's membrane
IX	$[\alpha 1(IX)\alpha 2(IX)\alpha 3(IX)]$	FACIT*	Cartilage, cornea, vitreous
X	$[(\alpha 1(X))_3]$	Network	Hypertrophic and mineralizing cartilage
XI	$[\alpha 1(XI)\alpha 2(XI)\alpha 3(XI)]$	Fibrillar	Cartilage, intervertebral disc
XII	$[(\alpha 1(XII))_3]$	FACIT*	Skin, tendon, cartilage

\* Fibril associated collagen with interrupted triple helices

Adapted from: Friess, 1998; Brinckmann, 2005.

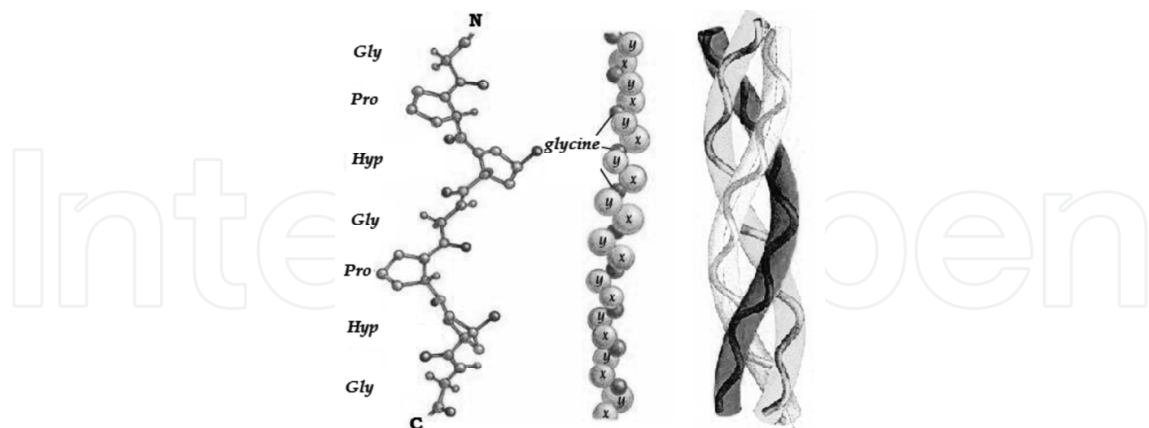
Table 1. Chain composition, subfamily and body distribution of the main collagen types found in animal tissue.



The most abundant and widespread family of collagens, it is represented by the fibril-forming collagens, especially types I, III and V (Sato et al., 1989). Their basic structure consists of three protein chains that are supercoiled around a central axis in a right-handed manner to form a triple helix, called tropocollagen, which is a cylindrical protein of about 280 nm in length and 1.5 nm in diameter. Each chain, called  $\alpha$  chain, contains about 1000 amino acids and it has a molecular weight of 100 kDa, depending on the source. Tropocollagen molecules may be formed by three identical chains (homotrimers) as in collagens II and III, or by two or more different chains (heterotrimers) as in collagen types I, IV, and V.

The three  $\alpha$  chains are perfectly intertwined throughout the tropocollagen molecule to form the triple helix except for the ends, where helical behavior is lost, since in these regions, called telopeptides, globular proteins that are involved with intermolecular crosslinking with other adjacent molecules are found (Engel & Bachinger, 2005). A structural prerequisite for the assembly of a continuous triple helix, the most typical conformation of collagen, is that every third position along the polypeptide chain is occupied by a glycine residue. Being glycine the smallest amino acid and lacking of a side chain, the collagen  $\alpha$  chains can coil so tightly because glycine can be easily accommodated in the middle of a steric smooth superhelix and form stable packed structures; this would be very difficult with the bulkier residues. Further stabilization of the triple helix is attained by the formation of hydrogen bonds that are formed between the amino groups of glycine residues and the carbonyl groups of residues from other chains.

The structural constraints that make the collagen triple helix unique among proteins are given by its unusual amino acid content. Besides glycine as the major residue, a repeated sequence that characterizes the collagenous domains of all collagens is the triplet (Gly-X-Y), where X and Y are often proline and hydroxyproline, respectively (Figure 2). Depending on the collagen type, specific proline and lysine residues are modified by post-translational enzymatic hydroxylation. These imino acids permit the sharp twisting of the collagen helix and are associated with the stability and thermal behavior of the triple helical conformation.



Source: Branden & Tooze, 1999; Voet & Voet, 1995.

Fig. 2. Spatial conformation of a typical tropocollagen molecule.

### 3.1 Sources and extraction

The main sources of industrial collagen are limited to the skin and bones of pigs and cattle. However, as a possible alternative to the problems associate to transmissible bovine

spongiform encephalopathy (BSE) and foot and mouth disease (FMD), as well as religious barriers, new alternatives are being sought for collagen sources (Jongjareonraka et al., 2005; Nagai, 2004). As a result, the extraction of collagen and their derivatives from marine sources has considerably increased in the last years since it represents an appropriate alternative source to land animals with promising functional properties (Shen et al., 2007).

