Global Veterinaria 21 (6): 368-372, 2019
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2019.368.372

Effect of Nano-Chitosan and Onion Extract as Coating Materials on the Quality Properties of Chicken Fillet Meat During Refrigeration

[1]F.A. Shaltout, [2] E.M. El-Diasty and [3]Asmaa M.A. Hassan

[1]Department of Food Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt
[2]Department of Mycology, Animal Health Research Institute Dokki, Giza (ARC), Egypt
[3]Student Campus, Benha University, Benha, Egypt

Absrtact: The present study examined the effects of nano chitosan alone and nano chitosan combined with crude onion extracts as coating materials for chicken fillets meat during refrigeration storage (7°C±1°C). Treatments included the following: The first portion was dipped in spore suspension 10^7 (control positive), 2nd portion was dipped in Nano chitosan alone (10 mg/ml), 3rd portion was dipped in Nano chitosan (10 mg /ml + crude onion extract, 4th portions was dipped in crude onion extract alone. Treatment of chicken breast fillets coated with nano chitosan could retard the growth of total mould more effectively, compared with crude onion extract due to the higher antimicrobial activity of nano-chitosan compared to onion extract due to their higher surface area per unit volume and charge density which provides interaction with the anionic cell membrane . The results indicated that nano chitosan and crude onion extract can be used for preservation of quality properties of chicken fillets meat samples. The nano chitosan was better than crude onion extract in reducing fungal count of fillets and the results indicated that nano chitosan can be used for preservation of quality properties of chicken fillets meat samples.

Key words: Nano-chitosan · Onion extract · Antifungal · Chicken meat

INTRODUCTION

Nano-chitosan is a natural material with excellent physicochemical properties. It is environmentally friendly and bioactive. Nano-chitosan have been prepared by several approaches, including physical crosslinking by ionic gelation between chitosan and specific negatively charged macromolecules such as pentasodium tripolyphosphate [1]. Moreover, chitosan and chitosan nanoparticle films and coatings can be used as a vehicle for incorporating natural or chemical antimicrobial agents, antioxidants, enzymes or functional substances such as plant extracts, probiotics, minerals or vitamins [2]. However, chitosan nanoparticles exhibited higher antimicrobial activity than chitosan during the storage period [3].

Onion (*Allium cepa L.*) is a multipurpose food plant that is used as traditional Egyptian spices. It has great health significance and is consumed for its putative nutritional and health benefits for centuries. Traditionally, onions and plants belonging to the *Allium* genus and *Allium* is the largest and important representative genus of the family *Liliaceae* which have been used as an herbal remedy for a wide range of ailments, due to their association with many pharmacological effects. Biological effects attributed to onions have been commonly ascribed to the volatile sulphur containing compounds, such as thiosulfinates, mainly responsible for the characteristic taste, aroma and lachrymatory effects [4, 5]. The antibacterial and antifungal properties reported to be possessed by flavanoids has increased the interest of the food industry in these natural compounds as components to improve food stability against microbiological spoiling agents [6]. Protection of food from pathogens and spoilage organisms has been traditionally achieved by chemical methods, but during recent years there has been an increase in consumer interest in developing foods which contain a low level or are free of chemical preservatives [7].

Corresponding Author: Eman M. El-diasty, Department of Mycology, Animal Health Research Institute Dokki, Giza (ARC), Egypt.

Changes in eating habits arising from the development of society, recently people search for affordable and healthier foods with satisfactory taste and pleasant appearance [8]. Chicken meat and their products can be contaminated by microorganisms from the environment, equipment and operator's hands where due to poor hygiene there is ample scope for contamination [9].

In processing plants, contamination of poultry meat products can occur throughout ideal processing, packaging and storage until the product is sufficiently cooked and consumed. Heavy microbial loads enter the processing operations with the living birds and these microorganisms can be disseminated throughout the plant during processing. Diseases can also result when these products are not properly cooked and post-processing contaminated [10]. Contamination of chicken products with different mould species as *Aspergillus*, *Fusarium* and *Penicillium* considers a real hazard as it affecting the quality of these products. It is generally accepted that the *Aspergillus*, *Fusarium* and *Penicillium* moulds are among the most important in producing mycotoxins. The mycotoxins of greatest concern include aflatoxin, which is generally produced by *Aspergillus* species such as *A. flavus*, *A. parasiticus* and the rare *A. nomius*, deoxynivalenol and zearalenone. Mycotoxins have direct potential health hazards to "human health and animals even with its low levels and severe economic losses [11].

