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The efficiency of Alginic acid, Sodium alginate and Egg white lysozyme in sustain the safety of beef burger

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Abstract

Alginates and its salts specially sodium and potassium and its oligosaccharides in addition to egg white lysozyme have attracted many interests in applied researches due to their positive impact on consumer health through potential antifungal and antibacterial, antioxidant, probiotic, antihypertensive, antidiabetic, antitumor, anticoagulant properties and many other benefits. The evidence is that it was used as a probiotic, feed supplements for aquaculture and poultry as well as cryoprotector for frozen foods. Hence, in this study, we tried to use alginic acid (AA), sodium alginate (SA) and egg white lysozyme (EWL) with different concentrations, either individually or collectively in beef burger. The obtained results showed that 2% AA was the best concentration that caused the most pronounced significant reduction ($P<0.05$) of *Staph. aureus* count (from 4.93 $\log_{10}$±0.01 as control to 4.14 $\log_{10}$±0.06 as treated) with reduction rate of 0.79 $\log_{10}$ (16.02%) as compared with 0.5 & 1% AA. In contrary, 2% AA showed weak reduction activity of *E. coli* count (from 4.95 log10±0.01 as control to 4.48±0.04 as treated) with 0.47 reduction rate (9.5%). The same effect was recorded for SA as 2% had the most pronounced reduction for *Staph. aureus* count which recorded 4.30 $\log_{10}$±0.04 with reduction rate and incidence of 0.63 (12.78%), while for *E. coli,* it was recorded 4.78 $\log_{10}$±0.01 with 0.17 $\log_{10}$ reduction rate (3.43%). By the same way, it was found that 200 ppm EWL was more effective in controlling of both *Staph. aureus* (from 4.93±0.01 to 3.98±0.03) with 0.95 reduction rate (19.27%) and *E. coli* from (4.95±0.01 to 4.45±0.02) with 0.5 $\log_{10}$ reduction rate (10.1%). The obtained results proved that a mixture of 200 ppm EWL + 2% SA was most effective concentration among all other treatments used in the present study which recorded 1.04 $\log_{10}$ reduction rate for *Staph. aureus* count (21.1%) and 0.75 (15.15%) for *E. coli*. and recommended to be used in food products as antimicrobial combination in competing both organisms used through the present study.

Keywords: *Staph. aureus, E. coli, Alginic acid (AA), Sodium alginate (SA), Egg white lysozyme (EWL), Natural antimicrobial activity, sustain safety product. beef burger.*

Introduction

Microbial food spoilage is responsible for deterioration of food as reduction in the sensory attributes, nutritional quality and subsequently, great economic losses. Furthermore, wide spread of the food-borne pathogens leads to food poisoning and damage to the consumer health and loss of food safety parameters. Multiple trends nowadays are encouraged to replacement of synthetic additives and use of new natural antioxidant and antibacterial substances with a possible role as nutritional agents. Multiple functionality seaweed extracts could be incorporated into foods as natural preservatives to enhance the food quality, safety and stability (**Vijayavel and Martinez, 2010; Gupta and Abu-Ghannam, 2011** and **Cox *et al.*, 2014).**

Consumers trust and prioritize to food products that have transparency in its contents ingredients/additives that are natural, with reliable names and are appropriate to be good for consumer health **(Brewer, 2011)**. In recent years,

there also have been concerns about food safety due to an increasing occurrence of food-borne illness outbreaks caused by pathogenic microorganisms (**Tajkarimi *et al.,* 2010**). In order to satisfy consumer's demands and retrieve its confidence in the safety of food products, those in charge of the food industry should search for natural alternatives to food additives that have strong antioxidant and/or antimicrobial properties. (**Fernandez-Lopez *et al.,* 2005** and **Ahmad *et al.,* 2015).**

Algins/alginates are available in both acid and salt forms, alginate is a natural anionic polysaccharide extracted from seaweed, which is composed of β-(1–4) linked D-mannuronic acid and α-L-guluronic acid units **(Zheng and Kohn, 2014** and **Aziz and Karboune, 2018)**.

