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REVIEW ARTICLE

Extraction and antioxidant activity of sericin, a protein from silk

Extração e atividade antioxidante da sericina, uma proteína da seda

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Abstract

Sericin is a globular protein that represents 20% to 30% of the silk fiber from *Bombyx mori* silkworm cocoon. This protein is usually removed from the raw fiber and discarded by silk producers, a process known as degumming. However, sericin possesses significant biological properties that allows its application in various fields. The antioxidant activity is one of its most relevant benefits. Several authors have reported its anti-tyrosinase activity, lipid peroxidation inhibition and free radical neutralization. The antioxidant potential of sericin protein varies according to the extraction method used. Even though a wide variety of extraction techniques have been studied, simple technics including water at high temperature have exhibited efficient results. Furthermore, this method does not interfere with the safety of sericin for subsequent applications in food.

Keywords: *Bombyx mori*; Degumming; Biological properties; Food application; Oxidative stress; Tyrosinase activity; Lipid oxidation.

Resumo

A sericina é uma proteína globular que representa entre 20% e 30% da fibra do bicho-da-seda *Bombyx mori*. Tem propriedades biológicas importantes, que permitem sua aplicação em vários campos, entre os quais se destaca a atividade antioxidante. Vários autores têm apontado que possui atividade anti-tirosinase, inibe a peroxidação lipídica e neutraliza os radicais livres nocivos ao corpo humano. A atividade antioxidante da sericina varia conforme o método de extração, conhecido como processo de desgomagem, e as condições de ensaio. Existem diferentes metodologias para este processo, algumas mais simples, que envolvem o uso de autoclave, e outras mais sofisticadas, como ultra e nanofiltração. A extração com água a temperaturas elevadas é eficiente e não interfere na inocuidade da sericina para aplicações subsequentes em alimentos.

Palavras-chave: *Bombix mori*; Desgomagem; Propriedades biológicas; Aplicação em alimentos; Estresse oxidativo; Atividade da tirosinase; Oxidação lipídica.

1 Introduction

Silk is a natural fiber derived from a variety of species, including silkworms and spiders. Among these, the most studied have been the silk fiber from the *Bombyx mori* worm (Gonzalez et al., 2014). Silk is a protein fiber that consists of sericin and fibroin (Pescio et al., 2009). Fibroin protein comprises approximately 70% to 80% of the silk fiber. It has amorphous and crystalline domains with short amino acidic chains that allow it to maintain its compact structure (Koh et al., 2015). Sericin is a globular protein that constitutes about 20-30% of the silk fiber. Its role is to coat and link the fibroin filaments together in the worm cocoons (Aghaz et al., 2015). Furthermore, the sericin protects the cocoon against UV radiation, wind, rain and low temperature (Cao & Zhang, 2016). Figure 1 shows silk fibers obtained via scanning electronic microscopy (SEM). In the figure, sericin and fibroin proteins are indicated to provide a clear comprehension about the structure of the silk fiber.