Therefore, the aim of the present assay was to investigate a comparative basis antifungal effect of nano-chitosan and onion extract coatings on quality of chicken fillet meat during refrigeration (7°C).

MATERIALS AND METHODS

Chitosan Nanoparticles: Nano chitosan solution was obtained from Nanotech Company (Gate3, Dreamland, 6th October, Cairo- Egypt.

The chitosan was prepared as the method described by Ojagh *et al*. [2]. Then Nanoparticles were achieved by the cross linking of chitosan (95% deacetylated, MW: 1000kDa)-sodium tripolyphosphate solution. Chitosan (2%) was dissolved in 1% acetic acid solution to form chitosan solution. Sodium tri-polyphosphate solution (2%, w/v) dissolved in distilled water. Under magnetic stirring at room temperature, 4 ml of tri-polyphosphate solution (2%) was added into 100 mL of chitosan solution. The mixture was stirred for 60 mins, then, treated with sonication at 1.5 kW for 10 mins before being used [12]. The nano chitosan concentrations solution (10 mg/ml).

Preparation of Onion (*Allium cepa*) Extract: One hundred gram of cleaned, air dried onions obtained were blended separately and individually soaked in 100mL of ethanol for 24 h in a sterile glass container. The pulp obtained was shaken vigorously to allow for proper extraction of active ingredients. The crude extract was then filtered using sterile Whatmans No. 1 filter paper. The filtered extracts were then stored in the refrigerator at 4°C Tagoe *et al*. [13].

Preparation of Spore Suspension of Fungal Culture (*A. flavus*): The present study carried out by using a strain of *Aspergillus flavus* (GenBank accession number: KP137699) isolated previously at the Mycology Department - Animal Health Research Institute, Giza, Egypt. The *A. flavus* subcultured and grown for 7 days on Czapek yeast extract. The *A. flavus* culture was washed with 10 ml sterile distilled water in 2% Tween 80 with the aid of glass beads to help in the spore dispersion. The spore suspensions were standardized by calculated using haemocytometer with dilution to reach to$1.1x10^7$ spores/ml.Sample preparation Raw fresh chicken breast fillets (boneless) were purchased from a local poultry shop. (at 254 nm) for 30 minutes each side. The samples were divided into four groups, each group weighted 200 g., then each group subdivided into two smaller portions (100g, for each).

Marinated Preparation: The first portion was dipped in spore suspension 10^7 (control positive), 2nd portion was dipped in Nano chitosan alone (10 mg/ml), 3rd portion was dipped in Nano chitosan (10 mg /ml +crude onion extract, 4th portions was dipped in crude onion extract alone, respectively and left for 30 minutes at room temperature.

Samples Inoculation: All samples were dipped in the prepared *A. flavus* (count of about 10^7). The incubated for 30 minutes at 25°C then kept for another 30 minutes at room temperature to enhance the fungal spore attachment. All samples were stored at 7°C ± 1°C for 12 days and fungal counts were done every 3 days.

Organoleptic Examination: The second group divided into four portions for organoleptic examination (colour, odour and texture) evaluation scales of muscle foods were defined by overall colour, odour and texture and evaluated by 3 trained panellists according to Collins and Huey[14], Hunt *et al*. [15], Grossklaus *et al*. [16] and Miller, [17].

Statistical Analysis: The obtained results were statistically analysed according to Feldman *et al.* [18].

RSULTS AND DISCUSSION

Chicken meat is favoured by consumers around the world because of its desirable nutritional qualities, such as low-fat content and a relatively high concentration of polyunsaturated fatty acids. Fresh meat products are usually marketed at refrigerated temperatures (2-4°C). Lipid oxidation and microbial growth may occur during refrigeration storage. Spoilage of fresh poultry meat is a financial burden to producers and requires the development of new methods to extend the shelf-life and overall safety/quality of the meat, which is the main problem faced by the poultry processing industry [19]. Mould and yeast comprise a large group of microorganisms which are ubiquitous in nature and affects our food supply as a result of their contamination. They are responsible for major protein deterioration in developing countries. Their presence in meat are regarded as an indicator of the hygienic conditions under which these products are handled and stored lead finally to either spoilage or foodborne mycotoxicosis [20].