Salts of alginic acid including Na^{+}, K^{+}, Mg^{2+} and Ca^{2+}out of which, sodium and calcium ions are considered the most effective cations and are commonly used as the gelling agents and they are extracted from brown seaweeds cell walls and also of the intracellular matrix of the brown algae (Phaephyceae) mainly**,** *Laminaria hyperborean*, *Macrocystis pyrifera, Ascophyllum nodosum*; lesser extent *Laminaria digitate, Laminaria japonica, Eclonia maxima, Lesonia negrescens, Sargassum* spp.**(Skurtys *et al.,* 2010; Kraan, 2012; Hay *et al.*, 2013** and **Vera *et al*., 2013)** and some bacteria including *Azotobacter vinelandii* and *pseudomonas* (**Thomas *et al*., 2013).** Furthermore, Alginate succeeded to be produced from Marine algae (**Peteiro, 2018**). The molecular weight of alginate ranges generally between 500 and 1000 kDa. (**Cha and Chinnan, 2004** and **Usov, 2013**).

In some researches, the term "algin" is used instead of alginate. The goal of the extraction process of sodium alginate is to obtain a product in a dry powdered form. The calcium and magnesium salts do not dissolve in water, while sodium salt is able to be dissolved in water and has a unique swelling, gelling, and mucoadhesive properties (**Cardoso *et al.,* 2016** and **Szkalska *et al.,* 2016**). Alginate Oligosaccharides (OLG) has been reported that they possess antioxidant, anti-inflammatory, and antibacterial properties (**Han *et al.,* 2019**).

Seaweed produces metabolites aiding in the protection against different environmental stresses. These compounds showed antiviral, antiprotozoal, antifungal, and antibacterial properties which aids in control of new diseases or multi-resistant strains of pathogenic microorganisms (**Perez *et al.*, 2016).**

Alginic acid (AA) and sodium alginate (SA) are widely used agents because of their high antimicrobial efficacy and cost effective. Their antimicrobial effects are based on the increase in proton concentration thereby, lowering the external pH. Furthermore, they may affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism, causing bactericidal effect **(Ricke, 2003).** Mixtures of alginate with organic acids or essential oils could exert a wider antimicrobial activity (**Theron *et al.,* 2010).**

New approaches with natural antimicrobial features, which characterized by i) its more potent ii) less hazardous to the consumers health iii) prolonged action are of very interest nowadays. Consequently, antimicrobial that based on natural origin, such as alginates and its salts which could be obtained from various agro-industrial sources are being studied increasingly. (**Andrade *et al.,* 2004** and **Kakita and kamishima, 2008).**

Many food products are perishable by nature as well as by the action of bacteria contaminating food during food production, preparation, processing, storage, distribution and handling which considered nowadays the major challenges facing food industry due to its effect on both food safety and quality, some of these microorganisms such as Escherichia coli, Staphylococcus aureus, Salmonella, Listeria monocytogenes and many other organisms that can potentially cause food-borne illness **(Lòpez-Malo *et al.,* 2005).** Lysozyme is a natural enzyme obtained from egg white which have a wide spectrum of antimicrobial activity against food-borne pathogens and spoilage bacteria (**Gutierrez *et al*., 2008 and 2009**)**.**

Lysozyme as a food preservative inhibits the growth of deleterious organisms, thus improve

the product safety and prolonging its shelf life. Lysozyme also has been used to preserve seafoods, meats, sausages, different kinds of cheese and fresh fruits **(Proctor and Cunningham, 1988).**

Lysozyme is one of the important proteins found in egg white which represent about 3.5% of total egg white proteins (**Ibrahim *et al.*, 2007).** There are many forms of lysozyme found in nature, but the one found in egg is considered as the most soluble and stable **(Benkerroum, 2008).** Enzyme can hydrolyze the β-linkage between N-acetylneuraminic acid and N-acetylglucosamine in bacterial cell walls.

Along with the antioxidant substances, the antimicrobial properties of different preservatives are required to fulfil the quality and safety parameters that compliant with consumer demands, satisfaction and their confidentiality, these substances or products including organic acids, alginic acid and its salts, egg white lysozyme, essential oils, herbal products and phenolic compounds (**Basuny *et al.*, 2012**).

