Evaluation of antimicrobial effect of Chitosan and acetic acid during thawing of frozen chicken

Shawkt m.Fathi[1], Osama A.Aattala[2], Bassam H.Mashat[2]
[1] Faculty of agriculture and veterinary medicine al qassim university
[2] The Custodian of the Two Holy Mosques Instittute for Hajj and Umrah Research

تقييم الشيتوزان وحامض الخليك كمثبطات لنمو الميكروبات أثناء عملية إذابة الدواجن المجمدة

شوكت محمد فتحى[١]، اسامة على عطاالله[٢]، بسام حسين مشاط[٢]
[١] كلية الزراعة والطب البيطرى جامعة القصيم
[٢] معهد خادم الحرمين الشريفين لابحاث الحج والعمرة - جامعة ام القرى

ملخص البحث (Abstract):

تعتبر عملية إذابة الدواجن المجمدة من النقاط الحرجة فى المنشات الغذائية خاصة بان العاملين فى تلك المنشات يضعون الدجاج المجمد فى احواض الماء الساخن للاسراع من عملية مما يتسبب عن ذلك فقد فى القيمة الغذائية للدواجن والمساعدة على نمو الميكروبات المسببة للفساد والتسمم الغذائى ، وتعتبر الاذابة بواسطة وضع اللحوم المجمدة فى الثلاجات عند درجة حرارة (5 -0 درجة مئوية)لمدة ٢٤ ساعة من اهم وافضل الطرق الصحية المتبعة ولكن للظروف الخاصة بالنسبة للمنشات الغذائية وخاصة (مطابخ الاعاشة - مطابخ المشاعر) فى مكة المكرمة والمشاعر المقدسة وعلى الرغم من ان هناك قواعد محددة لعملية الاذابة للحوم المجمدة الا انة هناك صعوبات تواجة العاملين فى تنفيذ تلك الاشتراطات وذلك لعدة اسباب منها ضيق المساحات فى المطابخ وعدم وجود منطقة مخصصة لعملية الاذابة ,عدم توافر ثلاجات او مبردات لعملية الاذابة الصحيحة والمطلوبة من الجهات ذات العلاقة (الامانات),ضيق الوقت وخاصة فى اوقات الذروة مثل مواسم الحج ورمضان (تحتاج عملية الاذابة الى حوالى ١٠-١٢ ساعة) وانة لابد من تجهيز وجبات الغذاء والعشاء لعدد كبير من الحجاج والمعتمرين فى اقصر وقت ممكن ولهذا اسلوب الاذابة المتبع فى الثلاجات لا يفى بتحضير تلك الوجبات فى المواعيد المطلوبة لذلك يمكن أن يكون استخدام المواد المثبطة لنمو الميكروبات فى احواض الاذابة للمساعدة على تقليل التلوث الميكروبى وخاصة السالمونيلا والاشيرشاى كولاى. ويمكن تقسيم تلك المواد المواد الطبيعة والكيميائية والبيولوجية.ويعتبر الشيتوزان من المواد الطبيعة والمستخلصة من الكيتين ولها تاثير مضاد للميكروبات ويمكن استخدامها لاطالة فترة صلاحية المنتجات الغذائية وتم استخدامها مع حامض الخليك وهو من المواد الكيميائية التى تستخدم بامان تام فى مجال الاغذية.وقد قامت هذة الدراسة للتحقق من تاثير الشيتوزان ومقارنتة مع حامض الخليك المضاف الى احواض التى تم فيها عملية الاذابة على السيطرة على ميكروبى السالمونيلا واى كولاى.وقد تم المعالجة بمحلول الشيتوزان بتركيزات مختلفة (٠,٥%- ١%)وحامض الخليك(٠,٥%- ١%) وخليط منهما (١%)لكلا منهما وتم فحص العينات للاختبارت الحسية والبكتيرية.وقد اظهرت النتائج ان الشيتوزان(٠,٥% - ١%) وحامض الخليك (٠,٥%-١%) والخليط منهما لم يختلف معنويا عن المجموعة الضابطة بالنسبة الى الاختبارات الحسية .اما بالنسبة للسيطرة على ميكروبى السالمونيلا واى كولاى فلم يكن هناك اختلاف معنوى بين العينات الضابطة وتلك التى عولجت بالشيتوزان اوحامض الخليك ٠,٥% بينما اختزلت محلول الشيتوزان وحامض الخليك ١% اعداد ميكروب السالمونيلا بمقدار ١لوخلية\جم وكذلك كان هناك فارق معنوى بين واضح بين استخدام الخليط من الشيتوزان وحامض الخليك بتركيز ١% على العدد الكلى للبكتيريا وميكروب الاشيرشاى كولاى واختزال اعداد ميكروب السالمونيلا بمقدار ١,٢لوخلية \جرام عن العينات الضابطة. وبالتالى توصى الدراسة باستخدام خليط من الشيتوزان وحامض الخليك بتركيز١% وذلك باضافتهما الى المياة المستخدمة فى عملية صهر الدواجن المجمدة وذلك للسيطرة على التلوث الميكروبى.