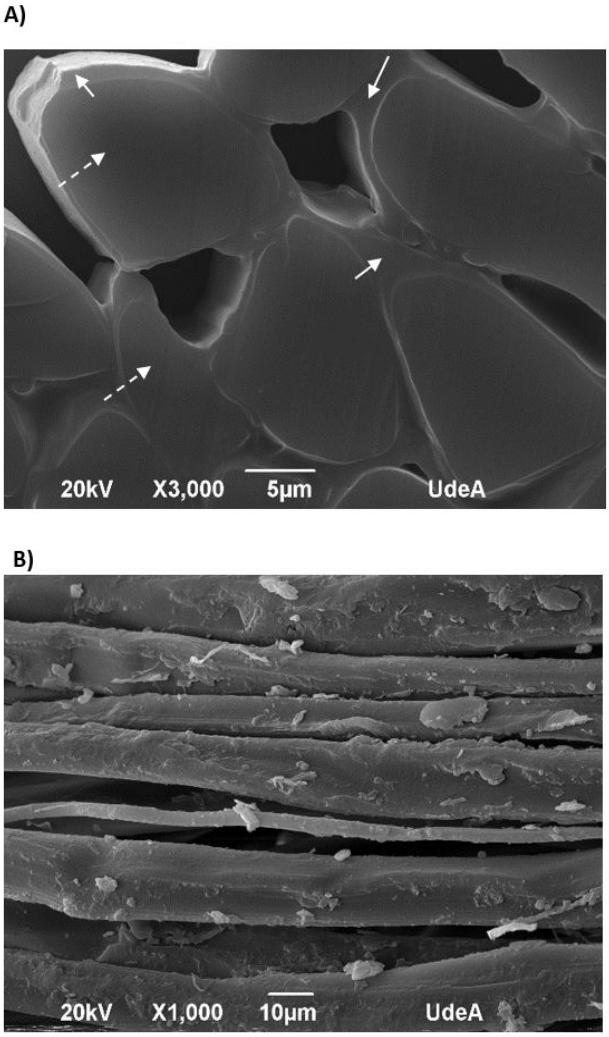

Figure 1. Silk fiber images obtained by scanning electron microscopy – SEM– in the Microscopy Department from Universidad de Antioquia (UdeA). (A) Transversal view; white solid arrows refer to the sericin which surrounds the fibroin fiber, while fibroin is indicated with white dashed arrows; (B) Longitudinal view.

The antioxidant potential of the sericin is related to its high content of amino acids with hydroxyl groups (mostly serine), which act as chelators as described in the following sections (Micheal & Subramanyam, 2014). The presence of phenolic and flavonoid compounds in the adjacent layers of the sericin protein not

only provide coloration to the cocoon, but they also contribute with the sericin's antioxidant activity (Aramwit et al., 2010a; Prasong, 2011; Zhao & Zhang, 2016; Napavichayanun et al., 2017).

In the silk industry, sericin is removed from the fibroin and subsequently discarded. However, it has been shown that this protein presents interesting properties which may allow its application in several fields. Due to its moisturizing and anti-wrinkling abilities, sericin is an interesting compound to the cosmetic industry (Sothornvit et al., 2010; Züge et al., 2017). Additionally, sericin has been studied with biomedical purposes, as biomaterial and drug delivery (Cao & Zhang, 2016; Lamboni et al., 2015; Srnivas et al., 2014; Suktham et al., 2018), mainly because it was reported to be immunologically inert (Lamboni et al., 2015). The sericin's antioxidant activity is one of the most significant property because it could provide positive effects on people health and in the food industry as a natural food preserver (Puangphet et al., 2015).

In the body, the antioxidants maintain a balance between formation and elimination of reactive oxygen species (ROS) and nitrogen. High ROS levels can be detrimental for the cell, affecting proteins, lipids and DNA, and consequently to the physiological functions of the organism. This process is known as oxidative stress (Micheal & Subramanyam, 2014) and is associated with neurodegenerative diseases, aging, arthrosclerosis and cancer (Schinella, 2015). The human body possess an antioxidant system that involves enzymatic complexes, vitamins and other specialized molecules. In addition, there are exogenous antioxidants provided by food such as fruits, vegetables and dietary supplements, which contribute to the body antioxidant defense. These type of antioxidants becomes more relevant when the person is exposed to high levels of oxidative stress (Pisoschi & Pop, 2015). Besides the concern of consuming antioxidants in terms of health benefits, there is a trend from the food industry to include natural antioxidants to foodstuff. This is possible because antioxidants can retard and prevent the lipid oxidation and therefore improve the food quality and its nutritional value. This approach substitutes the use of synthetic antioxidants, some of them associated to carcinogenic effects (Reddy et al., 2005; Caleja et al., 2017).

Therefore, sericin could have multiple attributes as a food ingredient due to its role in food preservation (Jassim & Alsaree, 2010; Sarovart et al., 2003; Doakhan et al., 2013) and in human health promotion as well. Furthermore, there are evidence about other positive effects due to sericin consumption. For instance, it has been reported that sericin ameliorates the constipation in rats, it increases the intestinal mineral absorption and it also possess a prebiotic function (Patel & Goyal, 2012). Further details about these effects are given below.

This review collects the different methods and conditions to extract sericin described in the literature and provides an updated overview of the antioxidant potential of the sericin in terms of human health and food preservation.

2 Sericin characteristics

Sericin protein consists of 18 different amino acids, most of them are characterized as polar. Table 1 shows the most prevalent amino acids in sericin reported in the literature. The differences observed among the authors regarding the amino acid proportion is due to the method of sericin extraction. Indeed, a study comparing the amino acid profile when using different extraction methods (alkali, acid, urea and temperature) found significant discrepancies in their ratio (Aramwit et al., 2010a). Nevertheless, all authors agreed that serine was the most abundant amino acid, followed by aspartic acid and glycine. Likewise, sericin is organized into layers: outer, middle and inner. The outer layer is the most soluble, meanwhile the layer adjacent to the fibroin can be only removed with high pressure, high temperature or alkalis compounds (Cao & Zhang, 2016). Under these treatments β-sheet structures are degraded and consequently the protein is denaturalized, increasing thereafter its water solubility (Lamboni et al., 2015). The extraction process of sericin from the silk fiber is known as *degumming*.