Collagen typically exists in a concentration from 3 to 11.1% in the mantle of some squid species like *Illex* and *Loligo* (Sikorski & Kolodziejska, 1986), whereas in *Dosidicus gigas*, collagen was found in a concentration up to 18.33% (Torres-Arreola et al., 2008). This variability among squid species may be attributed to the high degree of protein turnover that takes place in the muscle of cephalopods, which are fast-growing species as they usually reach their maximum maturity in one year or two. In general, the collagen from aquatic organisms, is highly soluble in salt solutions, dilute acids and acid buffers, unlike collagen from land mammals that are poorly soluble in such solvents (Kolodziejska et al., 1999). Consequently, in order to extract collagen from marine organisms, the most common solvent systems that have been found to be generally useful and convenient are described below; however, there is no single standard method for the isolation of collagen (Miller & Rhodes, 1982).

Prior to the actual collagen extraction procedure, it is a prerequisite to wash or digest the original tissue with a dilute alkali (e.g. 0.1 M NaOH) in order to remove non-collagenous proteins and to prevent the effect of endogenous proteases on collagen. This procedure can also be achieved by using caothropic solutes such as urea in high concentrations (6 M). These organic solutes are capable of increasing the ionic strength of the medium and breakdown the structure of water, which causes the disruption of the hydrogen bonds among the  $\alpha$  chains, which induces the unfolding and solubilization of hydrophobic residues inside the protein molecule, only affecting the soluble protein fractions (myofibrillar and sarcoplasmic) but leaving the stromal proteins intact (Usha & Ramasami, 2004). Once the connective tissue have been isolated, the following collagen extraction procedures are usually conducted at low temperatures (4-8°C) with the aim to minimize bacterial growth, enhance the solubility of native collagens, and to ensure the retention of native conformation on the part of the solubilized collagens (Miller & Rhodes, 1982):

- a. Neutral salt solvents (e.g. 1 M NaCl, 0.05 M Tris, pH 7.5): The high ionic strength of this solvent system allows to solubilize newly synthesized or young collagen molecules (Friess, 1998). Moreover, this solvent is also capable of solubilize more components beyond the stromal fractions, such as remaining myofibrillar proteins. Therefore, this solvent shows the least ability to solubilize pure collagen.
- b. Dilute acid solvents (e.g. 0.5 M acetic acid): Dilute acids such as acetic acid, hydrochloric acid, or citrate buffer are widely used to dissolve some pure collagen, although they are only limited to the portion of non-crosslinked collagen (Jongjareonrak et al., 2005). The pH of these solvents is about 3.0, which exhibits a sufficient capacity to induce swelling of most tissues promoting the solubilization of the triple helix junctions. Collagen extracted using these solvents usually have greater industrial applications in comparison with other soluble fractions because of the facility to incorporate the biomolecule with other polymers in an acidic medium (Garcia et al., 2007).
- c. Dilute acid solvents containing pepsin (e.g. 0.5 M acetic acid plus EC 3.4.23.1): These systems are one of the most versatile and widely used procedures for the extraction of collagen. The enzyme is usually added to the acidic solvent in sufficient quantities to

achieve a 1:10 ratio between the enzyme and the dry weight of the tissue to be extracted. The effectiveness of this system lies in its ability to solubilize native collagen (Miller & Rhodes, 1982). Despite its great efficacy, an essential and adequate control is needed for these systems, since pepsin is an exogenous proteolytic enzyme and if not controlled, it may contribute to the total disorganization or degradation of the collagen molecules.

After the different soluble collagen fractions are attained, the insoluble collagen remains. This fraction is characterized by numerous crosslinks within the molecule, which in turn prevent its disintegration and make it more resistant to proteolysis. Physically, insoluble collagen is an opalescent fibrous material that can only be degraded using either strong denaturing agents or mechanical fragmentation under acidic conditions (Friess, 1998). The insolubility of collagen is attributed to the modification of the forces that hold in together the  $\alpha$ -chains. Its presence is also related to the age of the animal and to its frozen storage, due to the protein aggregation that results from the removal of water molecules from the stromal proteins (Montero et al., 2000). Finally, once all the soluble and insoluble collagen fractions are collected, a selective neutral or dilute acid- salt precipitation procedure is usually performed in order to initially purify and recover the individual collagen types that may be present in extracts from various tissues. In the case that a final purification and resolution of the collagen molecules is required, different chromatographic techniques under non-denaturing conditions have been reported and found to be quite effective.

In the case of the mantle of *Dosidicus gigas*, Uriarte-Montoya et al. (2010) used urea 6M and the three solvent systems described above to isolate collagen from this species. They found that collagen from the mantle was 37% soluble in a neutral salt solvent, 23% in a dilute acid solvent and 25% in a dilute acid solvent containing pepsin, whereas the remaining 15% of the connective tissue was insoluble. They concluded that depending on the ultimate application, each collagen fraction warrants sufficient importance and might be used for different purposes rather than the traditional industrial collagen applications. As it will be mentioned in the next subsection, collagen has a wide variety of application, from food to medical and it is widely use in the form of collagen casings.

