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A Study on Different Hurdle Factors of Nano Metal Vitamin Complex for the Prevention of Microbial Spoilage in Seafood

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ABSTRACT

Hurdles that have a positive effect by inhibiting microorganisms may have a negative one on other parameters such as nutritional properties or sensory quality, depending on their intensity. In order to lower the preservative level, the hurdle technology concept has been developed, consisting in using combined hurdles to establish an additive antimicrobial effect, and even sometimes a synergetic one, thus improving the safety and the sensory quality of food. The antibacterial and anti-oxidant potential of copper oxide nano particles coupled with vitamin-E (CuONP_s+V_E) was investigated by applying the novel hurdle factors against seafood pathogens and by studying the cell viability using MTT assays. The hurdle method is proposed to explain the significance of combined use of different preservation factors as synergistic effects instead of using a large-intensity preservation factor. Effect of CuONPs+VE and chilling temperatures (-18°C and +4°C); and Effect of CuONPs+VE and brine salts at various concentrations (5%, 10%,) were evaluated. CuONPs+VE with different chilling temperatures and brine salt concentration showed significant results on compared to control temperatures. Thus CuONPs+VE due to their bacteriostatic activity can be efficiently used in hurdle technology which reduces the food spoiling organisms. Thus CuONPs+VE in combined with hurdle technology can be used as alternatives for chemical preservatives in preservation techniques.



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INTRODUCTION

Spoilage of seafood products is mainly due to enzymatic autolysis, oxidation and microbial growth. Proper handling, pre-treatment and preservation techniques can improve nutritional value, texture, flavour, freshness, quality in fish and fish products (Ghaly, 2010). Hence, low temperature storage, salting, canning, drying and chemical preservation for controlling water activity, enzymatic, oxidative and microbial spoilage are considered as common preservation methods (Abbas *et al.*, 2009).

Oxidative deterioration of lipids in seafood leads to the production of volatile compounds causing rancidity, changes in aroma and off-flavour production during the storage conditions. Lipid oxidation dur-

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ing storage is reported to be influenced by different intrinsic factor which leads to evident colour and odour changes in fishes (Simat *et al.*, 2017). (Ozogul *et al.*, 2010) reported that lipid oxidation of fatty acids can be reduced by adding natural and chemical antioxidants. Researchers added that vitamin-E found naturally in foods is available also as a dietary supplement. (Ohene-Adjei *et al.*, 2004) stated that vitamin E is a group of fat-soluble compounds with significant antioxidant activities by inhibiting the production of reactive oxygen species which was produced during the oxidation of lipids.

Nanoparticles and nanotechnology are the recent method of choice involved in the processing, packaging, and ensuring the safety of seafood during storage. Nanoparticles in the form of metal oxides, salts, and composites conjugated with natural polymers or bioactive agents are used in seafood industries. Metal and metal oxide nanoparticle based packaging could be an effective material in terms of their antimicrobial potential. It helps to release antioxidant and antimicrobial compounds through the direct interaction with the food as well as helps to improve food stability (Dasgupta *et al.*, 2015). Copper compounds (Cu) are one such metal which are hypotoxic and has a high antibacterial effect against bacterial species associated with fish spoilage (Raffi *et al.*, 2010). Copper nanoparticles have the potential to disrupt cell function and may reduce the ability of microorganisms to develop resistance against copper. Cu nanoparticles can penetrate the bacterial cell, damage the DNA, and release antimicrobial Cu⁺ ions leading to cell death (Harikumar, 2016).

Common hurdles in seafood preservations are salt, smoke, acids, temperature (high or low) and more recently redox potential (vacuum-packed products). In order to lower the preservative level, the hurdle technology concept has been developed. The process consists of using combined hurdles to establish an additive antimicrobial effect, inhibiting oxidative damages. This would in turn improve the safety and the sensory quality of food (Leroi, 2014).

Based on this seafood preservation concept, the antibacterial and anti-oxidant potential of copper oxide nanoparticles coupled with vitamin-E (CuONP_s+VE) was investigated by applying the novel hurdle factors against seafood pathogens and by studying the cell viability using MTT assays. The hurdle method is proposed to explain the significance of combined use of different preservation factors as synergistic effects instead of using a large-intensity preservation factor.

Hurdle technology method proposed in the present research work is considered as state of art of method

by evaluating, the effect of CuONP_s+VE and chilling temperatures (-18°C and +4°C); and the effect of CuONP_s+VE and brine salts at two different concentrations (5%, 10%) against total heterotrophic and total coliforms associated with the fish during storage conditions.