The present study was conducted to determine the efficiency of the antimicrobial activities of alginic acid (AA), sodium alginate (SA), egg white lysozyme (EWL) and a combination of EWL with SA by using different concentrations against *Staph*. *aureus* and *E*. *coli* experimentally inoculated in beef-burger.

Materials and Methods

preservatives used in the established experiment: Egg white lysozyme (EWL) AR, BIO BASIC CANADA INC., CAS:12650-88-3.**Alginic acid (AA)** AR, AVI-CHEM LAB., MUMBAI-INDIA, CAS: 9005-32-7. **Sodium alginate (SA)** AR, AVI-CHEM LAB., MUMBAI-INDIA, CAS: 9005-38-3

All preservatives which have been used in this study were of analytical grade and water-soluble ingredients and the doses of the preservatives used in the present study (0.5, 1 and 2% of both alginic acid and sodium alginate as well as 100, 150 and 200 ppm of egg white lysozyme) were recommended by several investigators who have used the same or even more than the concentrations in the present study as they suitable to preserve quality characteristics and does not alter the sensory attributes (firmness, color and odor) of food product (**Corradini and Innocente, 2002; Walewijk *et al.*, 2008**; and **Gammariello *et al.*, 2009).**

Sample preparation:

One beef meat sample weighted around 4500 g was purchased from butcher shop in Cairo to perform one experiment, where the experiment was repeated three successive times to obtain the mean ± SD, the bulk sample was transferred under strict hygienic measures to laboratory as soon as possible, minced with addition of ingredients required for production of beef-burger. Manufactured beef-burger was divided into five groups, each group contained six samples (a total of 30 samples of 150 g each); 15 samples were contaminated with 5 $\log_{10}$cfu/g *Staph. aureus* and the other 15 samples were contaminated with 5$\log_{10}$cfu/g *E. coli* and treated as follows:

The 1st group; three contaminated samples with *Staph. Aureus* and three contaminated samples with *E. coli* were kept as control positive to estimate the initial bacterial load of both organisms.

The 2nd group; three contaminated samples with *Staph. Aureus* and treated with 0.5, 1 and 2% of AA, separately and the other three contaminated samples with *E. coli*were treated with 0.5, 1 and 2% of AA.

The 3rd group contained six contaminated samples as mentioned in the 2nd group and treated with 0.5, 1 and 2% SA.

The 4th group contained six contaminated samples as mentioned in the 2nd group but treated with 100, 150 and 200 ppm EWL.

The 5th group contained six contaminated samples as in 2nd group but treated separately with a mixture of SA and EWL (0.5% & 100 ppm), (1% and 150 ppm) and (2% & 200 ppm).

The experiment was repeated three times to carry out the statistical operations.

Preparation of tested strains Working solution of *Staph. aureus* and *E. coli* were prepared from reference stock solution stored at -80˚C in cryovials. One bead was resuspended in brain heart infusion broth (Oxoid) and incubated overnight at 37˚C for 24 hour prior to the experiments to obtain a final viable count of about 10^9 CFU/ml, serial dilution was made using physiological saline to obtain approximately 10^5 CFU/ml which used to contaminate the ground beef used in the manufactured of. beef burger, while conducting the experiment under complete aseptic condition.

Preparation of the samples and serial dilution (APHA, 2001):
Twenty-five grams of each sample was transferred aseptically into stomacher bag and stomached with 225 ml of 0.1% sterile peptone water. Transfer by means of pipette 1 ml of the initial suspension into a tube containing 9 ml of sterile diluent. Mix thoroughly by using vortex for 5-10 seconds to obtain 1:100 dilution. Repeat this operation to obtain dilutions 1:1000, 1: 10000 and etc. dilutions.

Enumeration of *Staphylococus aureus* (FDA, 2001)
About one ml. of food homogenate was transferred and distributed over the surface of 3 plates of Baired-Parker agar (eg. 0.4 ml, 0.3 and 0.3 ml), using sterile bended glass spreader. The plates were retained in upright position until inoculum is absorbed by agar for about 10 mints, or placed in upright position in the incubator for about 1 hour. The plates were inverted and incubated for 24-48 hours at 35°C and examined for determination of *Staph. aureus* count.