### 3.2 Collagen as biomaterial and applications

Nowadays, collagen has several industrial and biomedical applications. In the former, collagen has been used from long time ago, whereas the latter have placed collagen as an object of intense research in the last years. Industrially, collagen has been used for leather processing and gelatin production. Both products consist mainly of collagen but they greatly differ in the chemical form of the collagen used (Meena et al., 1999). Leather is basically chemically treated animal skin, while gelatin is an animal connective tissue that is denatured and degraded by heat and chemicals to produce a soluble form.

Regarding the biomedical uses of collagen, probably one of the main attraction of collagen as a biomaterial is its low immunogenicity. Moreover, collagen can be processed into various presentations, such as sheets, tubes, sponges, powders, injectable solutions and dispersions, making it a functional component for specific applications in ophthalmology, wounds and burns, tumor treatment, engineering and tissue regeneration, among others areas (Kim & Mendis, 2006). Table 2 summarizes the main advantages and disadvantages of collagen as a biomaterial. Another recent application of collagen in biomedicine is found in the formulation of membranes and hydrogels, products in which collagen interacts with



another material to form a composite. The combination of collagen with chitosan is perhaps one of the most studied composites with practical emphasis on medicine, dentistry, and pharmacology (Lima et al., 2006).

Advantages	Disadvantages
Available in abundance and easily purified from living organisms	High cost of pure type collagen
Non-antigenic and non-toxic	Variability of isolated collagen
Biodegradable and biocompatible	Hydrophilicity may lead to swelling and more rapid release
Synergic with bioactive compounds	Variability in enzymatic degradation rate as compared with hydrolytic degradation
Formulated in a number of different presentations	Complex handling properties
Hemostatic	Possible side effects such as mineralization
Easily modifiable to produce other materials	
Plasticity due to high tensile strength	
Compatible with synthetic polymers	
Adapted from: Friess, 1998; Lee et al., 2001.	

Table 2. Main Advantages and disadvantages of collagen as a biomaterial.

#### 4. Chitosan

Chitosan, the main deacetylated derivative of chitin, is a biocompatible, non-toxic, edible, biodegradable polymer, and it possesses antimicrobial activity against bacteria, yeast and fungi, including toxigenic fungi (Cota-Arriola et al., 2011). This polymer is the only natural cationic polysaccharide, which special characteristics makes it useful in numerous applications and areas, such medicine, cosmetics, food packing, food additives, water treatment, antifungal agent, among others (Hu et al., 2009). At a commercial level, chitosan is obtained from the thermo-alkaline deacetylation of chitin from crustaceans (Kurita, 2006), and its production has taken great importance in the ecological and economic aspects, due to the use of marine by-products (Nogueira et al., 2005).

Chemically, chitosan is a linear polycationic hetero-polysaccharide (poly [ $\beta$ -(1,4)-2-amino-2-deoxy-D-glucopyranose]) (Figure 3) of high molecular weight, whose polymeric chain changes in size and deacetylation degree (Tharanathan & Srinivasa, 2007). The nitrogen forms a primary amine and causes N-acylation reactions and Schiff alkalis formation, while the hydroxyl (OH) and amino (NH<sub>2</sub>) groups allow the formation of hydrogen bonds (Agulló et al., 2004).

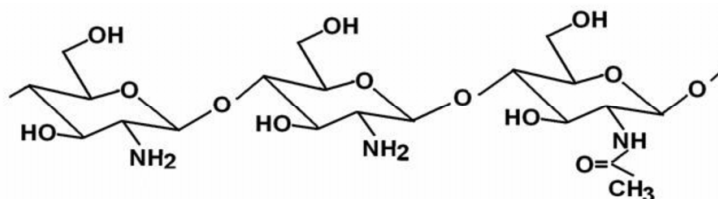


Fig. 3. Chemical structure of chitosan showing the characteristic amino (NH<sub>2</sub>) groups.

In its native form, the chitosan is insoluble in water and in the majority of organic solvents; nevertheless, at pH values <6.0 chitosan easily dissolve in diluted acidic solutions due to the protonation of the amino groups, turning it into a soluble cationic polysaccharide (Agulló et al., 2004; Raafat & Sahl, 2009; Sharma et al., 2009). Commercially, chitosan is available in several grades of purity, molecular weight, distribution and length of chain, deacetylation degree, density, viscosity, solubility, water retention capacity and the distribution of the amino/acetamide groups. All these characteristics affect the physicochemical properties and therefore, its application (Raafat & Sahl, 2009).

Among their biological properties, the antibacterial, antifungal, antiviral and insecticide activity of chitosan and its derivatives (Badawy & Rabea, 2005; Badawy et al., 2005) have been explored for agricultural applications (Daayf et al., 2010). Due to its peculiar characteristics, chitosan has potential applications in diverse fields (Table 3).