MATERIALS AND METHODS

The present research work was carried out in Department of Microbiology, Vivekanandha College of Arts and Science for women, Namakkal, Tamil Nadu, India. The research work was carried out during the period of November 2019 to February 2020. All chemicals and bacteriological media were procured from Hi Media Pvt Ltd (Mumbai, India).

Synthesis of copper oxide nanoparticles

Copper oxide nanoparticles were prepared using two prepared solutions separately. Solution - 1: About 6.9g of copper sulphate pentahydrate was dissolved in 100ml of distilled water. To dissolve completely the mixture was stirred in a magnetic stirrer kept at constant conditions (37°C and 200rpm). Solution-2: About 34.6g of sodium potassium tartrate and 12g of sodium hydroxide was dissolved in 100ml of distilled water. To dissolve completely similar stirring conditions were carried out using a magnetic stirrer kept at constant conditions (37°C and 200rpm). About 50ml of solution-1 and 50ml of solution-2 was mixed together with vigorous stirring and 5g of glucose was added and then the mixture was stirred vigorously was 10mins and then keep in boiling water bath at 60°C for 10mins. Then, the obtained mixture is centrifuged and washed with distilled water twice and with ethanol twice and it was air dried and the powdered substance was used for further analysis (Kooti and Matouri, 2010).

Preparation of Nanometal vitamin complex (CuONP_s+VE)

A series of Nanometal vitamin complex were synthesized according to the following general procedure: copper oxide nanoparticles were added gradually to magnetically stirred ethanol solution (20ml). Then, to the first reaction mixture, vitamin E oil was added and stirred carefully at about 60-80 °C till the reaction reached equilibrium. Then evaporation of the solvent (by placing the reaction mixture in a fume cupboard) and nanometal vitamin complex is synthesized (Fazary *et al.*, 2016).

Inhibitory effect of Nano-Metal Vitamin complex (CuONP_s +VE) on the viable cells using MTT assay methods

The inhibitory effects of Nano-Metal Vitamin com-

plex are determined on the viable bacterial cells (*Escherichia coli* *Salmonella* sp and *Vibrio* sp) using a standard MTT assay method. In brief, about 1ml of actively growing culture was taken and its absorbance indicating the prevalence of the organisms was calculated with help of UV-visible spectrophotometer at 600nm. At the development of cells on the culture a mixture of CuONPs+VE and MTT reagent is added and subjected to 10 minutes incubation. Centrifugation of the above sample at 8000rpm for 5mins resulted in crude pellet which was then redissolved in sterilized water.

The CFU was enumerated for both CuONPs+VE-MTT exposed and Control cells. The difference in CFU for test sample and control was calculated to express in percentage of cell inhibition (viable cells).

$$\text{Cell inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where A - Control cells, and B - MTT exposed cells.

Identification of effective hurdle factors that could retard the growth of pathogens during different seafood storage conditions

To investigate the effect of CuONPs+VE along with other factors (chilling temperature, brine salt concentrations and pasteurization temperature) for the growth inhibition of THB (total heterotrophic bacteria) and TC (total coliforms) was studied. The factors that were selected for the inhibition of the organisms were thus referred to as Hurdle Factors. The effective hurdle factor that could inhibit the increase in number of bacterial gut microbiota in the stored seafood products (Fish) was evaluated in the present research work. Two different hurdle factors with their respective storage conditions were analysed.

Effect of CuONPs+VE + Chilling temperature (°C)

About 400g of fresh marine fish was immersed in CuONPs+VE solution for 8h at room temperature (30°C). The excess CuONPs+VE suspension was removed by manual gentle shaking for 30 seconds and packed in a sterile food packing cling films. A separate pack of fishes (100g) were stored at 4°C in a refrigerator and the other pack of fishes (100g) was frozen at -18°C (freezer). Similarly, another set of fishes without immersing in the CuONPs+VE (Control) were packed and stored at 4°C and -18°C respectively.

Effect of CuONPs+VE + Brine salt concentrations (%)

To detect the effect of brine salt as one of the hurdle factor, sodium chloride at four different concentrations (5%, 10%) was selected. Each concentration of salt solution was prepared and the marine fishes

were immersed with and without CuONPs+VE suspension for 8h. Difference in the number of cells were enumerated as per microbiological standards and presented separately for total heterotrophic bacteria (THB) and total coliforms (TC).

