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STUDIES REGARDING ANTIOXIDANT PROPERTIES OF ANTIMICROBIAL BIOPRODUCTS FORMULATED WITH NATURAL POLYMERS

Mohammed Shaymaa Omar MOHAMMED[1], Nicoleta RADU[1], Mariana VOICESCU[2], Lucia Camelia PÎRVU[3], Narcisa BĂBEANU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Institute of Chemistry and Physics “Ilie Murgulescu”, 202 Splaiul Independentei, District 6, Bucharest, Romania
[3]National Institute of Pharmaceutical Chemistry R & D of Bucharest, 112 Calea Vitan, District 3, Bucharest, Romania

Corresponding author email: nicoleta.radu@biotehnologii.usamv.ro

Abstract

The antioxidant activity of antimicrobial bioproducts formulated with natural polymers most often depends on the activities of the active compounds from their composition. In the case of bioproducts with biopolymers, the antioxidant activities depend on the limonene source. In the present case, the antimicrobial activities of antimicrobial gels formulated with biopolymers were determined by the chemiluminescence method, using two sources of limonene, respectively the citrus essentials oils with 97% and respectively with 25% limonene. The results obtained from the chemiluminescence studies have shown that, if an essential oil (EO) with a high limonene content is used in the antimicrobial formulations, such as EO of Citrus sinensis (97% limonene), then the product will most often act as a prooxidant, due to the high limonene content. If the antimicrobial formulations are performed with less concentrated sources of limonene, such as the EO of bergamot (Citrus bergamia), then the resulting bioproducts, in addition to the maximized antimicrobial effect, will also act as antioxidants, the antioxidant activity obtained being 70%.

Key words*: antioxidant activities, antimicrobial gels.*

INTRODUCTION

The subproducts resulting from the food industry such as chitin, hides of cattle, and citrus peels can represent valuable raw materials, from which is possible to obtain products like hydrolyzed collagen, chitosan, or limonene, raw materials with huge applications in the pharmaceutical industry and not only. These raw materials can be functionalized with small quantities of antimicrobial reagents (quantities needed are less than 10 times in comparison with common products from the market) in order to obtain antimicrobial formulations such as gels (Mohammed et al., 2022). Formulations obviously exhibit antimicrobial activity, more or less specific, strongly influenced by the antimicrobial reagent type, respectively by synergies that can appear between the gel components. Considering that these formulations are intended for topical applications, it is important to know other properties of them. These properties, such as antioxidant/prooxidant activities, are important and obviously depend on the antioxidant/prooxidant activities of each component used in the gel formulations. According to Nurilmala et al., bioactive peptides with high antioxidant activity have a molecular weight of fewer than 10 kDa (Nurimala et al., 2020; Chen et al., 2020). Such a hydrolyzed collagen, obtained from the skin of yellowfin tuna, was investigated from the point of view of antioxidant activities with the ABTS method (2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acids)) and DPPH (2,2 -diphenyl-1-picrylhydrazyl). The hydrolyzed collagen obtained, (with a content of 3.27% arginine, 1.78% lysine, 1.41% threonine, 0.89% phenylalanine, 0.44% leucine, 0.29% histidine, 8.85% glycine, 4.09%

proline, 3.64% alanine, 3.35% glutamate, 1.93% aspartate, 1.35% serine, and 0.17% tyrosine), exhibit antioxidant properties expressed towards ABTS (90%) and respectively towards DPPH (40%) (Nurimala et al., 2020). Chen and col. (Chen et al., 2020), in their bibliographic studies carried out, showed that only peptides with a certain sequence of amino acids show antioxidant activity (i.e: GETGPAGPAGPIGPVGARGPAGPQGPRGD KGETGEQ; DGAR; LEELEEELEGCE; GPLLGFLGPLGLS, FDSG-PAGVL; NGPLQAGQPGER; DPALATEPDPMPF).
The same authors found that the skin healing properties possess only the peptides obtained from the cattle's Achilles tendon, which contain the following sequence of amino acids: GETGPAGPAGPIGPVGARGPAGPQGPRGD KGETGEQ (Chen et al., 2020). Leon Lopez et al. (Leon-Lopez et al., 2019), found that human collagen contains 33% glycine, and 22% (proline+hydroxyproline). The human collagen has a structure of a triple helix, with three alpha chains, and 1014 amino acids in each alpha chain. This type of collagen has an average molecular weight of about 100 kD. According to the same authors, only the collagen obtained by enzymatic hydrolysis from bovine tendons and respectively from pig skins (the last one obtained by aqueous hydrolysis and ultrafiltration) have antioxidant properties and contain peptides with a molecular weight between 1-10 kD (Leon-Lopez et al., 2019).
Regarding the antioxidant properties of chitosan, Yen et al., in their research on DPPH and OH- scavenging capacity, found that the antioxidant activity of chitosan increases in direct proportion with an increasing deacetylation degree of this (Yen et al., 2008).
Gumgumjee and col. reported that if the chitin exoskeletons obtained from marine species are previously calcinated at 160°C, colled, grinding, and dissolved in dimethyl sulphoxide (DMSO), then the scavenger properties for DPPH ions of these chitin solutions are higher than the chitosan solutions made with DMSO, with the chitosan obtained from the chitin of the same marine species, without calcination. These results can be due to the amino groups from the chitin macromolecules, which possess a pair of non-participating electrons, these acting as scavengers for hydrogen ions (Gumgumjee et al., 2018). The aim of this present study is to highlight the antioxidant/prooxidant properties of some raw materials or liquid formulations used in obtaining gels with antimicrobial activity.