Industry	Applications
Biomedicine	It promotes the growth of tissues, wound healing (bandages, sutures), flocculent, homeostatic, bacteriostatic/fungistatic, spermicide, antitumoral, anticholesterolemic, immunoadjuvant, sedative of the central nervous system, acceleration of the osteoblast's formation.
Pharmaceutics	Controlled release of medicines.
Agriculture	Preservation of fruits and vegetables, soils supplements, release of agrochemicals and nutrients supply, improves the seed's germination, protects against microbial damage.
Waste-water treatment	Bleaching of waste water, removal of heavy metals, water clarification.
Paper and textile	Flexibility and resistance. It improves stickiness of inks and clothes stains, stabilizes the color and resistance of cloths. It improves the paper sheen, the resistance to the microbial and enzymatic deterioration; improves the paper biodegradability and the antistatic of photographic paper, improves the paper air-tightness.
Cosmetics	Solar and moisturizing protector, decrease the expression lines, useful in contact glasses and hair conditioners.
Chromatography	Enzymes separation and gas chromatography.
Supplement or food additive	Water retention, reduces the lipids and cholesterol absorption, antiacid agent, food fibre, food formulation for babies, emulsifier, preservative, antioxidant, gellificant, clarficant of fruit juices, immobilization of enzymes.
Films and eatable recovering	It protects and preserves food, decreases the production of ethylene and CO <sub>2</sub> , antimicrobial, reduces the water permeability, controls enzymatic oxidation.
Preservative	Antibacterial, antifungal.

Adapted from: Agulló et al., 2004

Table 3. Applications of chitosan and chitosan composite films.

#### 4.1 Chitosan bio-based films

One of the properties of major importance of chitosan is its filmogenic capacity. The films can be prepared from moderately concentrated polymer solutions, in general at 3 % (w/w). Chitosan films possess acceptable mechanical and permeability properties, good adherence to different surfaces, are flexible, resistant to water and show excellent barrier properties against gases (O<sub>2</sub>, CO<sub>2</sub>, water vapor), that allows its application in the development of food packing materials (Agulló et al., 2004; Plascencia-Jatomea et al., 2010; Tharanathan et al., 2002).

There are different methods for the productions of chitosan films, as the evaporation of solvents (Casting) -the first developed and more used at present- and the extrusion with some polyesters, olefins or carbohydrates. The last one is the most used for the industrial production of polymeric materials (Bhattacharya et al., 2005; Pelissari et al., 2009). With regard to the edible covered films, these are obtained by direct application (spraying, immersion) of chitosan solutions on the food surface or in the medicine's tablets, forming a thin layer that covers and protects the product of the environment (Janjarasskul & Krochta, 2010). Nowadays, most of the films and chitosan composites are prepared by this method, changing the solvent and the component's concentration of the blends, according to the application. Recently, it has been reported that the electrospinning technique allows the preparation of ultrathin chitosan nanofibers with unusually high porosity in their nanometer scale architecture and large surface area (Chen, Z. et al., 2009; Martínez-Camacho et al., 2011; Ohkawa et al., 2004). As particular interests have been addressed in the tissue engineering, great efforts have been made to study electrospinning of biodegradable polymers (Schauer & Schiffman, 2008).

Due to its abundance in the nature and to its biocompatibility, chitosan is considered to be a promising polymer for the development of functional materials (Ohkawa et al., 2004; Westbroek et al., 2007). In contrast to other materials, it has been demonstrated that chitosan films possess antifungal properties (Martínez-Camacho et al., 2011; Plascencia-Jatomea et al., 2010), that make it a good alternative for food protection and food shelf life extension (Chien et al., 2007b; Chien et al., 2007a; Coma et al., 2003; El Ghaouth et al., 1992; Fornes et al., 2005; Li & Yu, 2001; No et al., 2007; Schnepf et al., 2000). In general terms, although the materials prepared with conventional synthetic polymers are functional, of easy production, and low cost, they hardly are degradable, which strongly impacts the environment (Tharanathan & Srinivasa, 2007). Nevertheless, the use of these biopolymers is limited due to problems related to its deficient mechanical properties (fragility, poor barriers against gases and moisture) and cost (De Azeredo, 2009).

The incorporation of natural compounds has allowed the appearance of new materials with good mechanical properties, which overcome those that possess the individual materials. Most of these films are prepared mainly thinking about its use as food packing and tissue engineering materials (Tharanathan & Srinivasa, 2007). Additionally, to improve chitosan blends elasticity a biodegradable materials might be added (Butler et al., 1996).

#### 4.2 Chitosan films and bio-plasticizer

Plasticizers are additives used to increase the flexibility or plasticity of polymers (Daniels, 1989). The most studied plasticizing agent in chitosan films has been glycerol and polyols and its efficacy in improving the properties of films has been well-documented (Table 4).

