DEVELOPMENT AND EVALUATION OF A WHEY AND GELATIN FILM FILLED WITH COPPER NANOPARTICLES SYNTHESIZED BY AN ECO-FRIENDLY METHOD

Pompilia Mioara Purcea Lopes*, Violeta Popescu

Physics and Chemistry Department, Technical University of Cluj-Napoca, 28 Memorandumului Str., 400114 Cluj-Napoca, Romania

Abstract

This paper aims to improve swelling properties by determining the optimal concentration of glycerin (Gly) in a nano-composite hydrogel based on whey and gelatin crosslinked with copper nanoparticles (CuNPs) obtained in-situ, by biosynthesis using ascorbic acid (AA) as a reducing agent. Swelling tests show that in the first few minutes, the swelling degree of the three samples with different Gly concentrations do not show significant differences, but throughout the swelling process, the sample with the lowest plasticizer concentration has shown better swelling capacity. All hydrogel samples kept their integrity after 24 hours with negligible mass dissolution in the swelling medium. Therefore, it's a quality that can be useful for food packaging systems. Fourier Transformed Infra-Red (FT-IR) and UV-Visible spectra confirmed changes in the polymer structure of the hydrogel samples due to the compound blending and crosslinking process. The results of this and the author's previous work encourage us to study and develop new hydrogel systems based on whey and gelatin crosslinked with metal nanoparticles as active packaging for the food industry.

Keywords: *nanoparticles, glycerin, whey and gelatin hydrogels, in-situ biosynthesis, swelling*

1. INTRODUCTION

Current trends in the production of hydrogels for pharmaceuticals, medicine, environmental protection, and food industry applications are aimed at using natural polymers. The sources of these natural polymers are virtually limitless, both from naturally occurring vegetation in different parts of the Earth and from by-products resulting from food processing, such as carbohydrate polymers (konjac glucomannan, cellulose, starch, alginate, agar-agar, etc) and proteins (albumins, globulins, serum proteins, collagen, and gelatin), all of them showing techno-functional characteristics, such as gel formation, absorption, and retention of a large quantity of water.

At present, most materials used to manufacture absorbent systems in food packaging come from non-biodegradable sources (Li *et al.*, 2020), which presents a risk to both consumer health and the environment (Batista *et al.*, 2019a). Natural resources have been studied for many decades to obtain biodegradable and biocompatible food packaging with the ability to reduce the proliferation of microorganisms, oxidative spoilage, weight loss, and extend the shelf-life of food (Ahmed *et al.*, 2017a). Meat, poultry, and seafood are highly perishable food categories, susceptible to spoilage under improper processing conditions, and represent an optimal environment for the growth of pathogenic microorganisms (Ahmed *et al.*, 2017a). Extensive research has documented the efficiency of CuNPs in composite hydrogel systems as active packaging systems concerning reducing microbial activity and thus prolonging shelf life for cheese (Conte *et al.*, 2013), fruit salads (Sportelli *et al.*, 2019), and fruit juices (Llorens *et al.*, 2012). Compared to other metals with antimicrobial properties (Au, Ag), copper compounds are widely used to obtain hydrogels (Longano *et al.*, 2012; Rai *et al.*, 2018; Batista *et al.*, 2019b; Sportelli *et al.*, 2019), being more affordable, and easier to prepare, even by eco-friendly methods (Chowdhury, Shaik and Chakraborty, 2015; Fathima *et al.*, 2018; Batista *et al.*, 2019b; Mostafavi and Zaeim, 2020). As precursors of copper nanoparticles can be considered copper sulphate pentahydrate ($CuSO_4 \times 5H_2O$) (Mandke and Pathan, 2012; Shankar and Rhim, 2014; Ramos *et al.*, 2019; Chandran *et al.*, 2020), copper acetate hydrate ($Cu(CH_3COO)_2 \times H_2O$) (Raspolli Galletti *et al.*, 2013a; Shankar and Rhim, 2014; Ramos *et al.*, 2019), copper chloride (Shankar and Rhim, 2014; Ramos *et al.*, 2019), copper nitrate hydrate ($Cu(NO_3)_2 \times 3H_2O$) (Raspolli Galletti *et al.*, 2013a; Sooch and Mann, 2021). Much research has shown significant interest in the antimicrobial activity of CuNPs