الكلمات المفتاحية: الشيتوزان - حمض الخليك - الدجاج المجمد – إذابة اللحوم - الاشيرشاى كولاى - السالمونيلا

Thawing process in chicken preparation is considered a critical step because handlers in food service establishment put frozen chicken in a Hot Water to enchantment the defrosting food material. Thawing by placing the frozen meat in the refrigerators at a temperature (0 - 5 °c) for 24 hours of the most important and best methods of health, but the special circumstances for the food and especially (kitchens – masahar kitchens) in Mecca and the holy sites and although There are specific rules for the process of melting frozen meat, but there are difficulties faced by the workers in the implementation of these requirements for several reasons and narrow areas in the kitchens and the absence of an area dedicated to the process of melting, the absence of refrigerators or coolers for the correct process required by the relevant authorities, Especially during peak times, such as the Hajj and Ramadan seasons (the process of thawing needs about 10-12 hours), and that meals and dinners should be prepared for a large number of pilgrims in the shortest time possible. Therefore, the method of thawing used in refrigerators is not sufficient to prepare these meals on the required time. The use of inhibitory microbial agents in melting ponds can help to reduce microbial contamination, especially salmonella and Eshrachia coli. The application of decontamination treatments during chicken processing specially during the thawing step could be highly useful to prevent the growth of food poising microorganisms causing spoilage of poultry meat. Several decontaminants have been reported to be effective in destroying pathogens; these treatments can be classified as natural and chemical decontaminants. Chitosan is a natural bioactive polysaccharide, derived from deacetylation of chitin with intrinsic antimicrobial activity and can be recognized as a natural alternative to synthesized antimicrobial for extending perishable food shelf life. Also organic acid with proven effectiveness as a decontaminant with different kinds of food, acetic acid is generally recognized as safe substance (GRAS).Therefore, the present study designed to investigate the effect of chitosan and acetic and their combination on the process of thawing of frozen chicken and decontamination of Salmonella typhimuium and E. coli. Addition of chitosan and acetic acid in water used for thawing process at a concentration of chitosan 0.5%, 1% and acetic acid 0.5%, 1% solutions beside their combination. Treated samples were examined for sensory attributes and bacterial load .Samples treated with chitosan 0.5%, 1% and combinations with acetic acid did not significantly differ from the control samples for sensory attributes. Using of acetic acid 1% reduces the bacterial load (Apc). Chitosan 0.5% and acetic 0.5% nearly had no effect on reduction of E. coli and salmonella. Combination of acetic acid 1% and chitosan 1% reduced E. coli and salmonella count.