Film/Plasticizer	Physicochemical properties	Reference
Chitosan Lignine	It improves the tensile force, the thermal degradation and the glass transition temperatures.	(Chen et al., 2009)
Starch-chitosan film/ Sorbitol; glycerol; polyethylene glycol	Increased flexibility and barrier properties; decreased rigidity; increased in solubility; affected color.	(Bourtoom, 2007)
Chitosan film/ Glycerol; ethylene glycol; poly (ethylene glycol); propylene glycol	Improves ductility, increase in strain and decrease in stress, and increases film hydrophilicity. Propylene glycol exposes antiplasticization phenomenon.	(Suyatama et al., 2005)
Chitosan film/ Glycerol; sorbitol; i-erythritol	Good flexibility and mechanical force.	(Fernández et al., 2004)
Chitosan film/ Poly (ethylene glycol)	Improve the elastic properties, enhanced the protein adsorption, cell adhesion, growth and proliferation.	(Zhang et al., 2002)
Chitosan film/ Poly(vinyl alcohol; sorbitol; sucrose	Decrease in thermal properties, increase in percentage of elongation and CO <sub>2</sub> and water permeability increase. High plasticizer contents caused decrease in both tensile and modulus.	(Arvanitoyannis et al., 1997)
Chitosan films/ Glycerol	Tensile strength values equal to that of HDPE and LDPE films	(Butler et al., 1996).

Table 4. Plasticizer effect on the properties of chitosan films.

Recently, the potential action of acid soluble collagen from jumbo squid (ASC) as a plasticizer in chitosan films was evaluated (Uriarte-Montoya et al., 2010; Arias-Moscoso et al., 2011). In general, the use of acid soluble collagen from jumbo squid by-products, in amounts equal or lower than 50 % in the production of chitosan biocomposites, produces films with reasonable tensile properties (Arias-Moscoso et al., 2011). More details about it will discuss in the following subsections.

## 5. Chitosan and collagen films

Biomaterials are increasingly being used in several fields, like packaging material (Tharanathan, 2003), tissue engineering (Nalwa, 2005), medical and pharmaceutical applications (Bures et al., 2001) due to their functional properties.

Collagen, as a natural polymer, has very weak mechanical properties, especially in aqueous media (Zhang et al., 1997). On the other hand, as it was mentioned previously, chitosan poses excellent film-forming property, antimicrobial activity, and unique coagulating ability with metal and other lipid and protein complexes due to the presence of a high density of amino groups and hydroxyl groups in the polymeric structure of chitosan (Dutta et al., 2002; Li et al., 1992; Shahidi et al., 1999). Although, mechanical properties of chitosan films are comparable to those of many medium-strength commercial polymers, it is considered that

this properties could be improved, mainly its elasticity (Suyatma et al., 2005). One strategy to increase chitosan elasticity is to associate it with biodegradable materials. However, the interactions that may occur between biopolymers are very important when the characteristics of any material are considered to be transformed. These interactions depend on the miscibility of its components.

Miscibility in polymer blends is attributed to specific interactions between polymeric components, which usually give rise to a negative free energy of mixing in spite of the high molecular weight of polymers (Shanmugasundaram et al., 2001). On the other hand, the interactions between natural polymers of different chemical structures, whether they are hydrogen bonding or electrostatic in nature, considerably improve the mechanical properties of the material obtained from such mixtures (Zhang et al., 1997). Although, most of polymers blends are immiscible with each other due to the absence of specific interactions, TEM micrographs of collagen-chitosan composites have shown that chitosan network can interpenetrate into the collagen network; the chitosan phase is wrapped in the collagen phase and is denser; besides, the amount of chitosan phase grows with the content of chitosan increasing (Zhang et al., 1997).

The main kinds of interactions that can give rise between the two polymers when they are in contact with water are: an electrostatic complex and an hydrogen bonding type of complex, in the presence of a great excess of chitosan (Taravel & Domard, 1993; Taravel & Domard, 1995). Hydrogen bonds between collagen and chitosan can be formed as follows: between either a carbonyl, hydroxyl, or an amino group from collagen, and either hydroxyl, amino, or a carbonyl group from chitosan. The formation of hydrogen bonds between two different macromolecules competes with the formation of hydrogen bonds between molecules of the same polymer (Mo et al., 2008). Apparently, interactions between collagen and chitosan depend on the structural organization of collagen and the amount and distribution of charges along the polymer chains. These properties are directly related to the pH of the medium, which is of fundamental importance for the study of interactions present between the biopolymers (Tohni, 2002).

### **5.1 Stability**

It is known that some environmental conditions may induce degradation of biopolymers, mainly affecting the mechanical properties and limiting their presentation, therefore the stability of the film is also a very important characteristic to evaluate. Lima et al. (2006) detected that a more stable film can be obtained when the chitosan in a collagen-chitosan film reaches 50% because chitosan increases the organization of the microscopic structure of the collagen. The effect of solar radiation on the properties of collagen, chitosan, and collagen-chitosan films, have been reported. Research of Sionkowska (2006), Sionkowska et al. (2006), and Sionkowska et al. (2011) indicated that collagen-chitosan blends are more sensitive to the action of UV irradiation or artificial solar light than pure collagen or pure chitosan films.

### **5.2 Applications of collagen/chitosan blends**

Biomaterials from collagen/chitosan blends with different applications have been successfully obtained (Table 5). However, it is important mentioned that the biological or mechanical properties, as well as stability of the product, depends of the compositions of the blend (Table 5).