Enumeration of β-glucuronidase - positive *Escherichia coli* according to (ISO 16649-2:2001) (TBX method):
This method for enumeration and isolation of β -glucuronidase–positive *Escherichia coli* in all kinds of food and feed of animal origin, by growing the organism on tryptone –bile-glucuronide medium (tbx) at 44°C for 24 h. Positive plates showed blue green colonies.

Statistical analysis: -
Statistical analysis of the obtained data was run in triplicate by using of Statistical Packaging for the Social Science (SPSS) Ver. 20.and the results were expressed as mean and standard deviation (Mean±SD). Data were analyzed using analysis of variance (one-way ANOVA). The results with p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

List of preservatives versus the concentration of each substance were listed in Table (A)

Table (A). Type and concentration of different preservatives used in the current experiment

Preservatives used	Concentration
Alginic acid (AA)	0.5 %
Alginic acid & Sodium alginate (AA)	1 %
Alginic acid & Sodium alginate (AA)	2 %
Sodium alginate (SA)	0.5 %
Sodium alginate (SA)	1 %
Sodium alginate (SA)	2 %
Egg white lysozyme (EWL)	100 ppm
Egg white lysozyme (EWL)	150 ppm
Egg white lysozyme (EWL)	200 ppm
Combination of both SA + EWL	0.5 % + 100 ppm
Combination of both SA + EWL	1 % + 150 ppm
Combination of both SA + EWL	2 % + 200 ppm

Results and Discussion

Most food products required strict protection against food poisoning and food spoilage bacteria which gained access to the food as a result of contamination during processing and storage operations. Also, consumers demand for safe product with natural preservatives if required, which promoting food producers and researchers to look for alternatives in order to get safe products and good storage practices which will result in improving product quality and mitigate microbial risk levels without causing nutritional losses and organoleptic changes. In this context natural preservatives are gaining a great interest from research and industry, due to the potential to provide quality and safety benefits, with a reduced consumer health hazard **(Lucera *et al*., 2012).**

It was observed from the data recorded in Table and Fig. (1) that the most pronounced reduction rate for *Staph. aureus* was in the samples of the 2nd group which treated with 2% of AA as compared with the other two concentrations (0.5, 1%) which used for treatment of the other samples in the same group, as the count was reduced to (4.14 $\log_{10}$±0.06) with reduction rate of 0.79 $\log_{10}$ (16.02%) as compared with the 1st control group (4.93 $\log_{10}$±0.01), followed by 1% AA which recorded *Staph. aureus* count of 4.60±0.02with reduction rate represented by 0.35 $\log_{10}$ (7.1%). On the other side, SA with 2% concentration represented for the 3rd group was also the most effective one in reducing *Staph. aureus* count to 4.30 $\log_{10}$±0.04 with reduction rate of 0.63 $\log_{10}$ (12.78%), followed by SA 1% who had achieved reduction rate of 0.15 $\log_{10}$ (3.04%). From the obtained results, it could be concluded that *Staph. aureus* counts were reduced significantly (P<0.05) in all treatments except, there was no significance difference (p>0.05) between control samples and that treated with 0.5% SA.

Despite these recorded results, there are only two concentrations assimilated by 2% of both AA and SA which had reduction rate exceeded more than 0.5 $\log_{10}$ (0.79 and 0.63 $\log_{10}$cfu/g) respectively, which cleared that 0.5% SA found to have the least antimicrobial activity against *Staph*. *aureus* among the other concentrations of AA of the 2nd group and SA of the 3rd group.

Table (1) also showed that *E. coli* was less affected by either AA or SA as compared with *Staph. aureus*, in which 2% concentration of AA resulted in reduction of *E. coli* by 9.5% (0.47 $\log_{10}$), followed by 1% (0.23 $\log_{10}$ / 4.67%). While 2% SA was recorded reduction rate of 0.17 $\log_{10}$ (3.43 %) followed by 1% (0.11 $\log_{10}$ / 2.22%).Statistical analytical results showed that there were an obvious significance differences (P<0.05) of *E. coli* counts between all samples, excluding, the difference was not significant (p>0.05) between treated samples with 0.5% SA and each of control samples and samples treated with 0.5% AA. Although, there were a significant differences between most of the different treatment concentrations of both AA and SA and control samples for *E. coli* count, the anti *E*. *coli* effect of all treatments as the microbial reduction rate has no tangible effect which does not exceed 0.5 $\log_{10}$ which is considered in all measures as a weak or low effect.