The results tabulated in table 1 show that the initial total count of *A. flavus* (day 0) value for the fresh chicken meat samples was $1.6x\ 10^7$ cfu /g then decreased gradually until 12 day and did not detect any colony by using Nano chitosan alone (10 mg/ml) and the reduction rate was (100%). While in treated samples with nano chitosan (10 mg/ml) + crude onion extract total mould values (*A. flavus*) was $1.4x10^3 \pm 3x10^2$ cfu /g with reduction rate by 99.1% at 3rd day, $1.1x10^2 \pm 1.2$ x10 CFU /g at 6th day with reduction rate by 99.9%, 0.8x10 ±0.4x10 cfu /g at 9th day with reduction rate by 99.9% and 0.7x10 ±0.2x10 cfu /g at 12th day with reduction rate by 99.9%. On the other hand, treated samples with crude onion extract, total mould values (*A. flavus*) was $1x10^5 \pm 7x10^4$ cfu/g with reduction rate by 90.6% at 3rd day, $6.1x10^4 \pm 2.1x10^3$ cfu /g at 6th day with reduction rate by 98.2%, $4.2x10^4 \pm 1.7x10^3$ cfu/g at 9th day with reduction rate by 99.7% and $4.1x10^4 \pm 1.1x10^3$ cfu/g at 12th day with reduction rate by 99.7%. Concerning to the control samples (untreated) decomposed at 6th day.

Treatment of chicken breast fillets coated with nano chitosan could retard the growth of total mould more effectively, compared with crude onion extract due to the higher antimicrobial activity of nano-chitosan compared to onion extract due to their higher surface area per unit volume and charge density which provides interaction with the anionic cell membrane. Antimicrobial function of chitosan is known against wide variety of Gram-positive, Gram-negative bacteria, mould and yeast. He polycationic property of chitosan exhibits antimicrobial activity due to its ability to interact with negatively charged cell membranes of these microbes [20].

Chitosan nanoparticles exhibit higher antifungal efficacy against *A. flavus* than onion extract chitosan based on the special character based on the special character of the nanoparticles such as nanoparticle´s longer surface area and higher affinity with fungal cell for a quantum-size effect. Nano-chitosan coating is more appropriate than onion extract coating to extend the shelf-life and delay the deterioration of chicken meat fillet during refrigerated storage. Goy *et al*. [21] reported that nano chitosan particles have been found to show antifungal activities with MIC values ranging from 10-500 ppm against variety of species. Fungal species such as Botrytis cinerea and *Drechstera sorokiana* exhibited very low MIC of 10 ppm while several other fungi such as *Candida albicans, Microsporum canis, Aspergillus fumigatus, Aspergillus parasiticus* have shown higher MIC between 1000 and 2000 ppm, while for *Trichophyton mentagrophytes* and Byssochlamys spp. the MIC values were found about 2000-5000 ppm. Regardless of antifungal activity of onion, it has been shown that crude extracts of onion may have potent antifungal properties [22]. Phenolic compounds such as quercetin and kaempferol present in onion may contribute to this activity [23]. In this study also onion-induced dose-dependent fungal growth inhibition may be attributed to the presence of phenols and secondary metabolites. In accordance to these findings, reports by Skerget *et al*. [24] can be quoted which recorded that the extracts of red onion's skin and edible part possess antifungal activity. Odour and flavour are important aspects of meat quality, sometime used as a determining criteria in the acceptance or rejection of the products [25]; while Miller [17] stated that texture or feel and colour of muscle food influence perceptions of acceptability and are the most reliable, rapid indicators of their quality.

Effect of concentration of Nano chitosan and crude onion extract on sensory attributes of treated chicken meat slices stored at refrigerated temperature 7 ± 1°C. The results in Table 2 reveal that the initial colour, odour and texture of the examined chicken meat samples were 4.9 ±0.05, 4.8 ±0.05 and 4.9 ± 0.04 of control samples then

Table 1: Antifungal activity of different concentration of Nano chitosan and onion extract against total mould count in chicken meat samples during storage at 7°C ± 1