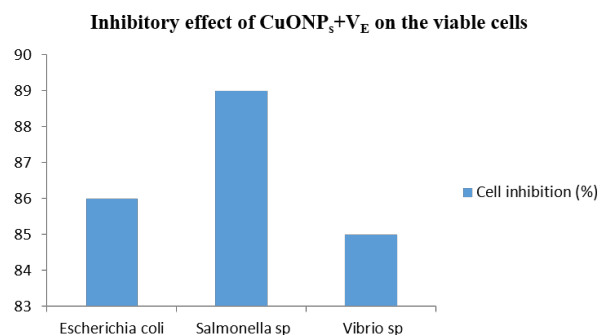


Figure 1: Inhibitory effect of CuONPs+VE on the viable cells

RESULTS AND DISCUSSION

Inhibitory effect of CuONPs+VE on the viable cells using MTT assay methods

The inhibitory effect of CuONPs+VE on the viable cells of food pathogens was evaluated using MTT assay methods. In this method, the CFU of the control cells was compared with the CFU of the CuONPs+VE exposed cells. During the analysis, all the three pathogens exposed to MTT showed maximum cell inhibition Figure 1. The CuONPs+VE exposed *Escherichia coli* reduced upto 86±0.57% after enumerating the CFU in a Plate Count Agar media. *Salmonella* sp and *Vibrio* sp exhibited 89±1.04% and 85±0.25% respectively. The obtained results were found to be well in accordance to the results of MIC and antibacterial activity values; where the CuONPs+VE in the well diffusion method showed good inhibitory zones against all the three test organisms. The cells were colorimetrically measured the by MTT assay, it is tetrazolium salt, which in the presence of fully functioned cell is reduced into a product possess for display of alive cells. The MTT assay reveals that the CuONPs+VE inhibited the metabolic activity of test organisms. The antimicrobial metal nanoparticles act on exterior region, tracked by lysis of cell there by reduction in biochemical reaction.

Identification of effective hurdle factors that could retard the growth of pathogens during different seafood storage conditions

Effect of CuONPs+VE + Chilling temperature (°C)

The effect of CuONPs+VE and chilling temperatures for preservation of seafood products is evaluated

Table 1: Effect of CuONPs+VE and chilling temperature at 4°C on Total heterotrophic bacteria (THB)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
Control 4°C	4x10 ² CFU/ml	6x10 ² CFU/ml	7x10 ² CFU/ml	8x10 ² CFU/ml	8x10 ² CFU/ml	10x10 ² CFU/ml
CuONPs+VE 4°C	2x10 ² CFU/ml	2x10 ² CFU/ml	3x10 ² CFU/ml	4x10 ² CFU/ml	4x10 ² CFU/ml	4x10 ² CFU/ml

and the chilling temperatures used are +4°C and -18°C. The inhibitory effect of CuONPs+VE is compared with the control i.e. the control sample at the respective temperatures. The results were observed weekly upto six weeks (34 days). The growth of both heterotrophic bacteria and coliforms are evaluated and tabulated.

Total heterotrophic bacteria (THB)

The growth of heterotrophic bacteria (THB) at 4°C was presented in Table 1. At 1st day, the control had 4x10²CFU per ml which was gradually increased upto 34th day. On 34th day, the growth was observed as 10x10²CFU/ml. About 6x10²CFU/ml were observed on 1st week (7th day). The cell count remained constant at 21st and 28th day (8x10²CFU/ml). Whereas, CuONPs+VE incorporated samples showed 3x10²CFU/ml in the 1st day. The Total Heterotrophic Bacterial count (THB) increased to 3x10²CFU/ml at the 14th day. About 4x10²CFU/ml were observed at 34th day. When compared to control significant heterotrophic bacterial growth difference was observed for CuONPs+VE exposed samples.

The growth of heterotrophic bacteria (THB) at -18°C was presented in Table 2. On 1st and 7th day, about 3x10²CFU per ml was observed for the control samples. The growth was slightly increased (4x10²CFU/ml) at 34th day due to -18°C. Samples in the presence of CuONPs+VE were found 1x10²CFU/ml in the 1st day. The cell count remained constant at 7th, 14th and 21st day (2x10²CFU/ml). At 34th day no CFU was evident, indicating that CuONPs+VE had significant inhibitory effect.