It was generally obvious from results in Table and Fig (1) that; (i) The higher the concentration, the more the antimicrobial activity (2% was the best concentration followed by 1% and finally, 0.5% which considered weak and non-significant). (ii) All tested concentrations of AA and SA had a marked reduction effect on *Staph. aureus* as compared with its very weak effect on *E. coli* (iii) Since the reduction rates of both AA and SA on either *Staph. aureus* as an example of Gram-positive bacteria or Gram -negative *E. coli* were not significant, it is not recommended to use either AA or SA alone as antimicrobials but it is preferred to be used in combination with another synergistic antimicrobial substances.This agreed with **Scott and Strong (1964)** as they indicated that the value of sodium alginate in controlling *Staph. aureus* food-poisoning microorganism in frozen food is questionable. Also, the obtained results were compatible with **Mhadhebi *et al.* (2012)** who found the extract of 24 screened organic fractions of 6 seaweeds from the Tunisian Mediterranean coast were exhibited moderate to weak activity against *Staph. aureus; Staph. epidermis*, *E. coli* and *Micrococcus luteus*. This also

substantiates the findings of **Kim *et al.* (2000)** and **Hay *et al.* (2013)** who concluded that Alginic acid and its salts may have other effective functions more important to food than being antibacterial as thickening agent, stabilizer, emulsifier, chelating agent, encapsulation, swelling, a suspending agent, or used to form gels, films and membrane.

Table (1). Mean count and reduction rate of *Staph. aureus* and *E. coli* using Alginic acid and sodium alginate.

Antimicrobials		Bacterial counts and reduction values (Log_{10}cfu/g)					
Group	Concentration	*Staph. aureus*			*E. coli*		
		Mean±SD	Reduction		Mean±SD	Reduction	
			Rate	%		Rate	%
Control	0.0	4.93^{a}±0.01	---------	--------	4.95^{a}±0.01	-------	-------
AA	0.5 %	4.84^{b}±0.02	0.09	1.83	4.90^{b}±0.01	0.05	1.01
	1.0 %	4.60^{c}±0.02	0.35	7.10	4.70^{c}±0.03	0.23	4.67
	2.0 %	4.14^{d}±0.06	0.79	16.02	4.48^{d}±0.04	0.47	9.50
SA	0.5 %	4.90^{a}±0.02	0.03	1.55	4.93^{ab}±0.01	0.02	0.40
	1.0 %	4.78^{e}±0.01	0.15	3.04	4.84^{e}±0.01	0.11	2.22
	2.0 %	4.30^{f}±0.04	0.63	12.78	4.78^{f}±0.01	0.17	3.43

Mean ± standard deviation (n=3);
Means in the same column with different superscripted letters are significantly different ($p<0.05$).