Composition	Main characteristics	Reference
Collagen/Chitosan composite hydrogel	Hydrogel system stable Capable of encapsulating T $\beta$ 4	(Chiu & Radisic, 2011)
Gelatin -Chitosan films	Water resistant Deformable Increase in breaking strength Rubbery semi-crystalline materials Antimicrobial activity Biodegradable films	(Gómez-Estaca et al., 2011)
Collagen/Chitosan scaffolds fabricated via thermally triggered cofibrillogenesis approach	Electrostatic interactions Intereconnected porous structure Excellent mechanical properties Collagen-chitosan scaffolds with superior characteristics	(Wang & Stegemann, 2011)
Electrospun 80 % Collagen/20 % Chitosan fibers	Fibrous membrane softer, flexible and elastics	(Chen, Z. et al., 2009)
Electrospun 20 % Collagen/80 % Chitosan fibers	Fibrous membrane inflexible, less compact	(Chen et al., 2009)
Anionic collagen /Chitosan sponges by freeze lyophilization	Polyelectrolytic interaction Low thermal stability Small porous size Promising feature to be processed into porous structures Application in cell transplantation and tissue regeneration	(Horn et al., 2009)
Chitosan/Gelatin copolymer	Very suitable for coating meat products	(Shane & Champa, 2009)
Collagen/Chitosan Scaffold	It accelerates of cellular proliferation; It reduces the biodegradation rate. Materials for tissue engineering.	(Tangsadthakun et al., 2007)
70% Collagen-30% Chitosan Films	Thermally stable Less mechanical stable	(Sionkowska et al., 2006)
50% Collagen-50% Chitosan Films	Low thermal stability Low mechanical stability	(Sionkowska, 2006)
Collagen/chitosan composite microgranules	Effective for the controlled release of transforming growth factor beta 1 Satisfy specifications for cartilage tissue engineering	(Lee et al., 2006)
Collagen /Chitosan Scaffold	Porous composite matrix Good tensile strength Biocompatible and biodegradable May be used as a chondrocyte carrier for cartilage tissue engineering	(Shi et al., 2005)
Complex membranes of collagen/chitosan.sodium hyaluronate	Material suitable for tissue-engineered cornea	(Chen et al., 2005)

Table 5. Properties of collagen/gelatin-chitosan blends.

## 6. Chitosan and acid soluble collagen from jumbo squid by-products blend films

It has been demonstrated that collagen/chitosan is miscible at any composition range in acetic acid solution and the compatibility will remain even when the solvent is absent, being possible the compatibility in a solid state (Dan et al., 2007). Collagen in acid solution exhibited positively charged groups and it has a molecular interaction with chitosan (Figure 4) with high potential to produce biocomposites with novel properties as was previously described (Liang et al., 2005; Lima et al., 2006; Sionkowska et al., 2006; Wang et al., 2005; Wess et al., 2004). Besides that, acid soluble collagen might be useful as a new source of plasticizer agent in the preparation of biofilms in composites with chitosan (Uriarte-Montoya et al., 2010).

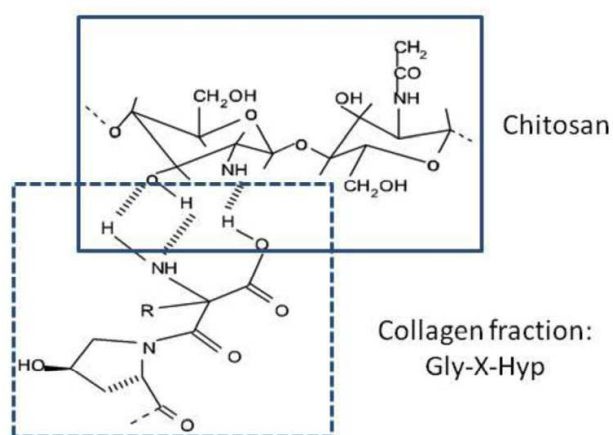


Fig. 4. Schematic presentation of chitosan-collagen fraction molecular interaction.

### 6.1 Molecular interactions in chitosan and acid soluble collagen from jumbo squid films

The molecular interaction between acid soluble collagen from jumbo squid (ASC) and chitosan was reported to be mainly due to hydrogen bonding between the two polymers (Uriarte-Montoya et al., 2010). These observations were based on the modification of the amplitude bands in infrared (FTIR) analysis. FTIR spectroscopy is an useful tool to study the hydrogen bonding and other interactions as well as the miscibility of polymer blends (Zhang et al., 2002).

The typical peaks of chitosan are around 2890, 1645, 1563, and 1414  $\text{cm}^{-1}$  which correspond to aliphatic groups ( $-\text{CH}_2$  and  $-\text{CH}_3$ ), amides I, II, and vibrations of  $-\text{OH}$  groups from primary alcohols, respectively. The amide I arises from  $\text{C}=\text{O}$  stretching; the amide II arises from  $-\text{NH}$  torsion groups. When a shoulder at 1645  $\text{cm}^{-1}$  is detected, it suggests that chitosan comes from a partial desacetylation process (Wess et al., 2004). The addition of ASC to commercial chitosan induced a decrease in the bands around 1645, 1563, and 1414  $\text{cm}^{-1}$  (Uriarte-Montoya et al., 2010) indicating that there is an interaction between the polymers and the compatibility between collagen and chitosan, which results in good film homogeneity.