in obtaining composite hydrogels with applications in medicine, food packaging (Ahmed *et al.*, 2017b), and wastewater treatment (Fathima *et al.*, 2018). No less, various hydrogel formulations mentioned CuNPs blended with both, synthetic and natural polymer, such as: polyethylene oxide (PEO) (Sportelli *et al.*, 2019), poly(propylene) carbonate (Indira Devi *et al.*, 2019), polyvinyl chloride (PVC) (Guzman *et al.*, 2015), polylactic acid (PLA) (Longano *et al.*, 2012; Conte *et al.*, 2013), gelatin (Ahmed *et al.*, 2017b; Arfat *et al.*, 2017), agar (Mostafavi and Zaeim, 2020), cellulose (Zhong, Oporto and Jaczynski, 2017), polycaprolactone (El-Naggar, Hasanin and Hashem, 2022), starch/sodium alginate (Mousavi *et al.*, 2021).

To improve the elastic properties of hydrogels, plasticizers are used in their formulations. Glycerin is by far the most widely used plasticizer for gelatin (Hosseini and Gómez-Guillén, 2018; Roy and Rhim, 2021) and whey (Min, Harris and Krochta, 2005; Hassannia-Kolaee *et al.*, 2016; Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020) based hydrogels.

This work aims to optimize the concentration of the plasticizer (glycerin) in the composite hydrogel with copper nanoparticles (CuNP) with the potential applications for active food packaging, from a copper (II) enriched whey-gelatin-based hydrogel that has already shown promising behavior in relation to swelling tests and antimicrobial activity (Purcea Lopes *et al.*, 2022). The synthesis of the CuNPs in this study was performed in situ, to avoid their tendency to aggregate in contact with air (Indira Devi *et al.*, 2019), with L-ascorbic acid as a reducing agent (Raspolli Galletti *et al.*, 2013a; Shankar, Teng and Rhim, 2014a; Fathima *et al.*, 2018; Ramos *et al.*, 2019) and copper sulphate as the precursor (Liu *et al.*, 2012a; Indira Devi *et al.*, 2019). On the other hand, with ascorbic acid having a low reducing action the intensity of the reaction is weak, therefore it is unlikely that copper nanoparticles would form aggregates (Liu *et al.*, 2012b). A peculiarity of copper-based nanocomposite hydrogel films is the lack of transparency, but this can be an advantage in obtaining packaging for photosensitive foods.

2. MATERIALS AND METHODS

2.1. Materials

The hydrogels were obtained from Instant Whey Protein Isolate (WPI) Active produced and distributed by Sly Nutritia SRL (Buzău, Romania), Gelatin Reagent (AMRESCO, LLC, Fountain Parkway Solon, Cleveland, OH, USA), glycerin (Sigma-Aldrich, Taufkirchen, Germany), $CuSO_4 \times 5H_2O$ (Sigma-Aldrich, Taufkirchen, Germany) and ascorbic acid packed in EU by Radziowi Sp. Z. o. o. (Brzozów, Poland) and distributed by SC Ropartner SRL (Piatra-Neamț, Romania).

2.2. Methods

2.2.1. Preparation of hydrogels

The whey solution was prepared with a magnetic stirrer AREX Heating Magnetic stirrer (VELP SCIENTIFICA, Usmate, Italy), for 2 hours at 800 prm and left until the next day in the refrigerator for a good dispersion of whey. The copper sulphate aqueous solution was obtained by weighting (*w/w*) in 5 % concentration.

Ascorbic acid (AA) solution was made in 33 % concentration by weighting (*w/w*) followed by heating the solution for a complete dissolution of ascorbic acid.

A number of three samples with the same amount of whey (WPI) solution 5 % (*w/w*) (6 g), gelatin (*g*) (1 g), copper sulphate 5 % (*w/w*) (1.5 g) and ascorbic acid 33 % (w/w) (0.48 g) were prepared. Glycerin solution 1:1 (*w/w*) was added in the amount of 1-1.5-2 g to the first, the second, and the third sample, respectively. All samples were brought to the same weight (11 g) with distilled water. For a good homogenization and dissolution of gelatin, the samples were kept in an ultrasonic bath at 45˚C in degas mode for 30 minutes. At the end of the process, the samples had a pH = 4.0. The reductant-to-precursor molar concentration ratio is 3:1.