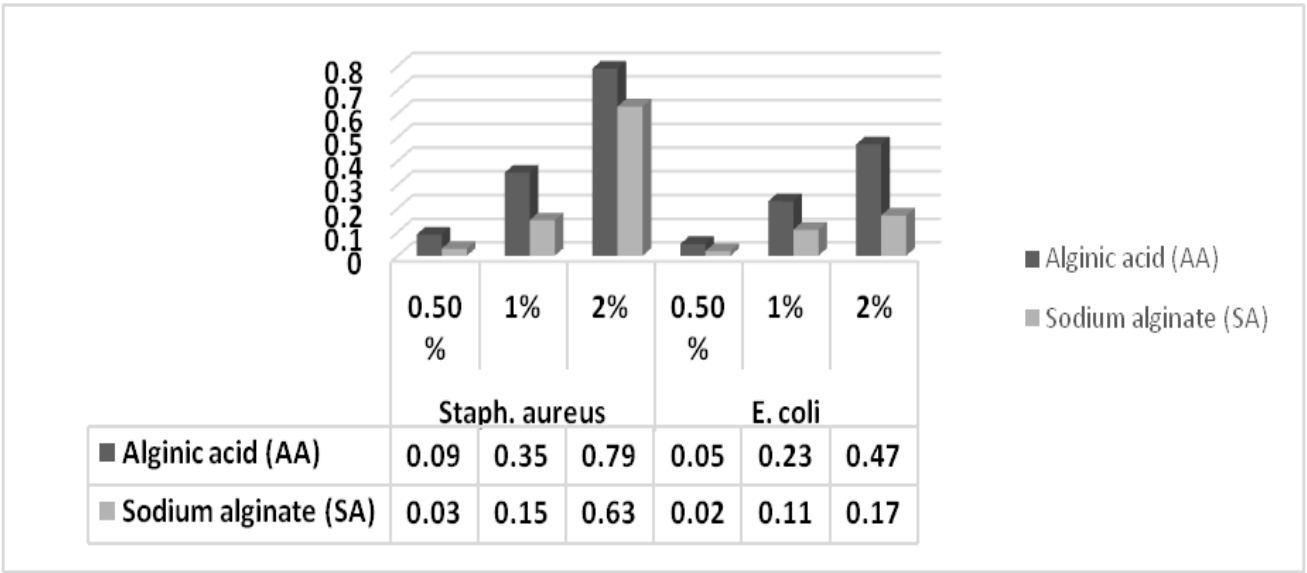

Fig. (1): Reduction rate (log_{10}cfu/g) of AA and SA on *Staph. aureus* and *E. coli*

Moreover, many investigators **(Nair *et al.*, 2005; Aguila-Ramírez *et al.*, 2012; Nogueira *et al.*, 2014; El Wahidi *et al.*, 2014**and **Karthikeyan *et al.*, 2015)** found that methanolic extract of AA and SA from seaweeds **(***L. johnstonii*, *D. flabellata* and *U. lactuca*) from the Gulf of California showed activity against *Staph. aureus*, while it poses no observed activity against *E. coli*. These results were compliant with that obtained in the present study as the AA and SA found to have a little or low antimicrobial activity against *E. coli* in comparison with their slightly to moderate effects on *Staph. aureus.* While, **Osman *et al.* (2010) and Dhanya *et al.* (2016)**observed the antimicrobial activities of crude extracts from the species of Rhodophyta, Chlorophyta, *Ulva reticulata* and Phaeophyta against *bothof Staph. aureus* and *E. coli*. Such differences in results may be attributed to the difference of in types of seaweeds used and the seasonal variation of antimicrobial activity of the extract. **(Padmakumar and Ayyakkannu, 1997).** Moreover, **Karbassi *et al.* (2014)** attributed the presence of antimicrobial activity of AA on *Staph. aureus* more than *E. coli* due to the difference in the cell wall structure and physicochemical characteristics of both microorganisms, in which *Staph. aureus* have an outer cytoplasmic membrane made from lipopolysaccharide which is easily adhered and penetrated by AA, while *E. coli* contained rigid peptidoglycan layer outside the cytoplasmic membrane which in unlikely to be penetrated by AA

The U.S. Food and Drug Administration (**US-FDA, 2018**) had classified food grade sodium alginate as GRAS (generally regarded as safe) substance in Title 21 of the Code for Federal Regulations (CFR) and listed its usage as an emulsifier, stabilizer, thickener and gelling agent**.** Furthermore, the European Commission (EC) listed alginic acid and its salts (E400–E404) as an authorized food additive **(Younes *et al.,* 2017**). Alginate is widely used in various industries such as food, beverage, textile, printing, and pharmaceutical (**Kim *et al.,* 2000 and Hay *et al.,* 2013).** Sodium alginate is the most common salt of alginic acid (**Yoo and Krochta, 2011)**.In this regard**,** European Food Safety Authority (**EFSA, 2017)** mentioned that Alginic acid and its salts (E 400–E 404) are authorized to be used in a wide variety of foods. Therefore, there is no fears of poisoning from consuming of alginic acid and its salts as food additives or may poses a risk to the consumer health. They added that, there is no hazards concerned the level of the exposure by using AA and its salts.

