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Manufacturing edible bio-films (EBF) from chicken feather keratin and study their effect on antioxidant status of cold storage broiler yields

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Abstract This study aimed to investigate using 2.5 and 5 g of purified feather keratin protein (PFKP) in edible bio-films (EBF) as package material to broiler yields with different cold storage condition periods (0, 7 and 14 days) at 4° C on the meat antioxidant status of these yields. 3×3 factorial treatments; un package, 2.5 and 5 g PFKP, as a first main factor, by 3 cold storage condition periods (0, 7 and 14 days) as a second main factor, with their interactions on meat antioxidant status (TBA test, Peroxide values, (PV) and free fatty acids concentration, (FFA) of broiler yields (breast, thigh and drumsticks). The results of this experiment revealed that increasing cold storage period to 14 days will significantly increase MDA concentration (TBA test), FFA concentrations and PV in all yields, whereas, packaging these yields with EBFs by two of PFKP will significantly decrease MDA, FFA concentrations and PV in all yields. The interactions between packaging and storage had the same effect, since, un package yields and stored for 14 days had the significant increase in MDA, FFA and PV values in comparison with packaging yields and stored for 14 days, in spite of, packaging yields store and without store in cold had no significant differences, and this revealed the ability of using packaging to extend shelf life of broiler yields without an effect on oxidant status of broiler yield meats store for 7 days.

1. Introduction

Keratin protein was a natural polymers, extract from chicken feathers used as a main compound of edible protein bio-films matrix [1], [2]explores edible bio-film (EBF) packaging as one of the most alternative methods used to preserve poultry meat instead of using chemical preservatives and more friendly to environment, also, EBFP extended shelf life and maintain poultry meat safety from degradation by microbes or another such as lipid oxidation in poultry meat that can generate detrimental compounds and cause unacceptability of consumer demands [3]. The oxidative equilibrium; between anti- and pro-oxidants and the rest compound of oxidizable substrates (including Poly unsaturated fatty acids, PUFA), cholesterol, proteins and pigments, leads to oxidative stability of poultry meat [4], so, peroxidation reaction of PUFA occur during storage at 2 to 4° C as most important biochemical changes happened followed post slaughter phase of poultry meat [5]. The unacceptability of poultry meat and its by-products could be due to unbalance of oxidative equilibrium causing changes in odors, texture, essential fatty acids contents leading to rancidity and more toxic ingredients produced [6].There are many standards could describe oxidant status; peroxidase and catalase enzymes, Free Fatty acids (FFA), peroxide value (PV), and Malondialdehyde (MDA) [7]. Free fatty acids (FFA) content increase with increasing storage time, FFA will be oxidized simply to produce H2O2, this product could affect the activities of peroxidase

(POD) and catalase (CAT), these enzymes used as indicators of antioxidant status during meat storage [8]. Hydroperoxide was one of compounds produced in the beginning of auto-oxidation, which could be determined in oldest commonly method, the peroxide value (PV) method, as milliequivalents of peroxides in one kilogram of lipids [9]. Malondialdehyde (MDA) is one of the major products produced after polyunsaturated fatty acids (PUFS) peroxidation, which react with Thiobarbuturic acid (TBA), so, the TBA method is one of the tests that used to determine the lipid peroxidation in meats and meat products [10]. Previous experiment was done by [11], he referred to the ability of manufacturing bio-edible films from chicken feathers and used it as packaging material by dipping broiler yields in EBF solutions, and the aim of this experiment was to explore the antioxidant status of broiler yields packaged with keratin EBFs during cold storage.

2. Materials and Methods

Firstly, keratin protein was extracted and purified according to [12] and modified method by [11], then, two levels of extracted keratin protein used in film solutions (2.5 g and 5 g protein separate / 30 ml of film solutions). Films solutions were prepared according to [13] and modified [11], hence 30 ml volume of films solution were prepared; 30 ml distilled water, ethyl alcohol (99.9%), 1 ml glycerol and 1 ml of ammonium hydroxide, then two level of keratin protein were added (2.5 and 5 g separate) to 30 ml of previous solution, finally one gram of methyl cellulose were added to solutions, then, the mixtures were heated in water bath to reach final temperature 70° C for 15 min.

Thirty-five broiler at 42-day old (1850 + 100 g mean live body weight) were slaughtered, the carcass was scalded, de-feathered, and eviscerated, then, carcasses were cut off to primary broiler yields, breast, thigh and drumsticks, after that, samples from different carcass yields were taken (120 + 1, 139+ 2, and 108 + 1 g from breast, thigh and drumsticks respectively). Broiler yields samples were un packaged (control group) and the others were dipped in two keratin EBF solutions (2.5 and 5 g keratin/30 ml, w/v) and then stored for three cold storage periods at 4° C (0, 7 and 14 days).