Table 1 presents the concentration of glycerin in composite hydrogels samples before drying

Table 1. The concentration of Glycerin in composite hydrogels samples before drying

Code sample	WGly1	WGly1.5	WGly2
Glycerin (%)	9.09	13.64	18.18

2.2.2. *Swelling tests*

Swelling tests were performed by immersing the samples in distilled water at room temperature. Dry samples were weighed and placed in previously moistened and heat-sealed tea bags. Then, at predetermined time intervals they were weighed after separation from the hydration medium and removal of excess water by blotting with paper towels as follows: at 5 minutes intervals in the first two hours, then at 15 minutes intervals in the next five hours.

The swelling degree (*SW*) was calculated with Equation (1) considering the weight of the teabag (W_1), the weight of the dry hydrogel sample (W_2), and the weight of the swelled hydrogel sample (W_3) (Popescu *et al.*, 2021; Purcea Lopes *et al.*, 2022).

$$SW = \frac{W_3 - W_2 - W_1}{W_2} \cdot 100 \qquad [\%] \qquad (1)$$

2.2.3 FTIR Spectroscopy

Both precursors and hydrogel samples were analyzed by Fourier transform infrared spectroscopy (FT-IR) with Spectrum BX FTIR spectrometer (PerkinElmer, Sunnyvale, CA, USA) provided with an Attenuated Total Reflectance (ATR PIKE MIRacleTM, Madison, WI, USA) with a diamond crystal plate in attenuated total reflection (ATR) mode. The background correction was made using the air spectrum.

2.2.4. UV-VIS Spectroscopy

UV-VIS spectra were performed on transmission mode by a double beam UV-VIS spectrometer (Lambda 35 UV-VIS spectrometer (Perkin Elmer, Waltham, MA, USA). The samples were dropped on glass slides to form a film approximately 1 cm in diameter.

3. RESULTS AND DISCUSSION

3.2. Swelling tests

When the ascorbic acid solution was added to the samples with copper sulphate their color turned immediately from blue to orange (Shankar and Rhim, 2014). The brown colour appeared after one day and intensified within five days (see figure 1 A) demonstrating the presence of copper nanoparticles (Raspolli Galletti *et al.*, 2013b).

As expected, it can be seen from Figure 1 that hydrogel samples kept their integrity (B) and released a negligible amount of their mass in the swelling medium (C) (Purcea Lopes *et al.*, 2022).

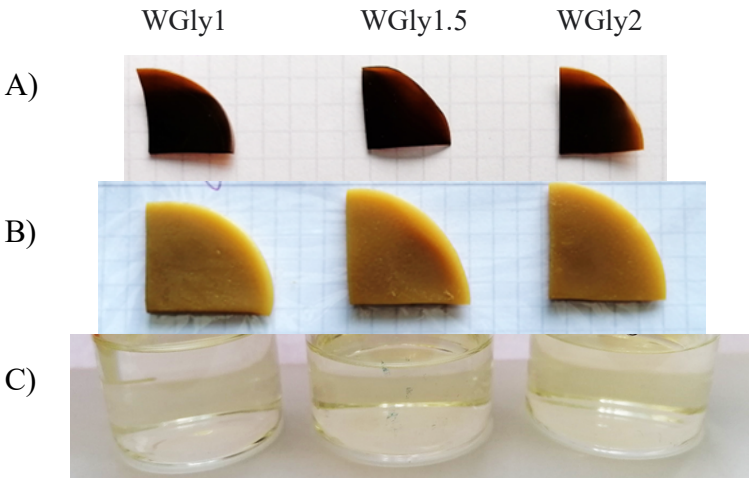


Figure 1. Hydrogels samples before the swelling process (A) and after 24 hours (B): WGly1 with 9.09 % glycerin, WGly1.5 with 13.64 % glycerin and WGly2 with 18.18 % glycerin. Swelling medium (distilled water) after 24 hours of swelling (C).

The influence of different concentrations of glycerin on the swelling degree SW (%) over the 24 hours is presented in Figure 2. It can be noticed that in the first phase of the swelling process there is very little difference in the absorption capacity of the three samples. This behavior can be explained by the fact that, in the beginning, the absorption of the liquid is mainly via the hydroxyl groups of the glycerin, which is in higher concentration in the WGly1.5 and WGly2 samples, and when these reached saturation, the swelling process was taken over by the other hydrophilic components of the hydrogel such as whey and gelatin. The hydrophilic groups of the latter, however, have better absorption capacity due to the complexity of the bonds formed in the process of obtaining the composite hydrogel.