Table 1. Amino acid composition of the sericin protein according to the literature.

Ser	Asp	Gly	Glut	Thr	Arg	Ala	Val	Leu	Lys	Reference
24.61	18.79	8.94	7.77	7.65	5.56	5.34	4.92	4.67	Na	Li et al. (2015)
32.55	14.42	12.17	4.81	7.48	4.45	5.71	6.31	2.03	0.60	Yang et al. (2013)
30.4	19.1	12.2	4.1	3.8	2.8	4.6	2.6	0.6	10.2	Sasaki et al. (2000)
31.0	17.8	19.1	4.4	8	3.9	3.8	3.1	0.8	2.7	Kato et al. (1998)
25.5	18.38	17.85	5.74	7.47	3.12	6.70	4.05	1.49	2.08	Cao & Zhang (2016)
28.89	19.81	10.75	6.98	7.81	4.39	4.65	3.12	1.12	3.51	Li et al. (2008)
32.74	17.64	9.89	7.31	5.51	6.16	3.86	3.14	1.44	3.05	Sothornvit et al. (2010)
21.56	14.0	23.20	3.30	7.04	11.95	Na	3.36	2.08	3.18	Züge et al. (2017)
33.73	20.82	9.53	4.98	8.57	5.22	1-3	1-3	Na	1-3	Puangphet et al. (2015)
25.55	17.33	8.23	5.79	5.04	3.48	2.80	2.57	0.92	3.14	Wu et al. (2008)
37.3	14.8	14.7	3.4	8.7	3.6	4.3	3.6	1.4	2.4	Gonzalez et al. (2014)

Values are shown in Mol (%). Ser = serine, Asp = asparagine, Gly = glicine, Glut = glutamine, Thr = threonine, Arg = arginine, Ala = alanine, Val = valine, Leu = leucine, Lys = lysine, Na = not available data.

3 Extraction methods of sericin

A great diversity of extraction methods has been studied, either from wastewater provided by silk industries or directly from the silk fiber. Sericin characteristics, such as purity, molecular weight, antioxidant activity and biochemical properties, vary according to the method used (Aramwit et al., 2010a, 2010b; Li et al., 2008). After extraction, molecular weights from 10 kDa to 310 kDa have been reported (Kumar et al., 2015). Most of the extraction methods existing in the literature are enumerated in Table 2.

Table 2. Methods and conditions described in the literature to extract sericin.

Extraction method	Condition of extraction	Outcome	References
Denaturing agents (boiling with detergents, alkaline compounds and chaotropic agents)	Urea (8M) for 30 min	10 to > 225 kDa sericin size, 18% to 20% yield.	Aramwit et al. (2010a) Aramwit et al. (2010b)
	Sodium carbonate (0.5%) for 30 min.	15 to 75 kDa sericin size, 6% to 12% yield.	
	Sodium oleate (0.3%) and sodium carbonate (0.2%) for 60 min.	High-purity sericin was recovered with calcium chloride	Yang et al. (2013)
	Calcium hydroxide (0.025%) for 40 min and neutralization with acids	< 20 kDa sericin size, more than 85% recovery	Zhao et al. (2018),
	Novel surfactant based on silk amino acids and lauryl chloride (0.2%) for 30 min.	Similar quality when using neutral soap, but eco-friendlier agent	Wang et al. (2015)
	Neutral soap (0.2%)	Longer processing time.	Wang et al. (2015)
	Coconut soap and sodium bicarbonate for 45 min. Precipitation with 75% ethanol after drying.	74.5% yield from silk wastewater, 63.8% sericin purity	Álvarez et al. (2013)
Enzymatic digestion	Commercial proteolytic enzymes for 5-240 min. at 50-65 °C	5 to 20 kDa sericin size with a weight-average molecular weight of about 12 kDa	Freddi et al. (2003)
	Novel protease isolated from *Bacillus sp.*	Efficient degumming, sericin was not characterized	Suwannaphan et al. (2017)
	Alcalase and Savinase at 55 °C		Arami et al. (2007)
	Alcalase/Savinase and ultrasound		Mahmoodi et al. (2010)
Infrared rays	IR heating extraction from silk wastewater	21% to 26% yield, a clean sericin is obtained, eco-friendly process	Gupta et al. (2013)