		3rd day		6th day		9th day		12th day	
Treatment	0 day	Count	Reduction rate by (%)	Count	Reduction rate by (%)	Count	Reduction rate by (%)	Count	Reduction rate by (%)
Control (*A.flavus* 10^7)	1.6x 10^7	2 x 10^8± 1.1 x 10^7	----	3.2 x 10^8 ±1.7 x 10^7	----	Decomposed			
Control (*A.flavus* 10^7)	1.6x 10^7	2 x 10^8± 1.1 x 10^7	----	3.2 x 10^8 ±1.7 x 10^7	----	Decomposed			
Nano chitosan alone (10 mg/ml + (*A. flavus* 10^7)	1.6x 10^7	ND	100	ND	100	ND	100	ND	100
Nano chitosan alone (10 mg/ml + (*A. flavus* 10^7) + crude onion extract	1.6x 10^7	1.4 x 10^3± 3 x 10^2	99.1	1.1 x 10^2 ± 1.2 x 10^1	99.9	0.8 x 10^1± 0.4 x 10	99.9	0.7 x 10± 0.2 x 10	99.9
Crude onion extract + (*A.flavus* 10^7)	1.6x 10^7	1 x 10^5± 7 x 10^4	90.6	6.1 x 10^4 ± 2.1 x 10^3	98.2	4.2 x 10^4± 1.7x 10^3	99.7	4.1 x 10^4± 1.1 x 10^3	99.7

Table 2: Effect of different concentration of Nano chitosan and onion extract on sensory attributes of treated chicken meat slices stored at refrigerated temperature (7 ± 1 °C)

	Control			Nano chitosan alone (10 mg/ml +(*A. flavus*10^7)			Nano chitosan alone (10 mg/ml +(*A.flavus* 10^7)+crude onion extract			Crude onion extract + (*A. flavus* 10^7)		
Preservation time	*Colour	*Odour	*Texture	*Colour	*Odour	*Texture	*Colour	*Odour	*Texture	*Colour	*Odour	*Texture
0 day	4.9± 0.05	4.8±0.05	4.9±0.04	5.0± 0.03	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	4.9±0.04	4.8±0.05	4.9±0.04
3rd day	3.5± 0.2	4.7±0.07	4.7±0.07	5.0± 0.03	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04
6th day	2.5± 0.16	3.0±0.12	3, 4±0.17	5.0± 0.03	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04
9th day	Decomposed		5.0± 0.03	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	
12th day			5.0± 0.03	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	4.9± 0.04	4.9±0.05	4.9± 0.04	

they gradually changed at 3rd day to 3.5±0.2, 4.7 ±0.07 and 4.7 ±0.07; 6th day to 2.5±0.16, 3. ±0.12and 3.4 ±0.17 and at 9th the sample decomposed, meanwhile in samples treated with Nano chitosan alone (10 mg/ml) the colour, odour and texture score were 5 ±0.03, 4.9 ±0.05 and 4.9 ± 0.04, respectively and there is no change in colour, odour and texture of the examined treated samples until day 12th while in samples treated with onion extract there is no change in colour, odour and texture from 0day to 12 days. Nano chitosan influence of packaging made of nano chitosan on colour stability in refrigerated ground beef has been studied previously [26].

The information given by the achieved results proved that treatment of chicken fillet meat by addition of Nano chitosan alone (10 mg/ml) and crude onion extract inhibited the mould growth and extends the shelf-life of refrigerated treated chicken fillet meat. It can be concluded that nano chitosan these plant extracts have the potential to be used in food as flavoring and natural preservatives to control food spoilage.

REFERENCES

1. Calvo, P., C. Remuñán-López, J.L. Vila-Jato and M.J. Alonso, 1997. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J. Appl. Polym. Sci., 63: 125-132.
2. Ojagh, S.M., M. Rezaei, S.H. Razavi and S.M.H. Hosseini, 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry, 120(1): 193-198.
3. Ramezani, Z., M. Zarei and N. Raminnejad, 2015. Comparing the effectiveness of chitosan and nanochitosan coatings on the quality of refrigerated silver carp fillets. Food Control, 51: 43-48.
4. Rose, P., M. Whiteman, P.K. Moore and Y.Z. Zhu, 2005. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Reports, 22: 351-368.
5. Lanzotti, V., 2006. The analysis of onion and garlic. Journal of Chromatogrphy, A 21: 1112(1-2): 3-22.
6. Taguri, T., T. Tanaka and I. Kouno, 2004. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biological & Pharmaceutical Bulletin, 27: 1965-1969.
7. Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez-Alvarez, 2008. Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. International Journal of Food Science and Technology, 43: 526-531.
8. Field, R.A., 1988. Mechanically separated meat, poultry and fish. In Pearson A.M. and Dutson, T.R. (Eds), Edible meat by-products. (83-128). London: Elsevier.
9. Kumar, H., S. Otta and I. Karunasagar, 2001. Detection of Shiga toxigenic Escherichia coli in fresh seafood and meat marketed in Mangalore, India by PCR, Lett. Appl. Microbiol., 32: 334-338.
10. Zhang, L., M.A. Davis and D.E. Conner, 2001. Poultry- borne pathogens: plant considerations. Poultry Meat Processing chapter 9. ISBN.