MATERIALS AND METHODS

Antioxidant activity was studied by the chemiluminescence method after a methodology presented in detail by Zaharie et al. (Zaharie et al., 2022), using the same device and the same reagents. The raw materials used in the formulation of solid biopolymer composites used in regenerative medicine were previously obtained by Babeanu and col. (Babeanu et al., 2022) and the bioproducts type gell, with antimicrobial properties previously obtained (Mohammed et al., 2022) were subjected to analysis. It is important to highlight the fact that until now, this methodology has not been used yet for the evaluation of antioxidant/prooxidant activity of the natural biopolymers (collagen, chitosan) or for gels formulations, which include them.

RESULTS AND DISCUSSIONS

Results obtained by chemiluminescence studies reveal that in the case of collagen, antioxidant activities are 42% after 5 s, and 44%; after 60 when the collagen concentration in the chemiluminescence matrix is 50 μL/mL. At concentrations less than 25 μL colagen/mL the system become prooxidant (Figure 1).
The IC50 of collagen is 46.29 μL/mL (Table 1), and according to Nurilama and col. (Nurilama et al., 2020) this value represents a very strong antioxidant reagent when this biomaterial is used alone. The prooxidant value obtained at a concentration less than 25 μL/mL is probably due to the source of this biomaterial (hides of cattle) which does not contain a proper content of peptide with amino acids sequence favorable for antioxidant activity (Chen et al., 2020). In the case of chitosan (Figure 2), at all concentration studied biomaterials act as a pro-oxidant, at all concentrations studied. This behavior is probably due to the moderate deacetylation grade of this biomaterial and to average molecular mass, and to its structure of wrapped

coil, unfavorable for antioxidant activities (Babeanu et al., 2022). Results obtained in the case of the essential oil of *Citrus sinensis*, which contains 97% Limonene (Schroder et al, 2022), results obtained reveal antioxidant properties at 5 s, for all concentrations studied (Figure 3). After 60 s, all systems become pro-oxidant.This behavior can be due to high levels of limonene, which does not have antioxidant activities.

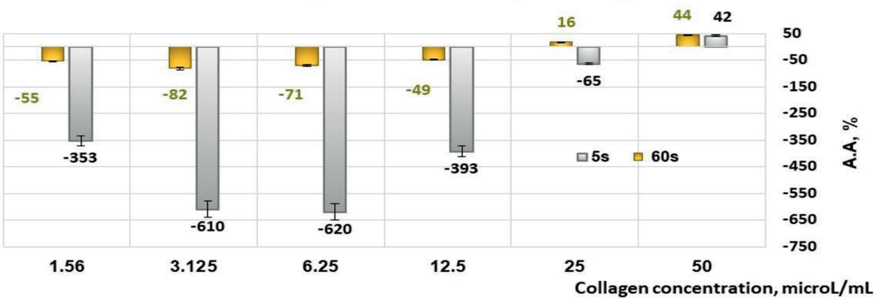

Figure 1. Antioxidant/prooxidant activity of the collagen obtained from beef hides, used as raw material for antimicrobial gels formulations

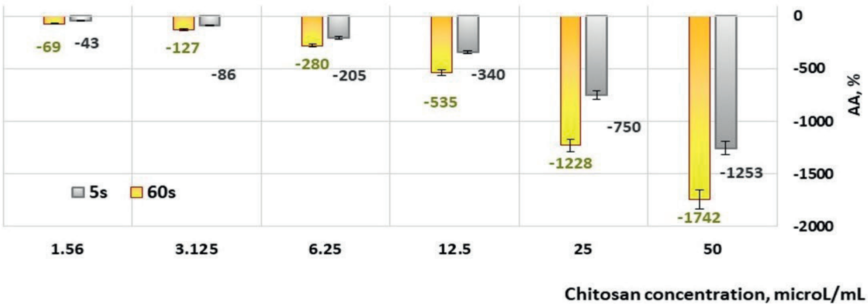

Figure 2. Prooxidant activity of the chitosan obtained from crab exoskeletons, used as raw material for antimicrobial gel formulations