Key words: Chitosan, Acetic acid, Chicken, Thawing, E.coil, Salmonella

Introduction

Poultry meat shelf life is affected mainly by two factors, storage temperature and initial microbial load. Consequently, refrigeration storage is a crucial step that significantly prevents microbial growth and maximizes both product safety and shelf life karadag and puhakka (2010).Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth ,including both spoilage and pathogenic microorganism ,therefore the microbial contamination during the poultry processing is very crucial(Jay el al., 2005).The application of decontamination treatments during processing specially during thawing process could be highly useful because growth of food poisoning microorganisms. Several decontaminants have been reported to be effective in destroying pathogens, these treatments can be classified as physical, chemical, and biological decontaminants Capita et al.(1999) .Thawing process is to melt the frozen food by extracting the food from the freezer and transfer it to the refrigerators at a temperature of(0 ° C to 5 °

C). Thawing process is a critical control point due to growth of microbes specially (E. coli and salmonella), It is considered to be one of the most important causes of food poisoning Mor-Mur,and Yuste(2010), especially since it has sufficient time and grow in a highly nutritive media (water and proteins), which needed to grow rapidly and secretion of toxins in those meat, which leads to damage and corruption (change in color and odor of meat) and deceased the nutritional value and sensory evaluation of poultry meat. The results of the previous research found that the survival of bacteria in food for two hours or more at the temperature of the area of danger zone (5-60°c) helps to damage and causing spoilage of those foods. Acetic acid is generally recognized as safe substance with no upper limit of daily intake for humans several of the organic acids investigated for application in poultry include acetic, formic, citric, lactic, and propionic acid. Although these acids can be effective antimicrobials, it has been reported that they could cause negative flavor and color changes Bagamboula et al. (2004). To avoid the negative quality changes associated with organic acids, the ideal approach is to combine antimicrobials. This will allow the use of lower levels of organic acids but maintain the antimicrobial efficacy of the compound. Chitosan is a natural carbohydrate polymer derived from the decetylation of chitin ,a main component of exoskeletons of crustaceans .different production methods are used to produce chitosan with different chemical properties ,which potentially reflect variations in the antimicrobial activity No and Mryers (2002).With increasing consumers awareness of food treated chemicals, food industry became highly interested in applying natural compounds that could achieve a high level of food safety Rauha et al.(2000) .Therefore , the present study was designed to investigate the effect of chitosan as a natural decontaminate and acetic acid as a chemical decontaminate on reduction of microbial load during thawing process.

Research aims

The present study was designed to investigate the effect of chitosan as a natural decontaminate and acetic acid as a chemical decontaminate on reduction of microbial load during thawing process.

Research methodology

The experiment was designed to investigate the effect of chitosan and acetic acid on microbial load during thawing of frozen chicken.

Preparation of samples:

Five groups of whole chicken samples obtained from local markets (average weight of each sample 1 kilo gram)

Preparation of treatments:

Chitosan: acid soluble chitosan (SIGMA ALDRICH) and used as solvent for 0.5% solution of acetic acid (v/v) in distilled water. Concentration of 0.5 and 1% chitosan were prepared (w/v).

Acetic acid: a solution of 0.5 and 1% of acetic acid in distilled water (v/v) was prepared.

Application of chitosan and acetic acid on water used thawing of frozen chicken:

Five treatments solutions were performed as follows : chitosan 0.5% ,chitosan 1% ,0.5% acetic acid 1% acetic acid and combination between chitosan 1% and acetic acid 1% . Each solution was used as a dipping solution for a group from previously prepared solution frozen chicken (100ml prepared solution added to two liter of water) in addition to control where dipped in water only.

Sensory examination:

Three raw samples were examined for its appearance, odor, consistency, and overall acceptability score according to Sumarmono and Raharrdjo(2008)using five point hedonic scale where 1 (very bad) to5 (very good) then the average was recorded as overall sensory score.

Bacteriological examination :

Bacterial counts- :

The technique recommended by Downes, Ito and Association (2001) was applied.