Total coliforms (TC)

The Total Coliform Growth (TC) on sample incorporated with CuONPs+VE and untreated at 4°C was presented in Table 3. On 1st day, the control had 3x10²CFU/ml. The bacterial cell count was increased at 7th and 14th day (4x10²CFU/ml) and started declining in the following weeks. At the 21st day the total coliform count was 3x10²CFU/ml and the bacterial count remained constant till 28th day (3x10²CFU/ml). About 2x10²CFU/ml was observed

at 34th day. Comparatively, the sample incorporated with CuONPs+VE had 2x10²CFU/ml at the 1st and 7th day and the cell count decreased on following days. About 1x10²CFU/ml growth was observed till 28th day respectively. There was no cell growth observed at 34th day.

Table 4 represents the Total Coliform growth (TC) on control sample and sample incorporated with CuONPs+VE at -18°C. On 1st day, the control had 1x10²CFU/ml. The bacterial cell count remained constant till 14th day. After 21 days, the total coliform count was 3x10²CFU/ml and CFU declined after 28th day (2x10²CFU/ml). No cell growth was obtained at 34th day. Comparatively, the sample incorporated with CuONPs+VE showed 1x10²CFU/ml from 1st day to 21st day. As coliforms are pathogenic bacteria which are found less in seafood, preserving under chilling temperatures incorporated with CuONPs+VE resulted in no growth from 28th day. Due to the effect of low temperature, no significant difference in the control and nanoparticles exposed samples were evident. The results showed that hurdle factor did not played any role with the effect of -18°C.

Effect of CuONPs+VE + Brine salt concentrations (%)

The effect of CuONPs+VE with brined samples was examined and optimization was done using different salt concentrations (5%, 10%, 15%, and 20%). The effect of CuONPs+VE is compared with the control i.e. the brined sample at different salt concentration. The results were observed weekly upto six weeks (34days). The growth of both heterotrophic bacteria and coliforms are evaluated and tabulated.

Total heterotrophic bacteria (THB)

Table 5 represents the growth of heterotrophic bacteria (THB) at 5% salt concentration. On 1st day, the control had 7x10²CFU per ml which was gradually decreased upto 21th day. The growth was observed as 5x10²CFU/ml at 34th day. About 4x10²CFU/ml was observed on 7th day for CuONPs+VE exposed samples. The cell count remained constant at 14th and 21st day (4x10²CFU/ml). About 3x10²CFU/ml were present at 28th day respectively. The Total

Table 2: Effect of CuONPs+VE and chilling temperature at -18°C on Total heterotrophic bacteria (THB)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
Control	3x10 ²	3x10 ²	4x10 ²	4x10 ²	4x10 ²	4x10 ²
-18°C	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml
CuONPs+VE	1x10 ²	2x10 ²	2x10 ²	2x10 ²	1x10 ²	0x10 ²
-18°C	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml

Table 3: Effect of CuONPs+VE and chilling temperature at 4°C on Total Coliforms (TC) by CuONPs+VE

Sample	1 day	7 days	14 days	21 days	28 days	34 days
Control 4°C	3x10 ²	4x10 ²	4x10 ² CFU/ml	3x10 ² CFU/ml	3x10 ²	2x10 ² CFU/ml
	CFU/ml	CFU/ml			CFU/ml	
CuONPs+VE	2x10 ²	2x10 ²	1x10 ² CFU/ml	1x10 ² CFU/ml	1x10 ²	0x10 ² CFU/ml
4°C	CFU/ml	CFU/ml			CFU/ml	

Table 4: Effect of CuONPs+VE and chilling temperature at -18°C on Total Coliforms (TC) by CuONPs+VE

Sample	1 day	7 days	14 days	21 days	28 days	34 days
Control	1 x10 ²	2 x10 ²	2 x10 ²	3 x10 ²	2 x10 ² CFU/ml	0 x10 ² CFU/ml
-18°C	CFU/ml	CFU/ml	CFU/ml	CFU/ml		
CuONPs+VE	1 x10 ²	1 x10 ²	1 x10 ²	1 x10 ²	0 x10 ² CFU/ml	0 x10 ² CFU/ml
-18°C	CFU/ml	CFU/ml	CFU/ml	CFU/ml		

Table 5: Effect of CuONPs+VE and 5% salt Concentration on Total heterotrophic bacteria (THB)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
5% Salt	7x10 ²	6x10 ²	5x10 ²	4x10 ²	5x10 ²	5x10 ²
	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml
CuONPs+VE	5x10 ²	4x10 ²	4x10 ²	4x10 ²	3x10 ²	2x10 ²
+ 5% Salt	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml

Heterotrophic Bacterial count (THB) decreased to 2x10²CFU/ml at 34th day which is significantly decreased when compared to that of control samples. The hurdle factor played an important role when the nanoparticles are coupled with 5% salt concentrations.