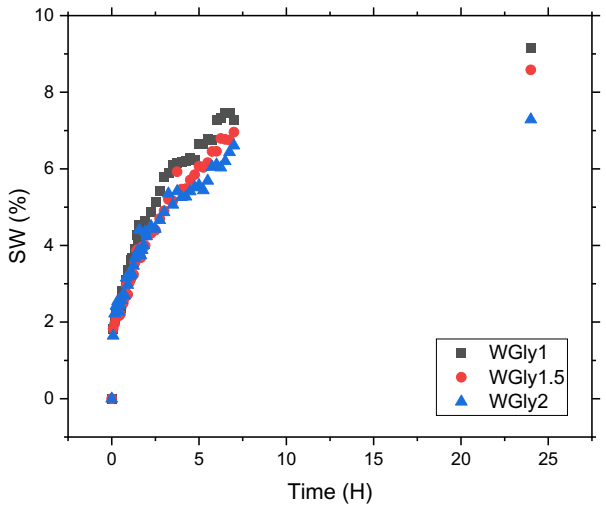


Figure 2. Swelling degree SW (%) as a function of time for hydrogel samples with different concentrations of glycerin.

3.3. FTIR Spectroscopy

The influence of different concentrations of glycerin in the nanocomposite hydrogels samples is reflected in the main vibration bands shown in the FT-IR spectra (Figure 3).

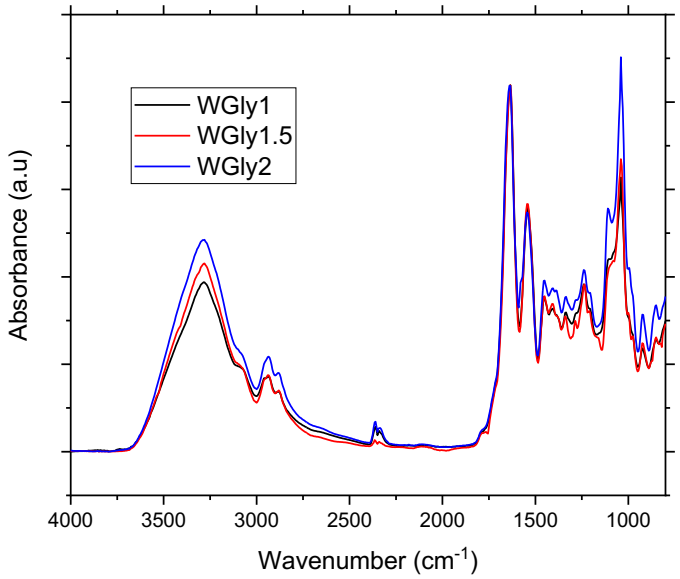


Figure 3. IR spectra of hydrogels samples with different concentrations of glycerin (WGly1; WGly1.5; WGly2)

In Table 2 are presented the main wavenumbers of hydrogen bonds, C=O, CN, NH bonds, and Amide (I, II, III) stretching vibrations in the nanocomposite hydrogels and their precursors. The corresponding glycerin, gelatin, and whey values were taken from a previous authors' paper (Purcea Lopes *et al.*, 2022). As can be seen, glycerin induces vibration bands shifts of gelatin and whey from 3273 cm^{-1} (Ge) and 3272 cm^{-1} (W) to 3285 cm^{-1} (WGly1 and WGly2) and 3280 cm^{-1} (WGly1.5) due to NH and hydrogen bonds formed between the hydrophilic functional groups and Amide A modifications (Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020). All interactions between precursors in the crosslinking process have induced the formation of new and stronger hydrogen bonds C=O—H-O and C=O—H-N reflected in higher wave numbers vibrations for Amide A and Amide I and II in the range of 1500 – 1650 cm^{-1} (Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020). Below wavenumbers 1450 cm^{-1} there is a shift towards lower wavenumbers of the absorption bands of Amide II and III. Crosslinking of hydrogels by in-situ formated CuNPs as a result of the reaction between copper sulphate and ascorbic acid resulted in further vibration band shifts corresponding to the modification of the secondary structure of gelatin and whey proteins, such as the shifts from 1080 cm^{-1} (Ge) and 1076 cm^{-1} (W) to 1045 cm^{-1} (WGly1) and 1040 cm^{-1} (WGly1.5 and WGly2) (Popescu *et al.*, 2021; Purcea Lopes *et al.*, 2022).