Preparation of sample homogenate-:

Ten grams of the chicken samples were weighed under aseptic condition into sterile stomacher polyethylene bag containing 90ml of a sterile ringer solution (OXIOD, BO0619), stomached in stomacher for 1 minute at room tempature,Then fivefold serial dilutions were prepared using 0.1 ringer solution.

Bacteriological analysis was performed according to the standard procedures for enumeration and identification of microorganisms (ICMSF 2005) as follows-:

Aerobic plate count.

Isolation and identification of Salmonella.

Isolation and identification of E. coli.

Statistical Analysis:

All data were analyzed using Statistical Analysis System SPSS Comparisons between treatments within each analysis were tested. Significance was determined by the F-test and least square means procedure. Main effects were considered significance at $P<0.05$.

Results and discussion

Sensory attributes (mean values ± SD) of different treated frozen chicken samples (TABLE 1)

treatments	control	0.5% acetic acid	0.5% chitosan	1% acetic acid	1% chitosan	1% acetic and 1% chitosan
appearance	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0
odor	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0
consistency	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	4.6a ±0.18
Overall acceptability	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	5.0a ±0.0	4.8a ±0.12

Sensory analysis:

Appearance is one of the most important sensory attributes of poultry meat as it can generally persuade a consumer's decision to purchase meat

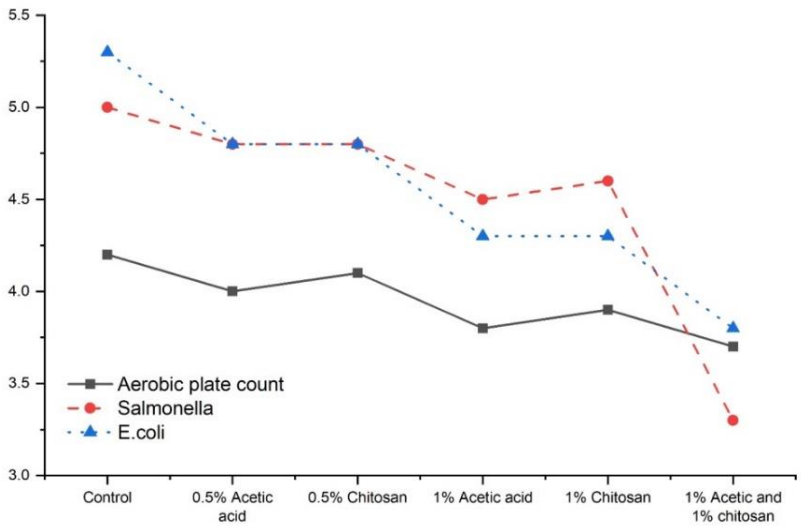
Application of acetic acid and chitosan (0.5%, 1%) has no significant effect on appearance, odor, consistency and overall acceptability compared with control samples was observed. The obtained results are in agreement with that reported by kross(2004)and Qiao(2002).Combination of acetic acid and chitosan treatment(1%)for each showed slightly slimness

(this could be attributed to the viscous nature of chitosan solution),while this combination had no effect on odor and overall acceptability. The results recorded by Ibrahim and El-khawas (2015) are in agreement of the obtained results.

Results of microbial counts of the treated frozen chicken samples (log cfu| g mean ± SD) (TABLE 2)

treatments	Aerobic plate count	Salmonella	E.coli
control	4.2a ±0.20	5.0a ±0.20	5.3a ±0.21
0.5% acetic acid	4.0a ±0.5	4.8a ±0.15	4.8b ±0.42
0.5% chitosan	4.1a ±0.25	4.8a ±0.15	4.8b ±0.36
1% acetic acid	3.8a ±0.26	4.5b ±0.20	4.3b ±0.35
1% chitosan	3.9a ±0.29	4.6b ±0.20	4.3b ±0.35
1% acetic and 1% chitosan	3.7b ±0.2	3.3b ±0.8	3.8c ±0.34

Results of microbial counts of the treated frozen chicken samples (log cfu/ g mean ± SD) Figure (1)



The control of foodborne pathogens in primary production, at hatchery and farm levels, is the first important step in the safety of poultry meat during the whole production chain. The microbiological condition of live birds influences the microbiology of the products as live animals are the principal source of microorganisms found in poultry carcasses European Union (1998).