From the above results it is clear that, increase in salt concentration increases the inhibitory effect. Moreover, the rate of inhibition of THB was higher in CuONPs+VE incorporated samples than the plain brined samples and the effective concentration was found to be 20%.

The growth of heterotrophic bacteria (THB) at 10% salt concentration is presented in Table 6. On 1st day, the control had 6x10²CFU per ml which was found to be increased in 7th day (6x10²CFU per ml). The growth was observed as 5x10²CFU/ml at 21st day and 4x10²CFU/ml at

28th day. About 5x10²CFU/ml was observed at 34th day respectively. Whereas in the presence of CuONPs+VE there were 2 x10²CFU/ml present in the 1st day. The heterotrophic bacterial count increased to 4x10²CFU/ml at 7th and 4th day. About 3x10²CFU/ml was observed at 21st day and 1x10²CFU/ml were present at 28th day. On 34th day there cell growth remained constant as they prevented the growth of heterotrophic bacteria.

Table 7 represents the growth of Total Coliforms (TC) at 5% salt concentration. On 1st day, the control had 5x10²CFU per ml which was gradually increased at 7th day (7x10²CFU/ml). The growth was observed as 6x10²CFU/ml at 14th day. About 5x10²CFU/ml was observed on 28th day. The cell count remained constant at 28th day and 34th day (4x10²CFU/ml) respectively. Whereas, CuONPs+VE incorporated samples showed 4x10²CFU/ml in the

Table 6: Effect of CuONPs+VE and 10% salt Concentrationon Total heterotrophic bacteria (THB)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
10% Salt	6 x102 CFU/ml	7 x102 CFU/ml	6 x102 CFU/ml	5 x102 CFU/ml	4 x102 CFU/ml	5 x102 CFU/ml
CuONPs+VE + 10% Salt	2 x102 CFU/ml	4 x102 CFU/ml	4 x102 CFU/ml	3 x102 CFU/ml	1 x102 CFU/ml	1 x102 CFU/ml

Table 7: Effect of CuONPs+VE and 5% salt Concentration onTotal Coliforms (TC)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
5% Salt	5 x102 CFU/ml	7 x102 CFU/ml	6 x102 CFU/ml	5 x102 CFU/ml	4 x102 CFU/ml	4 x102 CFU/ml
CuONPs+VE + 5% Salt	4 x102 CFU/ml	3 x102 CFU/ml	2 x102 CFU/ml	3 x102 CFU/ml	3 x102 CFU/ml	4 x102 CFU/ml

Table 8: Effect of CuONPs+VE and 10% salt Concentrationon Total Coliforms (TC)

Sample	1 day	7 days	14 days	21 days	28 days	34 days
10% Salt	5 x102 CFU/ml	6 x102 CFU/ml	6 x102 CFU/ml	5 x102 CFU/ml	4 x102 CFU/ml	3 x102 CFU/ml
CuONPs+VE + 10% Salt	3 x102 CFU/ml	3 x102 CFU/ml	2 x102 CFU/ml	1 x102 CFU/ml	2 x102 CFU/ml	1 x102 CFU/ml

1st day. The Total Coliforms (TC) decreased to 2x10²CFU/ml at 14th day. The TC remained constant at 21st at 28th day (3x10²CFU/ml) and about 4x10²CFU/ml was observed at 34th day respectively.

The growth of Total Coliforms (TC) at 10% salt concentration is presented in Table 8. On 1st day, the control had 15x10²CFU per ml which was found to be increased in the 7th day (6x10²CFU per ml). The growth was observed as 5x10²CFU/ml at 21st day and 4x10²CFU/ml at 28th day. The growth was decreased and about 3x10²CFU/ml was observed at 34th day respectively. Whereas in the presence of CuONPs+VE there were 3 x10²CFU/ml present in the 1st day. The bacterial count was 3x10²CFU/ml at 7th day and decreased to 2x10²CFU/ml at 14th day. About 1x10²CFU/ml was observed at 21st day and 2x10²CFU/ml at 28th day. On 34th day the total number of coliforms was found to be 1x10²CFU/ml.

From the above results it is clear that, the rate of inhibition of TC was higher in CuONPs+VE incorporated samples than the plain brined samples and the effective concentration was found to be 20%.

Salting is one of the oldest food preservation methods. Salting of seafood is done with salt. Chlorine and sodium ions are carried from brine to fish, and

water dipoles are carried from fish to the environment. The effects of brine and dry salting methods on the nutritional composition of chub and the changes on microbial that arise during storage were investigated by (Thorarinsdottir *et al*, 2004).