Table 2. Continued…

Extraction method	Condition of extraction	Outcome	References
Polymeric membranes	Membrane of polysulfone in by phase inversion with polydioxolane and polyethylene glycol	10 kDa - 250 kDa sericin size recovered from silk wastewater	Sonjui et al. (2009)
Steam (Autoclave)	120 °C for 60 min. The liquor ratio was 1:25. Sericin was concentrated by rotavapor.	A film made of glycerol/sericin was satisfactorily carried out to assess its use as a polymeric material.	Yun et al. (2016)
	82-120 °C (above 105 °C using autoclave) and time in the range of 10-60 min. Separation by hydraulic pressing at 2.5 MPa for 1 min and drying.	Extracted sericin exhibited mainly serine (18.24%) and 132 kDa molecular size. Effective to film formation.	Sothornvit et al. (2010)
	70-65 °C for 60 min.	Total phenolic compound correlated with amount of sericin extracted.	Prasong (2011)
	Autoclaved at 121 °C for 30 min/45 min/ 60 min.	16 to 44 kDa molecular size. Clean product. Lower yield compared to alkaline extraction.	Srnivas et al. (2014)
	Autoclaved 120 °C for 60 min and dried by spray- drying.	Sericin microparticles with an average diameter < 10 µm were obtained.	Chlapanidas et al. (2013)
	Autoclaved 120 °C for 60 min	Higher thermal stability compared with urea and alkaline extraction methods.	Aramwit et al. (2010b)
	Autoclaved 120 °C for 60 min and lyophilization or freezing-thawing precipitation	20 kDa to 400 kDa sericin size. Radical scavenging effect and antibacterial.	Rocha et al. (2017)
	Autoclaved at 121 °C for 30 min, filtered and dialyzed	Sericin as biomaterial is assessed.	Chirila et al. (2013)
	Autoclaved at 121 °C for 30 min, and a liquor ratio 1:30 (w/v), filtered and lyophilized.	Source of sericin extraction (cocoons or yarns) affects the final product.	Castrillón Martínez et al. (2017)
	Steam 120 °C for 60 min and precipitation with ethanol at various ratios.	Precipitation of hydrophobic SS and removal of low molecular weight SS, enhanced mechanical stability	Oh et al. (2011)
Ultrafiltration-Nanofiltration	Silk wastewater was centrifuged, crystallized and ultrafiltered with polyethersulfone membranes	Sericin is separated from fatty acids derived from soaps added during industrial degumming.	Capar et al. (2009)
	Hollow fiber nanofiltration membrane integrated with ultrafiltration	86% sericin from cocoon silk wastewater could be recovered. Isolated sericin was not characterized.	Li et al. (2015)
	Autoclaved and recovery by ultrafiltration with hollow fiber membrane of polyethersulfone	High molecular sericin is obtained	Silva et al. (2012), Gimenes et al. (2014)

3.1 Extraction with detergents and alkaline compounds

Detergents and soaps lead to protein denaturalization and partial hydrolysis of the silk filament chains (Cao & Zhang, 2016). Sodium carbonate (Aramwit et al., 2010b; Yang et al., 2013), calcium hydroxide (Zhao et al., 2018) and non-ionic detergents (Mahmoodi et al., 2010) have been utilized for the degumming process. Even though this method is widely used by silk processing industries, it is considered as a non- desired approach due to the presence of these alkaline compounds in the residual water (Wang et al.,

2015; Mahmoodi et al., 2010). Furthermore, the subsequent isolation of the sericin from the detergent is a complex process (Lamboni et al., 2015).

3.2 Extraction with steam using autoclave

This process consists of the removal of sericin through high temperature and pressure. Due to the high temperature applied, hydrogen bonds between hydroxyl groups become unstable allowing the water to interact with polar amino acids of the sericin (Silva et al., 2012). The molecular weight of the samples obtained is in the range of 27 to 200 kDa (Rocha et al., 2017). This method is quite simple, likewise a good quality and clean product is attained (Lamboni et al., 2015). However, some studies argue that this method could degrade the sericin protein. Furthermore, factors such as the temperature and the extraction time are involved in the variation of the molecular weight obtained (Oh et al., 2011; Silva et al., 2012). The yield obtained through the use of steam is lower compared to extraction by alkaline compounds (for instance, 0.5% calcium carbonate) (Srnivas et al., 2014). On the other hand, the absence of chemical compounds and the lower water consumption that required this method, contribute positively to the environment and consequently, to the sustainable feature of the method (Wang et al., 2018).