Table (2) and Fig. (1) represents a marked decreased in aerobic plate count (log cfu|g) was seen in all examined samples treated with (0.5%, 1%) acetic acid or chitosan compared with control .Combination of acetic acid and chitosan at a concentration 1% revealed a significant reduction in aerobic plate count than control samples ,the obtained results generally agree with that obtained by Chounou et al.(2013).

Poultry meat ranked first as cause in food poisoning with incidence of 29.32%, followed by meat with incidence15.33% Altabari and Ai-dughaym(2002).The food poisoning microorganisms causing outbreaks were mainly salmonellae and E.coli .These bacteria are considered indicators of microbiological quality.

Table(2)represents the initial count of Salmonella Typhimurium in control and treated samples with 0.5% acetic acid and 0.5% chitosan were 5 ,4.8,4.8 log(cfu|g),respectively with no significance difference between these groups, while samples treated with 1% chitosan and 1% acetic acid showed reduction in salmonella count(4.6,4.5 log cfu|g).On other hand treated samples with combination of acetic acid and chitosan at 1% showed significantly reduction 3.3 log cfu|g, these results agreed with Wang (1992) and Knowles and roller(2001).Antibacterial activity of chitosan involves binding of the amino group of chitosan to the surface components of bacteria ,thereby inhibiting their growth, chitosan bind to the negative charged bacterial surface to disturb the cell membrane and cause cell death due to leakage of intracellular components Sudarshan el al (1992).

E.coli counts were determined on broiler chicken carcasses visibly and not visibly contaminated with fecal material during commercial slaughter practice Jimenez et al (2003).

Table (2) and Fig.(1)showed significant reduction in E.coli count could be observed in samples treated with chitosan 1% and 1% acetic acid than control and chitosan and acetic acid at a concentration 0.5%

Reduction of E.coli count may be attributed to acetic acid at 1% decreases the ionic concentration within the bacterial cell membrane of the exterior cell wall of the bacterial organism. This leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm and inhibition of substrate transport Praveen et al (2007).

conclusion

From the obtained results, it could be concluded that treatment with chitosan1% and 1% acetic acid can slightly reduce bacterial load specially Aerobic plate count and E. coli, while treatment with 1% acetic acid and 1% chitosan combination between them proved significantly reduction in Salmonella and E.coli count by 1- 1.5log cfu|g and has no adversely effect on odor, consistency, and overall acceptability.

Recommendations

The application of decontamination treatments during chicken processing specially during the thawing step could be highly useful to prevent the growth of food poising microorganisms causing spoilage of poultry meat. Especially during peak times, (Hajj and Ramadan seasons) the process of thawing needs about (10-12 hours), and that meals and dinners should be prepared for a large number of pilgrims in the shortest time possible. Therefore, the method of thawing used in refrigerators is not sufficient to prepare these meals on the required time. The use of inhibitory microbial agents in melting ponds can help to reduce microbial contamination, especially salmonella and Eshrachia coli.

References

- Altabari, G,and Al-Dughaym,A.M., (2002): The role of sanitary inspection of meat in relation of food poisoing .Annual Scientific Meeting For Environment Hygiene (Meat Hygiene) ,Riyadh,pp.180-203.
- Bagamboula, C. F., Uyttendaele, M., and Debevere,J.,(2004): Inhibitory effect of thyme and basil essential oils,carvacrol,thymol ,estragol, linalool towards SHIGELLA and S. flexneri.Food Microbiology.21:33-42.
- Capita R.C., Alonso-Calleje C, Garcia-Arias MT, Garcia-Fernandes MC and Moreno B (1999): Decontamination of poultry :Chemical treatments and decontamination situation within the Europen Union .Alimentaria, 303.