It was determined that crude protein, lipid, crude ash and salt amounts in the group where dry salting method was applied were higher than the group where brine salting occurred, in addition protein and lipid values decreased as storage period was longer. It was determined that there is an increase in total aerobic mesophilic, psychrophile bacteria and enumeration of yeast and mould as storage period increased, while coliform bacteria decreased.

The Effect of CuONPs+VE and chilling temperatures (-18°C and +4°C) and effects of CuONPs+VE and brine salts at different concentration (5%, 10%, 15%, 20%) were studied. CuONPs+VE with different chilling temperatures showed significant results on compared to control temperatures.

Similarly CuONPs+VE with brine salts at different concentration showed better results than the control sample containing brine salts.

CONCLUSIONS

Effect of CuONPs+VE and chilling temperature (-18°C and $+4^{\circ}\text{C}$); and Effect of CuONPs+VE and brine salts at various concentrations (5%, 10%) were evaluated. CuONPs+VE with different chilling temperatures and brine salt concentration showed significant results on compared to control temperatures. Thus CuONPs+VE due to their bacteriostatic activity can be efficiently used in hurdle technology which reduces the food spoiling organisms. Thus CuONPs+VE in combined with hurdle technology can be used as alternatives for chemical preservatives in preservation techniques. The suggested process would address antimicrobial activity as well as destructive oxidation of the desired lipids and fats. However, more efforts are required to understand the role of proximate composition of fish, post-harvest history, environmental conditions, initial microbial load, type and nature of bacteria and their interaction in order to optimize the shelf-life of fish.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

- Abbas, K. A., Saleh, A. M., Lasekan, M., O 2009. The relationship between water activity and fish spoilage during cold storage: A review. *Journal of Food Agriculture and Environment*, 7:86–90.
- Dasgupta, N., Ranjan, S., Mundekkad, D., Ramalingam, C., Shanker, R., Kumar, A. 2015. Nanotechnology in agro-food: From field to plate. *Food Research International*, 69:381–400.
- Fazary, A. E., Ju, Y.-H., Rajhi, A. Q., Alshihri, A. S., Alfaifi, M. Y., Alshehri, M. A., Saleh, K. A., Elbehairi, S. E. I., Fawy, K. F., Abd-Rabboh, H. S. M. 2016. Bioactivities of Novel Metal Complexes Involving B Vitamins and Glycine. *Open Chemistry*, 14(1):287–298.
- Ghaly 2010. Fish Spoilage Mechanisms and Preservation Techniques: Review. *American Journal of Applied Sciences*, 7(7):859–877.
- Harikumar, P. S. 2016. Antibacterial Activity of Copper Nanoparticles and Copper Nanocomposites against Escherichia Coli Bacteria. *International Journal of Sciences*, 2(02):83–90.
- Kooti, Matouri, L. 2010. Fabrication of nanosized cuprous oxide using fehling's solution. *Transaction F. Nanotechnology*, 17(1):73–78.
- Leroi, F. 2014. Role of bacteria in seafood products. In Kim, A. S. K., editor, *Seafood Science: Advances in Chemistry, Technology and*, pages 458–482. CRC Press.
- Ohene-Adjei, S., Bertol, T., Hyun, Y., Ellis, M., McKeith, F. K., Brewer, M. S. 2004. Effect of vitamin E, low dose irradiation, and display time on the quality of pork. *Meat Science*, 68(1):19–26.
- Ozogul, Y., Ayas, D., Yazgan, H., Ozogul, F., Boga, E. K., Ozyurt, G. 2010. The capability of rosemary extract in preventing oxidation of fish lipid. *International Journal of Food Science & Technology*, 45(8):1717–1723.
- Raffi, M., Mehrwan, S., Bhatti, T. M., Akhter, J. I., Hameed, A., Yawar, W., ul Hasan, M. M. 2010. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. *Annals of Microbiology*, 60(1):75–80.
- Simat, V., Ficovic, M., Cagalj, M., Skroza, D., Ljubenkovic, Mekinac, Ig 2017. Preventive effect of herb extracts on lipid oxidation in fish oil. *Croatian Journal of Food Technology*, 12(1):30–36.
- Thorarinsdottir, K. A., Arason, S., Bogason, S. G., Kristbergsson, K. 2004. The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). *International Journal of Food Science and Technology*, 39(1):79–89.