3.3 Extraction with enzymes

This process comprises the elimination of sericin from the silk fiber assisted with proteolytic enzymes. Alkaline and neutral proteases have shown an efficient degumming. After removed, sericin is recovered by drying. The enzyme dosage and treatment time influence the kinetics of the process. Moreover, chemical properties of soluble sericin peptides varies as according to the enzyme utilized. Peptides in the range of 5 to 20 kDa are obtained and these are free of alkali and fatty acids (Freddi et al., 2003). Whereas this approach is slightly more expensive than the technics described above, less energy is required. Consequently, this process becomes more sustainable (Wang et al., 2018). The combined use of enzymes (savinase and alcalase) and ultrasound to extract the sericin from the silk fiber was also assessed. In this case, the efficacy of the degumming process increased along the treatment time. However, the integrity of the sericin isolated have not been studied by the authors (Mahmoodi et al., 2010). Interestingly, an extracellular protease isolated from *Bacillus sp.* has shown a high specificity to remove sericin from the silk at a pH slightly alkaline. The peptides obtained from the proteolytic activity by the purified enzyme consists in 10 to 12 kDa (Suwannaphan et al., 2017). Similarly, a thermostable alkaline serine protease from a bacterium (*Bacillus hallodurans*) capable of remove sericin from the silk was recently isolated. This novel protease has exhibited a higher degumming ability than commercial alcalase proteases (Yakul et al., 2019).

3.4 Other methods

More sophisticated technics have also been studied. For instance, a process that combines acid precipitation, ultrafiltration and nanofiltration revealed an efficient performance. The sericin from industrial degumming water was recovered up to 86% through this complex technique (Cao & Zhang, 2016; Li et al., 2015). Other techniques use infrared rays and presents additional advantages such as the use of lower temperatures, it does not use as much water as other methods, and superior yield is obtained. However, if greater amounts need to be recovered, the process costs are higher. Further extraction methods such as sonication and the use of polymeric membrane have also been studied by a few authors (Mahmoodi et al., 2010; Sonjui et al., 2009).

4 Antioxidant activity of sericin protein

several studies have demonstrated that isolated sericin prevents oxidative stress and lipidic peroxidation (Kato et al., 1998; Micheal & Subramanyam, 2014; Chlapanidas et al., 2013; Takechi et al., 2014; Khyade,

2016; Napavichayanun et al., 2017; Deori et al., 2016; Sangwong et al., 2016; Kumar & Mandal (2017); Fan et al., 2010; Kaewkon et al., 2012; Zhaorigetu et al., 2001; Dash et al., 2008), and inhibits tyrosinase activity (Aramwit et al., 2010a; Kato et al., 1998; Thongsook & Tiyaboonchai, 2011; Puangphet et al., 2015; Wu et al., 2008; Manosroi et al., 2010). Table 3 provides an overview of studies supporting the sericin's antioxidant activity.

4.1 The role of sericin against lipid oxidation

The reaction between a polyunsaturated lipid and oxygen is known as lipid peroxidation. The secondary products, which result from this process are not only harmful for the cellular metabolism, but they also alter the quality of food, modifying the texture, flavor and even the color in some occasions (Samaranayaka & Li-Chan, 2011).

Several studies were carried out to investigate the protective role of the sericin in cell tissues. Kato et al. (1998), studied the sericin's antioxidant activity by observing the inhibition of the lipid peroxidation in homogenized brain extracts from mice treated with sericin. They found that those tissues incubated with sericin for longer time, presented a low number of products derivate from lipid peroxidation when comparing with control, where higher oxidation was observed (Kato et al., 1998). A similar study was performed by Khyade (2016), by using feline fibroblasts previously treated with hydrogen peroxide to induce oxidative stress. The samples incubated with sericin also exhibited a remarkable lower concentration of products from lipid peroxidation in this experiment when comparing with the non-sericin treated tissue (Khyade, 2016).

The protective function of sericin on lipid oxidation in foodstuff was likewise investigated. Fan et al. (2009) studied the effect of sericin in the inhibition of lipid peroxidation, specifically in linoleic acid. For this purpose, sericin was extracted by hot water, filtered and lyophilized. They found an inhibition of approximately 80% of peroxides from linoleic acid, being this effect potentially higher than the synthetic antioxidant butylated hydroxytoluene (BHT) (Fan et al., 2009). These findings suggest that sericin prevents the oxidation process in which lipids are involve. Furthermore, as it commented in the following section, it inhibits the enzyme tyrosinase, responsible of the oxidation process that causes the browning of some fruits and vegetables.