On the other hand, the differential scanning calorimetry (DSC) analysis has been used by different authors to elucidate how the collagen associates to other macromolecules (Privalov & Tiktopoulo, 1970). The glass transitions temperatures ( $T_g$ ) is an important criteria for the miscibility of the components. In a completely miscible blend of two polymers, only one  $T_g$  will appear in the DSC thermograms (Suyatma et al., 2005). Uriarte-Montoya et al. (2010) and Arias-Moscoso et al. (2011) detected that in chitosan-ASC films at 85-15 and 50-50 respectively, the  $T_g$  value of was lower than that of the chitosan film. According with the theory of plasticization, those results confirm that chitosan-ASC have good miscibility (Suyatma et al., 2005), and suggests that collagen may act as plasticizer in chitosan film structure.

## 6.2 Characterization of chitosan/acid soluble collagen from jumbo squid blends

Films produced by mixing collagen from jumbo squid and chitosan are easily obtained by using a casting plate (Arias-Moscoso et al., 2011). These films usually are opaque, soft, with porous structure, and hygroscopic (Figure 5), which also poses poor water barrier properties and slight acidic smell (Arias-Moscoso et al., 2011). The last mentioned properties may produce films not suitable to prevent deterioration in some kinds of products; however, might be suitable for medical or pharmaceutical applications and, to avoid the acidic smell, a neutralization process may be applied.

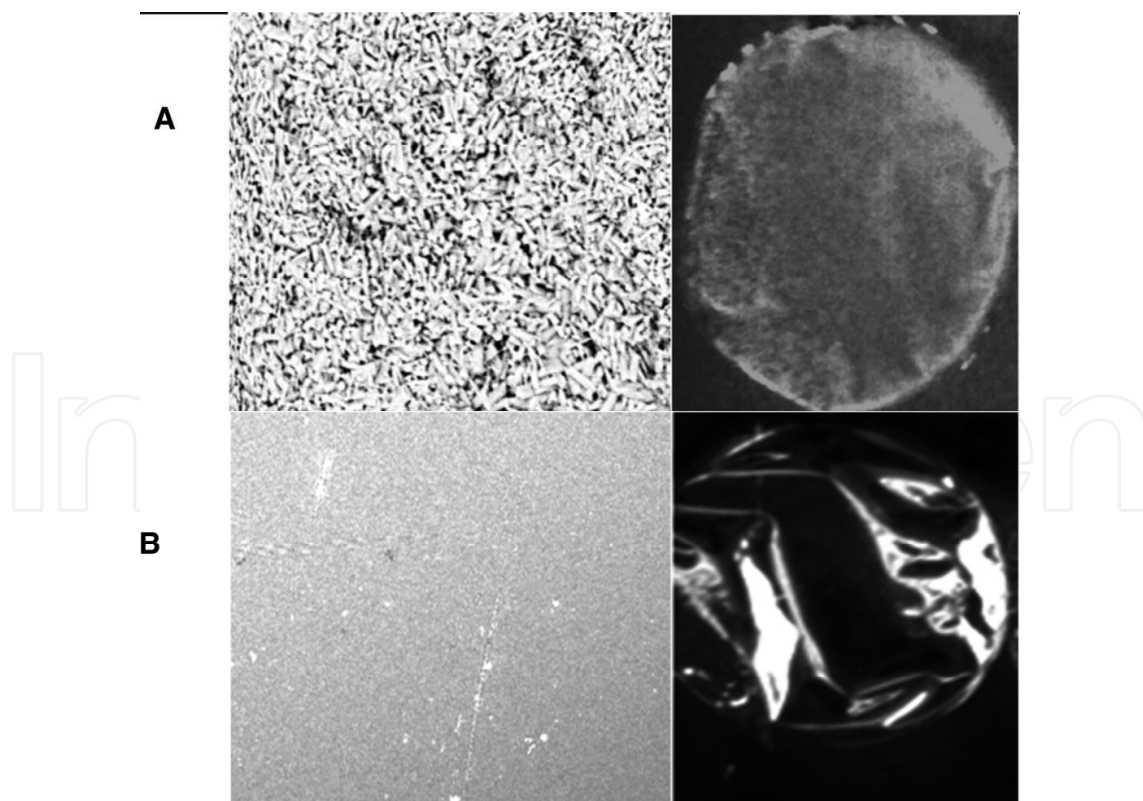


Fig. 5. Micrograph and picture of acid soluble collagen obtained from jumbo squid by-products-chitosan (A) and chitosan (B) films.