Table 2. The main vibrations of hydrogels samples and their precursors

Glycerin	Gelatin	Whey	WGly1	WGly1.5	WGly2	Attributions
			(cm^{-1})			
3286	3273	3272	3285	3280	3285	Amide A, tensile vibrations NH, hydrogen bonds (Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020; Popescu *et al.*, 2021; Purcea Lopes *et al.*, 2022)
-	1631	1632	1635	1635	1640	Amide I, C=O and NH vibrations, H bonds coupled with COO^- (Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020)
-	1527	1516	1540	1545	1545	Amide II, CN vibrations/stretching, NH bending (Piccirilli *et al.*, 2019; Huntrakul and Harnkarnsujarit, 2020)
1414	1445	1446	1445	1445	1450	Amide II, CN vibrations, NH bending (Huntrakul and Harnkarnsujarit, 2020)
-	1080	1076	1045	1040	1040	Amide III, CN and NH vibrations (Popescu *et al.*, 2021; Purcea Lopes *et al.*, 2022)

3.4. UV-VIS Spectroscopy

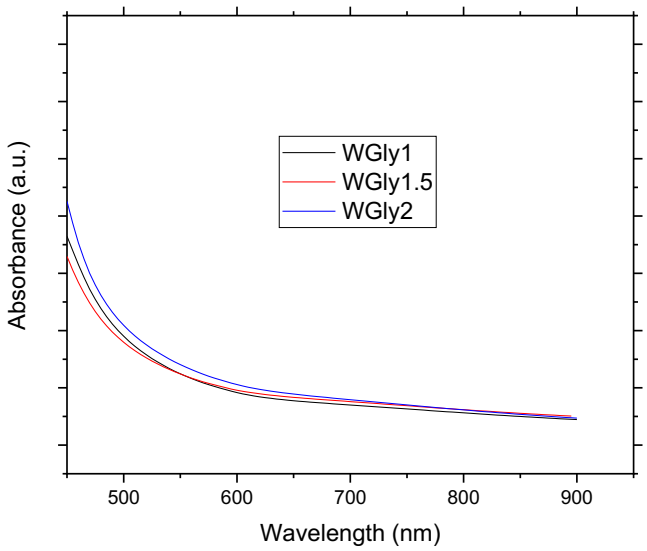


Figure 4. UV-VIS spectra of hydrogel samples with different concentrations of glycerin.

As optical properties are influenced by the morphology and dimensions of the nanoparticles (Longano *et al.*, 2012), from Figure 4 it can be noticed the low visible light absorbance of nanocomposite hydrogels which can be explained by the monodispersitivity and nanosized particles in the hydrogel samples (Shankar, Teng and Rhim, 2014b; Musa *et al.*, 2016) or by the thin layer of the hydrogel samples on the glass slides.

4. CONCLUSIONS

The low mechanical strength and rather a rapid dissolution of hydrogels in water leads to a limited application of hydrogels in the food packaging industry. This, in turn, opens up unlimited research openings for new hydrogel formulations.

Glycerin has shown its high effectiveness as a plasticizer for gelatin and whey-based hydrogels in the smallest concentration (9.09 %). This is explained by the presence of numerous hydroxyl groups that allow the formation of hydrogen bonds with the hydrophilic groups on the chains of protein polymers (gelatin and whey) giving optimal hydration properties to the nanocomposite hydrogels. The swelling results in this work, demonstrate that whey and gelatin hydrogels crosslinked with copper compounds should be able to maintain their structural integrity over the fresh foods' shelf-life. The FT-IR, and UV-VIS spectra demonstrate a good compatibility of whey, gelatin, glycerin, and copper compounds reconfirmed by the author's previous works. Therefore, the authors consider that whey and gelatin-based composite hydrogel formulations cross-linked with copper compounds present valuable characteristics for obtaining moisture absorbents for active food packaging.

ACKNOWLEDGMENTS

This paper was financially supported by the Project "Entrepreneurial competencies and excellence research in doctoral and postdoctoral programs - ANTREDOC", a project co-funded by the European Social Fund financing agreement no. 56437/24.07.2019.