4.2 Effect of sericin in the inhibition of tyrosinase activity

Among the antioxidant mechanism of the sericin, the inhibition of the tyrosinase activity has been widely studied. Tyrosinase enzyme, also known as poliphenoloxidase, catalyzes the hydroxylation from monophenols to diphenols and the oxidation of these to quinones. This process results in the formation of melanin, which causes the browning effect observed in some fruits and vegetables (Xing et al., 2016). The fact that tyrosinase is also implicated in diseases such as cancer and Parkinson, causes that compounds with possible anti- tyrosinase effects are being widely investigated either for biomedical or food applications (Aramwit et al., 2010a; Xing et al., 2016).

Several studies have reported anti-tyrosinase activity of sericin from cocoons belonging to different strains and from different extraction methods as well. Aramwit et al. (2010a) compared the inhibition of the tyrosinase when sericin from *Bombyx mori* was extracted by four different methods: urea, temperature, acid and alkaline compounds. The extraction with urea exhibited the highest anti-tyrosinase activity. This might be caused by the large amounts of valine and arginine amino acids that results from this extraction, which are known to have more affinity to bound to tyrosinase enzyme and inhibit it. On the contrary, peptides with larger proportion of tyrosine, act as a substrate for the enzyme, thus promoting its oxidative activity (Aramwit et al., 2010a). Kato et al. (1998) measured the tyrosinase activity from a mushroom enzyme when incubating with 1% and 0.5% sericin, which was previously extracted by hot water, filtered and spray dried. They found an inhibition of 50% and 25% of the tyrosinase activity with 1% and 0.5% of sericin respectively

(Kato et al., 1998). Interestingly, the influence of worm's feed, either artificial or natural, and the worm strain on the sericin anti-tyrosinase activity, was further investigated by Chlapanidas et al. (2013). 20 strains were analyzed. Among these *Nistari, ADPR, Sajaku Green BG* and *Daizo* presented the highest antioxidant activity with a 60% to 80% of tyrosinase inhibition compared to positive control (100%). Regarding to the type of feed, worms fed with fresh mulberry leaves showed to increase this potential. Therefore, both strain and feed have an effect on the anti-tyrosinase activity (Chlapanidas et al., 2013).

Similarly, biopeptides of sericin, obtained by enzymatic hydrolysis, exhibited a higher antioxidant potential compared to non-hydrolyzed sericin (Wu et al., 2008; Puangphet et al., 2015, 2018). According to Puangphet et al. (2018), sericin biopeptides containing larger amounts of serine and asparagine present the most inhibitory effect (Puangphet et al., 2018). The type of protease used for the hydrolysis also influences the effect, where alcalase showed the best performance (Fan et al., 2010). The authors suggest that the reason behind the increase of the anti-tyrosinase activity observed with hydrolyzed sericin, is the metal-chelating ability. Since tyrosinase enzyme requires copper ions to be active (Puangphet et al., 2015), the sericin binds to the copper atoms of tyrosinase enzyme, and therefore its oxidant activity is blocked. This effect is due to the presence of a high content of amino acids with hydroxyl groups, such as serine, asparagine and threonine acting as chelators (Micheal & Subramanyam, 2014). Furthermore, the hydrophobic section of these amino acids might contribute to their binding in the hydrophobic pocket near the active site of tyrosinase, causing its inhibition (Puangphet et al., 2018). These findings make the sericin an interesting candidate to retard the browning effect in foodstuff without the addition of synthetic antioxidant compounds.

4.3 Sericin effect in Reactive Oxygen Species (ROS)

It has been shown that sericin has a scavenger effect, stabilizing free radicals, which are responsible of oxidative damage (Micheal & Subramanyam, 2014; Chlapanidas et al., 2013; Takechi et al., 2014; Li et al., 2008). Chlapanidas et al (2013) found a ROS-scavenging activity of 80% exerted by sericin extracted from *Bombyx mori* cocoon when compared to a positive control (100%) (Chlapanidas et al., 2013). Li et al. (2008) observed that sericin partially reverted the oxidative stress induced by alcohol in mice liver (Li et al., 2008). Manosroi et al. (2010) compared the sericin scavenger ability when it was extracted using autoclave and through a hydrolysis with alkaline compounds. The most effective effect was shown with the last named technique where the antioxidant activity was even higher than those obtained with C and E vitamins (Manosroi et al., 2010). Moreover, there was found an inverse relation between the sericin molecular size and the antioxidant activity. When sericin size decreased by the action of proteases, the scavenger activity increased (Sangwong et al., 2016). In a recent study, the radical scavenging activity of a sericin extract obtained by a novel isolated bacterial protease was compared with a sericin extract produced by a commercial alcalase. The antioxidant potential from the bacterial purified protease was significantly higher than those treated with a commercial protease (Yakul et al., 2019).