To estimate TBA in lipid tissues of broiler yields (breast, thigh and drumsticks) [14] method was used to determined MDA values as a major product of lipid peroxidation, whereas, Peroxide Value (PV) was measured according to [9], however, free fatty acids (FFA) were evaluated as referred by [15].

Two – ways ANOVA analysis were used to assess the effect of un package treatments and using two levels of keratin protein in EBF solutions as a first factor and different periods of cold storage periods as a second factor, and their interactions between them on antioxidant status of three broiler yields sample (breast, thigh and drumsticks). The analysis was applied by using General Linear Model (GLM) procedure of statistical software package SAS version 9.1[16], P-values less than 0.05 were considered to be significant for the main effects. Results were presented as mean/SEM (pooled).

3. Results and discussion

MDA concentrations in broiler yield meats by TBA test were illustrated in table (1), it's obvious from table (1) that increasing storage period will significantly increase MDA concentrations in all broiler yields, whereas, packaging these yields with EBFs by two protein concentrations will significantly decease MDA concentration in broiler yields. The interactions between packaging and storage had the same effect , since, un package yields and stored for 14 days had the significant increase in MDA values in comparison with packaging yields and stored for 14 days, in spite of , packaging yields store and without store in cold had no significant differences, and this revealed the ability of using packaging to extend shelf life of broiler yields without an effect on MDA values, or , without any effect on oxidant status of broiler yield meats store for 7 days. These results were in accordance with [17] when they noticed that packaging poultry meat with chitosan reinforced with two different montmorillonites (tradable product) and stored under cold conditions (5° C) for 15 days will decrease the lipid peroxidation to the half values in broiler breast meats.

Table (1).MDA concentrations (mg MDA/kg meat) in broiler yields (breast, thigh and drumsticks) after of no packaging and packaging with EBFs and stored for different periods using TBA test.

treatments		Breast	Thigh	Drumstick
0 d stored		0.18 c	0.17 c	0.18 c
7 d stored		0.38 b	0.85 b	0.54 b
14 d stored		0.64 a	1.24 a	1.29 a
Prob.		*0.0001*	*0.0001*	*0.0001*
Un package		0.52 a	1.12 a	1.06 a
Package (2.5 g protein/30 ml)		0.39 b	0.59 b	0.49 b
Package (5 g protein/30 ml)		0.29 c	0.56 b	0.45 b
Prob.		*0.0001*	*0.0001*	*0.0001*
0 day stored	Un package	0.20 d	0.19 e	0.17 e
	Package (2.5 g protein/30 ml)	0.19 d	0.17 e	0.19 e
	Package (5 g protein/30 ml)	0.16 d	0.16 e	0.17 e
7 d stored	Un package	0.49 bc	1.31 b	0.78 bc
	Package (2.5 g protein/30 ml)	0.41 c	0.69 d	0.41 de
	Package (5 g protein/30 ml)	0.25 d	0.57 d	0.45 cde
14 d stored	Un package	0.87 a	1.89 a	2.24 a
	Package (2.5 g protein/30 ml)	0.58 b	0.93 c	0.88 b
	Package (5 g protein/30 ml)	0.47 c	0.91 c	0.74 bcd
Prob.		*0.0004*	*0.0001*	*0.0001*
Total mean		0.40	0.76	0.67
Pooled SEM		0.044	0.109	0.124

Un packaging and increase cold storage periods for 14 days causes significant increase in FFA concentrations in broiler yields meat as showed
in table (2), also, significant decrease were noticed in broiler yields meat during packaging and without any differences between un stored and store for 7 days, that means, packaging broiler yields could

increase shelf life for 7 days in cold storage without any determined changes in FFA concentrations, [18] refers to the strong indicative factor that could predict the oxidative status by lipid composition.

Table (2).FFA concentrations (percentage) in broiler yields (breast, thigh and drumsticks) after of no packaging and packaging with EBFs and stored for different periods.