REFERENCES

1. Ahmed, I. *et al.* (2017a) 'A comprehensive review on the application of active packaging technologies to muscle foods', *Food Control*, 82, pp. 163–178.
2. Ahmed, I. *et al.* (2017b) 'A comprehensive review on the application of active packaging technologies to muscle foods', *Food Control*, 82, pp. 163–178.
3. Arfat, Y.A. *et al.* (2017) 'Thermo-mechanical, rheological, structural and antimicrobial properties of bionanocomposite films based on fish skin gelatin and silver-copper nanoparticles', *Food Hydrocolloids*, 62, pp. 191–202.
4. Batista, R.A. *et al.* (2019a) 'Hydrogel as an alternative structure for food packaging systems', *Carbohydrate Polymers*, 205, pp. 106–116.
5. Batista, R.A. *et al.* (2019b) 'Hydrogel as an alternative structure for food packaging systems', *Carbohydrate Polymers*, 205, pp. 106–116.
6. Chandran, N. *et al.* (2020) 'Structural and optical properties of copper nanoparticles synthesized via wet chemical route', *AIP Conference Proceedings*, 2244(1), p. 070020.
7. Chowdhury, P.P., Shaik, A.H. and Chakraborty, J. (2015) 'Preparation of stable sub 10nm copper nanopowders redispersible in polar and non-polar solvents', *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 466, pp. 189–196.
8. Conte, A. *et al.* (2013) 'A novel preservation technique applied to fiordilatte cheese', *Innovative Food Science & Emerging Technologies*, 19, pp. 158–165.
9. El-Naggar, M.E., Hasanin, M. and Hashem, A.H. (2022) 'Eco-Friendly Synthesis of Superhydrophobic Antimicrobial Film Based on Cellulose Acetate/Polycaprolactone Loaded with the Green Biosynthesized Copper Nanoparticles for Food Packaging Application', *Journal of Polymers and the Environment*, 30(5), pp. 1820–1832.
10. Fathima, J.B. *et al.* (2018) 'Synthesis of eco-friendly copper nanoparticles for augmentation of catalytic degradation of organic dyes', *Journal of Molecular Liquids*, 260, pp. 1–8.

11. Guzman, A. *et al.* (2015) 'Synthesis and characterization of copper nanoparticles/polyvinyl chloride (Cu NPs/PVC) nanocomposites', *Procedia Materials Science*, 9, pp. 298–304.
12. Hassannia-Kolaee, M. *et al.* (2016) 'Development of ecofriendly bionanocomposite: Whey protein isolate/pullulan films with nano-SiO2', *International Journal of Biological Macromolecules*, 86, pp. 139–144.
13. Hosseini, S.F. and Gómez-Guillén, M.C. (2018) 'A state-of-the-art review on the elaboration of fish gelatin as bioactive packaging: Special emphasis on nanotechnology-based approaches', *Trends in Food Science & Technology*, 79, pp. 125–135.
14. Huntrakul, K. and Harnkarnsujarit, N. (2020) 'Effects of plasticizers on water sorption and aging stability of whey protein/carboxy methyl cellulose films', *Journal of Food Engineering*, 272, p. 109809.
15. Indira Devi, M.P. *et al.* (2019) 'Biodegradable poly(propylene) carbonate using in-situ generated CuNPs coated Tamarindus indica filler for biomedical applications', *Materials Today Communications*, 19, pp. 106–113.
16. Li, F. *et al.* (2020) 'Copper sulfide nanoparticle-carrageenan films for packaging application', *Food Hydrocolloids*, 109, p. 106094.
17. Liu, Q. *et al.* (2012a) 'Preparation of Cu nanoparticles with ascorbic acid by aqueous solution reduction method', *Transactions of Nonferrous Metals Society of China*, 22(9), pp. 2198–2203.
18. Liu, Q. *et al.* (2012b) 'Preparation of Cu nanoparticles with ascorbic acid by aqueous solution reduction method', *Transactions of Nonferrous Metals Society of China*, 22(9), pp. 2198–2203.
19. Llorens, A. *et al.* (2012) 'Study of the antifungal potential of novel cellulose/copper composites as absorbent materials for fruit juices', *International Journal of Food Microbiology*, 158(2), pp. 113–119.
20. Longano, D. *et al.* (2012) 'Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging', *Analytical and Bioanalytical Chemistry*, 403(4), pp. 1179–1186.
21. Mandke, M.V. and Pathan, H.M. (2012) 'Electrochemical growth of copper nanoparticles: Structural and optical properties', *Journal of Electroanalytical Chemistry*, 686, pp. 19–24.
22. Min, S., Harris, L.J. and Krochta, J.M. (2005) 'Antimicrobial effects of lactoferrin, lysozyme, and the lactoperoxidase system and edible whey protein films incorporating the lactoperoxidase system against Salmonella enterica and Escherichia coli O157: H7', *Journal of Food Science*, 70(7), pp. m332–m338.
23. Mostafavi, F.S. and Zaeim, D. (2020) 'Agar-based edible films for food packaging applications - A review', *International Journal of Biological Macromolecules*, 159, pp. 1165–1176.
24. Mousavi, S.N. *et al.* (2021) 'Starch/alginate/ Cu-g-C3N4 nanocomposite film for food packaging', *Materials Chemistry and Physics*, 267, p. 124583.
25. Musa, A. *et al.* (2016) 'Effect of gelatin-stabilized copper nanoparticles on catalytic reduction of methylene blue', *Nanoscale research letters*, 11(1), pp. 1–13.
26. Piccirilli, G.N. *et al.* (2019) 'Effect of storage conditions on the physicochemical characteristics of edible films based on whey protein concentrate and liquid smoke', *Food Hydrocolloids*, 87, pp. 221–228.
27. Popescu, V. *et al.* (2021) 'The Influence of Enzymatic Hydrolysis of Whey Proteins on the Properties of Gelatin-Whey Composite Hydrogels', *Materials*, 14(13), p. 3507.
28. Purcea Lopes, P.M. *et al.* (2022) 'New Composite Hydrogel Based on Whey and Gelatin Crosslinked with Copper Sulphate', *Materials*, 15(7), p. 2611.