4.4 Effect of sericin in colon cancer

Sericin protein is resistant to human proteases, subsequently, it reaches intact to the rectal zone (Kaewkorn et al., 2012). Because of this characteristic, the influence of sericin in colon cancer was investigated. Two studies performed in mice found a correlation between the ingestion of sericin and a positive effect in the incidence of colon tumors (Zhaorigetu et al., 2001; Kaewkon et al., 2012). Zhaorigetu et al. (2001) histologically investigated the anti-tumoral function of sericin in colon cell and its relationship with the antioxidant activity of sericin. For this purpose, colon tumorigenesis was induced in mice, and the animals were treated orally with sericin. The cellular proliferation and the oxidative stress analyses of the target tissue revealed a lower tumor incidence and a minor oxidative stress compared with the control group, where mice were fed with casein protein instead of sericin (Zhaorigetu et al., 2001).

Similarly, Kaewkorn et al. (2012) found that 5 out of 14 control mice with induced colon cancer developed tumors. Meanwhile 1 out of 14 mice, which were fed with a diet rich in sericin, presented tumors in colon. Furthermore, they found a reduction of colonic lipid oxidation. The authors suggest that these findings might be due to a suppression activity exerted by sericin of the initiation and promotion stages of colon tumorigenesis (Kaewkon et al., 2012). The effect was also evaluated in human carcinogenic colon cells by an incubation of these cells with increasing concentrations of sericin varying in its molecular size as well. As a consequence, a lower cell viability in samples treated with smaller size of sericin was observed. According the authors, this result can be awarded to an anti-proliferative effect of the sericin by inducing cell apoptosis (Kaewkorn et al., 2012).

These studies suggest a positive influence by the sericin in colon cancer, even though more studies are necessary to establish a solid relation between sericin treatment and incidence of colon cancer.

4.5 Effect of sericin on oxidative stress in epidermis tissues

Skin cells are in constant contact with oxygen and frequently exposed to solar radiation. This fact gives rise to the increase of ROS and consequently the incidence of skin cancer (Zhaorigetu et al., 2003). Zhaorigetu et al. (2003) and Dash et al. (2008) investigated the possible role of the sericin in controlling oxidative stress in epidermis cells. For this aim, they used as *in vivo* model mice exposed to skin carcinogenic agents and feline fibroblasts with damage induced by a pro-oxidant, such as H_2O_2, as *in vitro* assay (Zhaorigetu et al., 2003; Dash et al., 2008; Khyade, 2016). It was found that only 10% of mice treated topically with sericin developed tumors after being exposed to carcinogenic agents (Zhaorigetu et al., 2003). When analyzing the effect of the sericin on H_2O_2–induced DNA damaged in feline fibroblasts, an increase in the cellular viability for sericin-treated cells was obtained. The survival observed was proportional to the amount of sericin used (Dash et al., 2008). Moreover, an attenuated release of catalase, enzyme that catalyzes the decomposition of H_2O_2, was found in sericin-treated samples. These findings suggest a protective effect from sericin protein against oxidative stress (Dash et al., 2008). Similar results were obtained by Kumar & Mandal (2017) when analyzing the protective function of sericin against oxidative stress in mouse fibroblasts with H_2O_2-induced cell damage. The influence on the antioxidant effect of the extraction method of sericin from the silk was also studied. The extraction by using steam exhibited a better protection and cell recovery as well, compared with other methods such as urea, alkali, acid and conventional (hot water) treatments (Kumar & Mandal, 2017).

Overall these experiments, both *in vitro* and *in vivo*, suggests that sericin protein has a suppressor role in skin tumorigenesis by a reduction of the oxidative stress in the epidermis cells. Thus, sericin topically treatments could be an interesting point to research with biomedical application purposes.

Table 3. Literature about antioxidant activity of sericin protein.