- Chounou,N.,Chouliara, E., Mexis ,S.F., Stavros,K.,Georgantelis,D.and Kontominas,M.G:(2013). Shelf life extension of ground meat stored at 4 c using chitosan and an oxygen absorber. International Journal of Food Science and Technology, 48, 49-95.
- Downes ,F, P.,Ito,K., Association,A.P.H.(2001):Compendium of Methods for the Microbiological Examination of Food .American Public Health Association ,DC USA .
- European Union (1998) Benefits and Limitations of Antimicrobial Treatment for poultry carcasses. Report of the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH .(
- Ibrahim, S.and El-khawas,K.M.(2015): Extending the shelf life of chilled fillet using developed active packaging material .J.EGYPT .VET. Med.Assoc.75 (4):625-629.
- ICMSF(International Commission on Microbiological Specifications of Food)(2005):Poultry products in Microorganisms in foods 6:108-173.
- Jay .J., M., loessner,M.j., Golden, and David, A. (2005).Modern food Microbiology. New York: Springer Science inc .
- Jimenez, S.M., Tiburzi,M.C., Salsi, M. S., Pirovani, M.E. and Moguilevsky, M.A.(2003):The role of visible faecal material as a vehicle for generic E.coli and other enterobacteria contaminating poultry carcasses during slaughtering .J.Appl .Microbiology. 95:451-456.
- Karadag, D.and Puhakka,J.A. (2010):Effect of changing temperature on anaerobic hydrogen production and microbial community composition in an open-mixed culture bioreactor. Int .J Hydrogen Energy, 35:10934-10959.
- Knowles, J.and Roller, S.(2001):Efficacy of chitosan ,carvacrol, and a hydrogen peroxide based biocide against foodborne microorganisms in suspension and adhered to stainless steel . J.Food Prot.64 (10):1542-1548 .
- Kross,R.September 2004. Methods for reducing meat discoloration.U.S.patent 20040175476 AL.
- Mor-Mur,M., and Yuste,J.(2010): Emerging bacterial pathogens .Meat and Poultry : An Overview ,Food Bioprocess and Technology,3,24-35.
- No,H.K., PARK,N.Y., Lee,S.H.,and MEYERS, S.P.(2002):Antibacterial activity of chitosan and oligomers with different molecular weights .Int.J.Food Microbiology.(74)65-72.
- Parveen, S.,Taabodi M.,Schwarz J.G., Oscar T.P., Harter-Dennis J.,and White D.G.(2007): Prevalence and antimicrobial resistance of salmonella recovered from processed poultry.J.Food Prot.(70):2466-2472.
- Qiao, M., Fletcher D. L., Northcutt J. K., and Smith D. P. (2002): The relationship between raw broiler breast meat color and composition .Poult. Sci. 81:422-427.
- Rauha, P., Remes , S., Heinonen ,M., Hopia,A.,Kahkonen,M.,Kujala,T. Pihlaja, K. Vuorela, P.(2000):Antimicrobial effect of finnish plant extracts containing flavonoids and other phenolic compounds.Inc. Food Microbiology .56:3-12.
- Sudarshan, N.R., Hoover,D. G., and Knorr,D.(1992):Antibacterial action of chitosan .Food Biotechnol.6257-6272.
- Summarmono, J., Agustins, D. and Rahardjo, H.D.(2008): Effect of decontamination using organic acids on total microbial number and qualities of poultry carcasses , Animal Production,4:129-139 .
- Wang,G.(1992):Inhibition and inactivation of five species of foodborne pathogens by chitosan.J.Food Prot.(55):916-919.