### 6.2.1 Mechanical properties

The acid soluble collagen from jumbo squid induces modification of crosslinking reaction in chitosan films affecting their mechanical properties (Table 6). In general, the presence of acid soluble collagen from jumbo squid on chitosan films induces the formation of more flexible and less rigid films (Arias-Moscoso et al., 2011; Uriarte-Montoya et al., 2010).

According with Arias-Moscoso et al., (2011) the general trends of the stress-strain curves of acid soluble collagen-chitosan films are characteristic of elastic films. However, these tensile properties vary with the collagen content on the film. It was detected that the tensile strength values of most of ASC/chitosan films evaluated were within the acceptance range of low density polyethylene food packaging (4 and 78.6 mPa<sup>42</sup>). Meanwhile, the elongation at the break of chitosan films with 20% of ASC was comparable to cellophane and also comparable to polypropylene when contained 50% of ASC, which for some specific applications is a desirable attribute.

Properties	Films	
	CH	ASC/CH
Tensile strength (MPa)	46.5-53.7	1.4-39.6
Elongation at break (%)	4.9-5.5	11.0-4.3
Elastic modulus (Mpa)	2210-2514	660-2430

Adapted from Arias-Moscoso et al. (2011)

Table 6. Mechanical properties of acid soluble collagen from jumbo squid-chitosan (ASC/CH) and chitosan (CH) films.

It is known that the mechanical properties are dependent on the distribution and intensity of inter- and intramolecular interaction; therefore, when the content of ASC increases, the collagen molecules also increases, and this may reduce chitosan interaction, inducing high possibilities of movements, producing films more flexible and less rigid.

## 7. Conclusion

It is possible to recover and utilize compounds from squid wastes, which may represent a potential for increased business and more ways to make a more environmentally sounded use of natural resources from this seafood product. From this wastes or by-products collagen can be obtained.

ACS can be extracted from the muscle jumbo squid with 0.5 M acetic acid with an average yield of 15% from the total muscle protein, and showed comparable biochemical characteristics to the collagen of skin from squid species, but it presented higher transition temperature, similar to type I collagen from bovine skin.

Novel biomaterials from collagen-chitosan blends, with excellent biocompatibility and antibacterial properties can be successfully prepared. One of the applications of these blends is the elaboration of films. Moreover, the interactions between collagen and chitosan may exhibit great potential in areas not much exploited such as plasticizers.

Chitosan, a polysaccharide that is produced by deacetylation of naturally occurring chitin, has a great potential for a wide range of applications due to its biodegradability,

biocompatibility, antimicrobial activity, non-toxicity, and versatile chemical and physical properties. Acid soluble collagen/chitosan blends are miscible and interact at the molecular level, although pure chitosan, as a film material, poses low percentage of elongation, it is possible to improve its elasticity with the addition of biodegradable materials. The film properties of the chitosan can be enhanced by adding collagen to the blend, where both polymers interact by electrostatic and hydrogen bonding.

ASC from jumbo squid and chitosan blends are miscible and interact at the molecular level, being hydrogen bonding the most abundant interaction forces between the polymers. These interactions affect the chitosan films properties. Opaque and more elastic chitosan films are obtained by the incorporation of acid soluble collagen.

The general trend of the stress-strain curves of acid soluble-chitosan films was characteristic of elastic films where collagen showing a conventional action of plasticizers (increase in elongation and decrease in strength). Therefore acid soluble collagen from jumbo squid is suitable to be used as additive to improve the elastic properties of the chitosan films.

This chapter only covers a limited introduction to collagen as bio-plasticizer. However, in view of the advances in technologies for recovering collagen from seafood catch and processing discards that should environment friendly and the continue need within the seafood industry to find alternative products, further research efforts should also be directed toward ways to evaluated the industrial potential of collagen as an alternative to traditional petroleum-based plasticizer, and provide its comparison as a chitosan or other polymer plasticizer with other bio-plasticizer.

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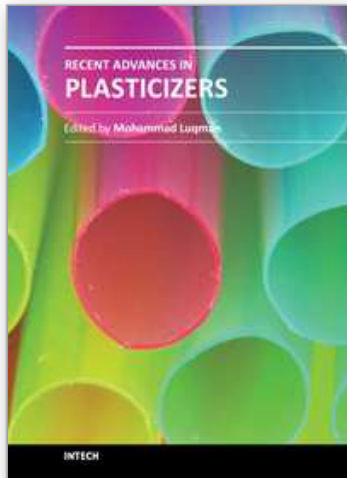
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Plasticizers are used to increase the process-ability, flexibility, and durability of the material, and of course to reduce the cost in many cases. This edition covers introduction and applications of various types of plasticizers including those based on non-toxic and highly effective pyrrolidones, and a new source of Collagen based bio-plasticizers that can be obtained from discarded materials from a natural source; Jumbo Squid (*Dosidicus gigas*). It covers the application of plasticizers in plastic, ion-selective electrode/electrochemical sensor, transdermal drug delivery system, pharmaceutical and environmental sectors. This book can be used as an important reference by graduate students, and researchers, scientists, engineers and industrialists in polymer, electrochemical, pharmaceutical and environmental industries.

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