The value obtained for IC5 (154.32 µL/mL) indicates that the essential oils of *Citrus sinensis* have average antioxidant activity (Nurilama et al., 2020). Lin et al. (Lin et al., 2021), in their studies with the DPPH technique, reported an IC50 ranging between (0.02-30) mg/mL, for more essential oils derived from different citrus varieties.Values obtained in the case of essential oils of *Citrus beragamia* (Figure 4, Table 1) indicate an antioxidant activity, probably due to the low content of limonene (30.41%) (Schroder et al., 2022). The value of IC50 obtained in this case (32.54 µL/mL) indicates it is a very strong antioxidant biomaterial. These observations are in agreement with the conclusions of other scientists (Sicari et al, 2016; Da Pazzo et al, 2018) which found for bergamot juice an IC50 between 8.77-17 mg GAE/g and respectively of (19.6-27.3) µL/mL, the last one measured by DPPH method. Analyses performed on the two gels with antimicrobial properties (Mohammed et al., 2022) and the chemiluminescence studies reveal the prooxidant activities for both of them (Figures 5 and 6). This behavior can be due to the fact that the properties of the final bioproducts are given by the raw materials which represent the majority, in this case, the chitosan and collagen. In addition, limonene, chitosan, and clotrimazole have prooxidant activities, and is much more probable that these raw materials imprint their properties on the end products. The antibiotic reagent used in antimicrobial formulations (an imidazole derivative named clotrimazole) does not exhibit antioxidant activities but the formulation made in the presence of plant extract or essential oil derived from plants (Elshaer et al., 2022; Carbone et al., 2019; Ouédraogo et al., 2012). Oh et al., in their studies of antioxidant activities regarding DPPH free radical and hydroxyl free radical scavenging by a different type of chitosan, reported that the scavenger activities of chitosan increase with decreased average molecular mass of chitosan used. For example, chitosan with an average molecular mass of 3 kDa exhibits antioxidant activity greater than chitosan with an average molecular mass of 10 kDa, 20 kDa, 50 kD, or 100 kDa (Oh et al., 2019).

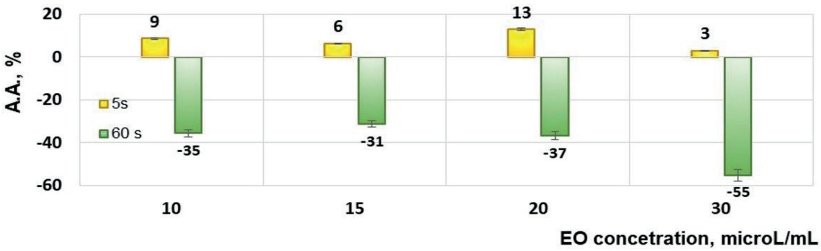

Figure 3. Antioxidant/prooxidant activity of the limonene obtained from *Citrus sinensis* (Life supplier), used as raw material for antimicrobial formulations

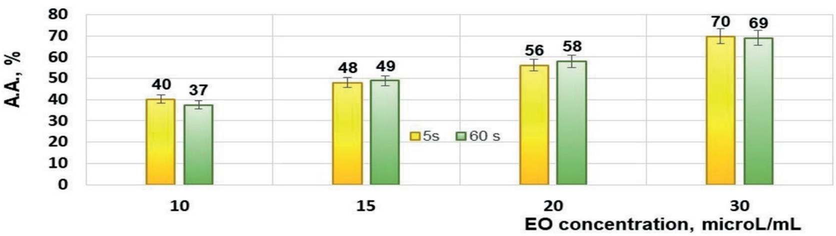

Figure 4. Antioxidant activity of the limonene obtained from *Citrus bergamia* (Life supplier), raw material in antimicrobial formulations

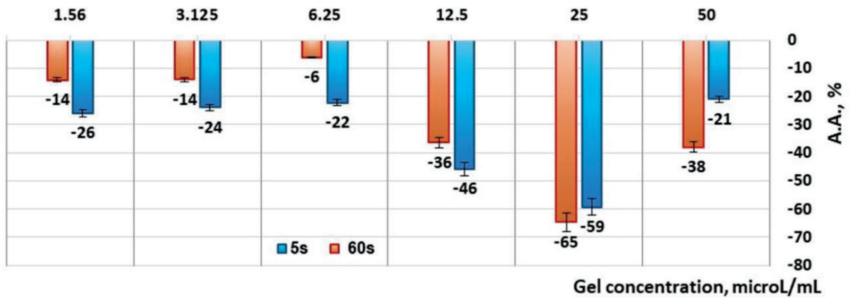

Figure 5. Prooxidant activity of the antimicrobial gel with a mass ratio of COL:CHIT:LIM:CT = 1:0:1:0.1