11. Varshney, J.L., D.K. Agrawal and A.K. Sarbhoy, 1991. Mycotoxin contamination of food, its influence of animals, human and plants and its management. Proc. Symposium Mycotoxin incidence and human Health, Bhagalpur, pp: 147.
12. Du, W.L., S.S. Niu, Y.L. Xu, Z.R. Xu and C.L. Fan, 2009. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. Carbohydrate Polymers, 75: 385-389.
13. Tagoe, D.N.A., H.D. Nyarko and R. Akpaka, 2011. A Comparison of the Antifungal Properties of Onion (Allium cepa), Ginger (Zingiber officinale) and Garlic (Allium sativum) against *Aspergillus flavus, Aspergillus niger* and Cladosporium herbarum. Research Journal of Medicinal Plants, 5(3): 281-287.
14. Collins, D.S. and R.J. Huey, 2015. Gracey's Meat Hygiene. Eleventh edition published by John Wiley & Sons, Ltd.
15. Hunt, M.C., J.C. Actorn, R.C. Benidict, C.R. Calkins, D.P. Comforth, L.E. Jeremiah, D.G. Olson, C.P. Salm, J.W. Savel and S.D. Shivas, 1991. Guidelines for meat color evaluation. Reciprocol. Meat Conf., 44: 232.
16. Grossklaus, D., W. Bruhann, R. Levetzow and U. Gotze, 1979. Geflugelfleisch-hygiene Tierhaltung, Schlachtung, Lebendtier und Fleischuntersuchung, Erzeugisse Rechtsgrund-lagen. Verlag Paul Parey. Berlin und Hamburg.
17. Miller, R.K., 1994. Quality characteristics. In: Kinsman, K. M.: Kotula, A. W. and Breidenstein, B. C. (Eds.). Muscle Foods. Chapman & Hall New York. London.
18. Feldman, D., R. Hoffman and J. Simpson, 2003. The solution for data analysis and presentation graphic. 2nd ed., Abacus lancripts, Inc., Barkeley, CA, USA.
19. Zhang, L., Z. Yang, J. Wei, P. Su, W. Pan, X. Zheng, K. Zhang, L.Lin, J. Tang, Y. Fang and D. Zhiyun, 2017. Essential oil composition and bioactivity variation in wild-growing populations of *Curcuma phaeocaulis* Valeton collected from China. Industrial Crops and Products, 103: 274-282.
20. Kwon-Chung, K.J. and J.E. Bannet, 1992. Medical mycology. Lea and Febiger. Philadelphia-London.
21. Goy, R.C., D. Britto and O.B.G. Assis, 2009. A review of the antimicrobial activity of chitosan. Polímeros: Cienciae Techologia, 19: 1-7.
22. Elnima, E.I., S.A. Ahmed, A.G. Mekkawi and J.S. Mossa, 1983. The antimicrobial activity of garlic and onion extracts. Pharmazie, 38(11): 747-758.
23. Rauha, J.P., S. Remes and M. Heinonen, 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56: 3-12.
24. Skerget, M., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry, Barking, 89: 191-198.
25. Mottram, D.S., 1994. Some aspects of the chemistry of meat flavour. In: The Flavour of Meat and Meat Products (Ed. F. Shahidi). Chapman and Hall, London, pp: 210-230
26. Suman, S.P., R.A. Mancini, P. Joseph, R. Ramanathan and M.K. Konda, 2010. Packaging-specific influence of chitosan on colour stability and lipid oxidation in refrigerated ground beef. Meat. Sci., 86: 994-9.