treatments		Breast	Thigh	drumstick
Un stored		0.34 c	0.33 c	0.32 c
7 d stored		0.44 b	0.46 b	0.44 b
14 d stored		0.51 a	0.53 a	0.53 a
Prob.		*0.0002*	*0.0001*	*0.0001*
Un package		0.44 b	0.53 a	0.51 a
Package (2.5 g protein/30 ml)		0.51 a	0.38 b	0.38 b
Package (5 g protein/30 ml)		0.38 b	0.41 b	0.40 b
Prob.		*0.001*	*0.0001*	*0.001*
Un stored	Un package	0.35 c	0.33 d	0.31 e
	Package (2.5 g protein/30 ml)	0.35 c	0.31 d	0.33 de
	Package (5 g protein/30 ml)	0.33 c	0.35 cd	0.33 de
7 d stored	Un package	0.54 ab	0.60 a	0.54 b
	Package (2.5 g protein/30 ml)	0.39 c	0.37 cd	0.37 cde
	Package (5 g protein/30 ml)	0.39 c	0.42 bc	0.41 cde
14 d stored	Un package	0.64 a	0.65 a	0.67 a
	Package (2.5 g protein/30 ml)	0.44 bc	0.46 b	0.44 bcd
	Package (5 g protein/30 ml)	0.44 bc	0.48 b	0.48 bc
Prob.		*0.05*	*0.0038*	*0.0264*
Total mean		0.43	0.44	0.43
Pooled SEM		0.021	0.023	0.024

Alterations in PV of broiler yields un packaged and packaged under cold storage and non-cold storage conditions were illustrated
in table (3), it's seemed that 14 days cold storage significantly increase PV in comparison with non-storage or store for only 7 days, the same significant increase were noticed in un packaged broiler yields in comparison with packaged broiler yields in PV, whereas, interaction between two major factor (storaged X packaged) showed that packaged broiler yields with EBFs decreases significantly PV in

broiler yields during cold storage for 7 days and 14 days in comparison with no packaging treatment and cold store for the same periods.
Antioxidant status of meat can effect meat properties via reaction with proteins leading to meat values and finally changes in sensory traits of meat [19], and these changes magnify especially during cold storage for long periods, so, to decrease antioxidant changes on meat quality some preserver technique were used, one of these, were packaging meat to extend shelf life of it. Using traditional packaging materials causes cumulative effect of these materials on environment because of low degradable rate, so, efforts were done to replace traditional materials (e.g. nylon, cartoon …. etc) by EBFs.

Table 3. Peroxide values (meq O2 /kg lipid) in broiler yields (breast, thigh and drumsticks) after of no packaging and packaging with EBFs and stored for different periods.

treatments		Breast	Thigh	drumstick
Un stored		1.21 c	1.28 c	1.45 c
7 d stored		1.86 b	1.95 b	1.79 b
14 d store		2.43 a	2.80 a	2.60 a
Prob.		*0.0001*	*0.0001*	*0.0001*
Un package		2.52 a	2.63 a	2.54 a
Package (2.5 g protein/30 ml)		1.52 b	1.74 b	1.71 b
Package (5 g protein/30 ml)		1.46 b	1.65 b	1.59 b
Prob.		*0.0001*	*0.0001*	*0.0001*
Un stored	Un package	1.32 def	1.26 e	1.45 d
	Package (2.5 g protein/30 ml)	1.26 fe	1.39 de	1.38 d
	Package (5 g protein/30 ml)	1.06 f	1.19 e	1.52 d
7 d stored	Un package	2.46 b	2.66 b	2.39 b
	Package (2.5 g protein/30 ml)	1.59 cd	1.65 d	1.46 d
	Package (5 g protein/30 ml)	1.52 cde	1.52 de	1.51 d
14 d stored	Un package	3.78 a	3.98 a	3.79 a
	Package (2.5 g protein/30 ml)	1.72 c	2.18 c	1.92 c
	Package (5 g protein/30 ml)	1.79 c	2.24 c	2.11 c
		0.0001	*0.0001*	*0.0001*
Total mean		1.83	2.01	1.95
Pooled SEM		0.155	0.168	0.144

Antioxidant status changes during cold storage for long periods causes significantly effect on meat qualities, especially, on some antioxidant parameters (TBA, FFA, and PV), these parameters were an indicator for peroxidation initiate in lipid meat [5], so, decrease levels of these parameters will improve meat qualities to acceptable ranges to consumer's needs. TBA values represent MDA reactive compound which bound to –SH and –NH2 groups of proteins and nucleic acids, and any increase of TBA values means increase of MDA levels and consequently significant changes will occur in acceptability of meat [20]. [21] referred that the ending of product shelf life was correlated with sharp increases of PV values in samples, also, [22] found that cold storage (4° C) for 15 days in turkey breast meat packaged with chitosan. The previous results of research's revealed the agreement of the results of this experiment on PV values, the significant increase in PV values in broiler yields meat storage for long period (14 days) in comparison with no stored and stored for 7 days, also, packaged these yields and stored for long period illustrate the significant decrease of PV values in packaged meats. The same trend was happened in FFA concentrations in long stored broiler yields and packaged yields with EBFs.

4. Conclusion:

The goal of this research was to use keratin protein derived from chicken feathers in the packaging of broiler cuts and the effects of it on the oxidation characteristics during the cooling process.

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