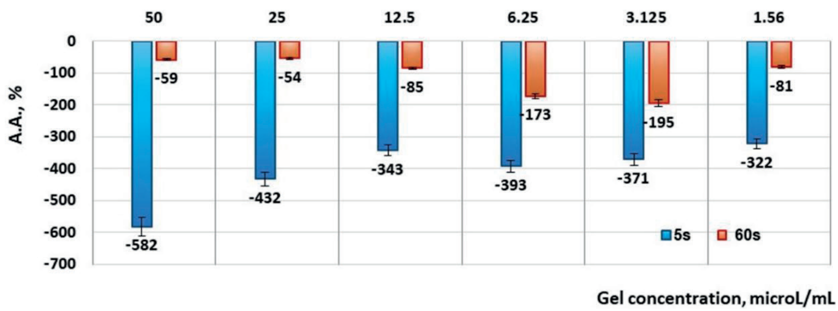

Figure 6. Prooxidant activity of the antimicrobial gel with a mass ratio of COL:CHIT:LIM:CT = 1:1:1:0.1

According to other scientists (Ivanova & Yaneva, 2020; Muthu et al., 2021) the presence of chitosan, increases the level of reactive oxygen species (ROS) inside the tumors cells, under the presence of these species the mitochondrial membrane is depolarized, is released the de cytochrome C, increase the expression of Caspase 3, the pro-inflammatories cytokines are inhibited and finally the tumor cell proliferation decrease (tumor cells have a mitochondrial redox metabolism changed).

In normal cells, (cells with normal mitochondrial redox metabolism) chitosan plays the role of ROS scavenging, increases the expression of antioxidant enzymes, preventing cell destruction due to oxidative stress (Ivanova & Yaneva, 2020; Muthu et al., 2021).

Table 1. Main characteristics of biomaterials tested by chemiluminescence for antioxidant/prooxidant activity

Product/Sources	Biologic activity	IC 50 µL/mL	Observation	Bibliographic sources
Collagen (beef hides)	Decrease cytotoxicity in the magistral formula-tion; Improve tissue regeneration	46.29	Antioxidant activity at high concentra-tion. Low concen-trations act as prooxidants.	Babeanu et al., 2022; Ioan et al., 2020; Radu et al., 2016; Schroder et al., 2022; Nurilama et al., 2022
Chitosan (crabs exoskeleton)	Local antimicrobial activity for *E. coli, S. aureus*, *C. albicans*	-	Prooxidant acti-vities that increase over time	Babeanu et al., 2022; Schroder et al., 2022; Nurilama et al., 2022
Essential oil of *Citrus sinensis*	Antimicrobial activity against *E. coli* and *P. aeruginosa*	154.32	Low antioxidant activity; after 5s it becomes pro-oxidant;	Schroder et al., 2022; Nurilama et al., 2022
Essential oil of *Citrus bergamia*	High antimicrobial activity for *S. aureus; S. aureus* MRSA; *P. aeruginosa*	32.54	High antioxidant activity	Schroder et al., 2022
Bioproduct COL:CHIT:LIM:CT = 1:0:1:0.1	Local antimicrobial activity for *E. coli*. Moderate antimicrobial activity for *S. aureus*. Significant antimicro-bial activity for *S. aureus* MRSA; *C. albicans*	-	Prooxidant activities	Mohammed et al., 2022
Bioproduct COL:CHIT:LIM:CT = 1:1:1:0.1	Local antimicrobial activity for *E. coli*. Moderate antimicrobial activity for *S.aureus MRSA*. Significant anti-microbial activity for *S. aureus*; *C. albicans*	-	High prooxidant activities	Mohammed et al., 2022
IC50 = inhibition concentration of 50% of free radicals from the system. Quantification of antioxidant activities from IC 50 value, expressed in mg/mL (after Nurilama et al., 2020): IC50<0.05: very strong; 0.05≤IC50<0.1 strong; 0.1≤IC50<0.15 average; 0.15 ≤IC50<0.2 weak; IC50>0.2 wery weak				

CONCLUSIONS

The antioxidant activity of some biomaterials such as collagen, chitosan, two essential oils obtained from citrus peels (*Citrus sinensis*; *Citrus bergamia*), and two gels with antimicrobial properties obtained with them have been highlighted for the first time, using the chemiluminescence method. The results obtained in the case of biomaterials used as raw materials, in gels formulations, confirmed the existing results in the specialized literature with the DPPH or ABTS method. Regarding the finished products (two gels with antimicrobial activities formulated with chitosan, collagen, clotrimazole, and limonene), the chemiluminescence studies carried out showed that they have pro-oxidant activities, properties most likely generated by major components from the system, which imprint their own properties in end products.

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