29. Rai, M. *et al.* (2018) 'Copper and copper nanoparticles: role in management of insect-pests and pathogenic microbes', *Nanotechnology Reviews*, 7(4), pp. 303–315.

30. Ramos, A.R. *et al.* (2019) 'Effects of reaction temperatures and reactant concentrations on the antimicrobial characteristics of copper precipitates synthesized using L-ascorbic acid as reducing agent', *Journal of Science: Advanced Materials and Devices*, 4(1), pp. 66–71.

31. Raspolli Galletti, A.M. *et al.* (2013a) 'Novel microwave-synthesis of Cu nanoparticles in the absence of any stabilizing agent and their antibacterial and antistatic applications', *Applied Surface Science*, 280, pp. 610–618.

32. Raspolli Galletti, A.M. *et al.* (2013b) 'Novel microwave-synthesis of Cu nanoparticles in the absence of any stabilizing agent and their antibacterial and antistatic applications', *Applied Surface Science*, 280, pp. 610–618.

33. Roy, S. and Rhim, J.-W. (2021) 'Gelatin-Based Film Integrated with Copper Sulfide Nanoparticles for Active Packaging Applications', *Applied Sciences*, 11(14), p. 6307.

34. Shankar, S. and Rhim, J.-W. (2014) 'Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles', *Materials Letters*, 132, pp. 307–311.

35. Shankar, S., Teng, X. and Rhim, J.-W. (2014a) 'Properties and characterization of agar/CuNP bionanocomposite films prepared with different copper salts and reducing agents', *Carbohydrate Polymers*, 114, pp. 484–492.

36. Shankar, S., Teng, X. and Rhim, J.-W. (2014b) 'Properties and characterization of agar/CuNP bionanocomposite films prepared with different copper salts and reducing agents', *Carbohydrate Polymers*, 114, pp. 484–492.

37. Sooch, B.S. and Mann, M.K. (2021) 'Nanoreinforced biodegradable gelatin based active food packaging film for the enhancement of shelf life of tomatoes (Solanum lycopersicum L.)', *Food Control*, 130, p. 108322.

38. Sportelli, M.C. *et al.* (2019) 'A new nanocomposite based on LASiS-generated CuNPs as a preservation system for fruit salads', *Food Packaging and Shelf Life*, 22, p. 100422.

39. Zhong, T., Oporto, G.S. and Jaczynski, J. (2017) '19 - Antimicrobial food packaging with cellulose-copper nanoparticles embedded in thermoplastic resins', in A.M. Grumezescu (ed.) *Food Preservation*. Academic Press (Nanotechnology in the Agri-Food Industry), pp. 671–702. Available at: https://doi.org/10.1016/B978-0-12-804303-5.00019-5.