Antioxidant assay	Target	Comments	Reference
Lipid peroxidation	Feline fibroblasts	Locally treated (pre-incubation) for 24 hs. Effect observed at 100 ng/mL.	Khyade (2016)
	Brain and peripheral rat tissues	Oral via, 0.25 and 0.5 gm/kg body weight (b.w.)/day of CSE for a period of 28 days.	Deori et al. (2016)
	Rat brain	Locally treated (pre-incubation), 0.3% sericin for 30 to 200 min.	Kato et al. (1998)
	Mouse fibroblast (L929) cells	Locally treated (pre-incubation), 10 and 100 µg/mL sericin.	Kumar & Mandal (2017)
	Skin fibroblast cell line (AH927) damaged by H_2O_2	Locally treated (pre-incubation) for 24 h. Positive effects in cell viability is observed	Dash et al. (2008)

Table 3. Continued…

Antioxidant assay	Target	Comments	Reference
		for *B. mori* and lipid peroxidation for *A. mylitta.*	
	Larvae mid-gut cell homogenate	Locally treated (pre-incubation).	Micheal & Subramanyam (2014)
Tyrosinase activity	Tyrosinase mushroom solution (100 Units/mL)	0.8 mg sericin. Sericin extracted by Urea presented the highest antioxidant effect.	Aramwit et al. (2010a)
	Tyrosinase mushroom solution	Hydrolyzed sericin at 5%.	Puangphet et al. (2015)
		0.5% and 1% sericin.	Kato et al. (1998)
		Sericin at doses of 6.4, 3.2, 1.6 mg/mL.	Chlapanidas et al. (2013)
		Hydrolyzed sericin at 1.2 to 10 mg/mL.	Wu et al. (2008)
		Sericin extracted by basic hydrolysis exhibited the best performance.	Manosroi et al. (2010)
	Tyrosinase mushroom solution, Tyrosinase apple solution, Tyrosinase banana solution, Tyrosinase bean sprouts solution	Hydrolyzed sericin at 5%.	Puangphet et al. (2018)
ROS (Reactive oxygen species)	Skin fibroblast cell line (AH927) damaged by H_2O_2	Locally treated (pre-incubation) for 24 h.	Dash et al. (2008)
	Catalase activity (CAT)	Sericin at 10 and 100 µg/mL	Kumar & Mandal (2017)
	Brain and peripheral rat tissues	Glutathione (GSH) and superoxide dismutase (SOD) analyzed as oxidative stress markers.	Deori et al. (2016)
	Larvae mid-gut cell homogenate	CAT and SOD analyzed.	Micheal & Subramanyam (2014)
FRAP (Ferric ion reducing antioxidant power assay)	Biochemical assay	Sericin at 1 mg/mL.	Kumar & Mandal (2017)
		Mulberry and non-mulberry strains were analyzed.	Butkhup et al. (2012)
		Sericin hydrolysate with bacterial protease	Yakul et al. (2019)
ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)	Biochemical assay	Hot water extraction at different times was studied.	Sangwong et al. (2016)
		Mulberry and non-mulberry strains were analyzed.	Butkhup et al. (2012)
		Sericin hydrolysate with bacterial protease	Yakul et al. (2019)
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Biochemical assay	Mulberry and non-mulberry strains were analyzed.	Butkhup et al. (2012)
		Alkali sericin extraction presented the best antioxidant activity.	Kumar & Mandal (2017)
		Sericin at 1 to 9 mg /mL.	Fan et al. (2009)
		Sericin and protease-hydrolyzed sericin is studied	Sangwong et al. (2016)
ORAC (oxygen radical absorbance capacity)	Bread with and without sericin	8 g sericin per 300 g bread.	Takechi et al. (2014)
Flavonoids quantification	Sericin from different strains	Extraction by different solvent were studied.	Napavichayanun et al. (2017)
		Sericin at 1 mg/mL. Different extraction methods analyzed.	Kumar & Mandal (2017)

5 Conclusion

Regarding to the methods reported in the literature to extract sericin, the use of high temperature, either by boiling water or using autoclave and posterior filtration, is efficient and a clean product is obtained. This technique is also economically convenient when compared to others more sophisticated methods of similar efficiency.

The antioxidant activity of sericin is mainly attributed to intrinsic properties of the protein, such as the amino acidic profile, which allows it to inhibit the tyrosinase enzyme, the lipid peroxidation and the oxidative stress and are associated to diseases such as cancer. Furthermore, sericin could act as natural food preservative due to the antioxidant potential commented, retarding the oxidative processes that affect the shelf life and quality of food. Therefore, sericin protein is an attractive multifunctional compound that might have not only benefits to the health when consuming, but also a protective effect to the food.

Further investigation is though required in terms of safety, to evaluate its use as functional food ingredient in the scope of regulatory authorities. Finally, considering that the sericin is a by-product from the silk manufacture, this protein has potential value from the economic and environmental point of view with promising biomedical and food applications.

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