The results recorded in Table 2 and Fig. (2&3) revealed that 200 ppm of EWL (4^{th} group) was the most effective concentration and had more pronounced reduction rate for *Staph aureus* as compared with the other concentrations in the 4^{th}group, as the count was reduced to (3.98 $\log_{10}$cfu/g±0.03) with reduction rate of 0.95 $\log_{10}$ (19.27%) as compared with the 1^{st} control group (4.93 $\log_{10}$±0.01), followed by 150 ppm EWL (4.4 $\log_{10}$cfu/g ±0.01) with reduction rate of 0. 53 $\log_{10}$ (10.75%). On the other side, 200 ppm EWL + 2% SA (5^{th} group) was also more effective in reducing *Staph. aureus* count to 3.89 $\log_{10}$±0.01 with reduction rate of 1.04 $\log_{10}$ (21.1%), followed by 150 ppm EWL + 1% SA which recorded reduction rate of 0.79 $\log_{10}$ (Table 1). From the obtained results, it could be concluded that *Staph. aureus* counts were reduced significantly ($P<0.05$) in all treated groups as compared with control group except those samples treated with both 100 and 150 ppm of EWL, did not show any significant differences ($P>0.05$). It means that those two concentrations (100 ppm and 150 ppm) were found to produce the lowest reduction rates (0.48 and 0.53 log10cfu/g), respectively. Meaning of low antimicrobial activity against *Staph. aureus*. While, the highest reduction rate (1.04 $\log_{10}$cfu/g) was recorded for EWL 200 ppm + 2% SA, followed by 200 ppm of EWL alone (0.95 $\log_{10}$cfu/g) then 150 ppm 0f EWL + 1% SA (0.79 $\log_{10}$cfu/g) and finally, 100 ppm EWL + 0.5% SA (0.7 $\log_{10}$cfu/g).

Table 2 and Fig. (2&3) also showed that *E. coli* was less affected by either EWL or a combination of EWL + SA, as compared with *Staph. aureus*, in which only 200 ppm concentration of EWL (4^{th} group) resulted in reduction of *E. coli* count from 4.95 $\log_{10}$cfu/g ±0.01 (1^{st} control group) to 4.45±0.02 which represented by

0.5 log_{10} reduction rate (10.1%), while the other two concentrations (100 ppm and 150 ppm) achieved a weak reduction rates (0.14 and 0.37 log_{10}), respectively. Moreover, a combination of200 ppm of EWL with 2% SA (5th group) was able to reduce *E. coli* count to 4.2±0.08 with reduction rate of 0.75 log_{10} (15.15%), while the other two concentrations (100ppm EWL + 0.5% SA & 150ppm EWL + 1% SA) were resulted in reduction rates of 0.19 (3.84%) and 0.42 log_{10} (8.49%), respectively. Also, Table (2) revealed a significance differences ($P<0.05$) of *E. coli* count in all treated samples, except the difference was insignificant ($P>0.05$) between samples treated with 100 ppm EWL (4th group) and that treated with a combination of 100 ppm EWL + 0.5% SA (5th group). Also, there was no significance difference between those treated samples by150 ppm of EWL and that treated with combination of EWL and SA (150 ppm + 1%).

Through the overall results, it could be concluded that the antimicrobial effect of either EWL alone or EWL in combination with SA were not exceeded 0.5 log_{10}cfu/g. Meaning that either EWL or combination of with SA had a weak antimicrobial activity against *E. coli*, except only those samples treated with 200 ppm EWL + 2% SA which recorded reduction rate of (0.75 log_{10}cfu/g) which considered the only effective concentration against *E. coli.*

The obtained results in the present study complied with **Shelef and Seiter (1993)** and **Branen and Davidson (2004**) as they concluded that susceptibility of Gram-negative bacteria to lysis by lysozyme can be increased by the use of outer membrane disrupting agents**.** this is what we tried to apply in the current study by using sodium alginate (2%) as distrupting agent which proved its ability to inhibit *Escherichia coli* as a gram-negative bacterium along with lysozyme (200 ppm) more than using lysozyme alone. It means the existence of synergistic effect between them. Also, **Aminlari *et al.* (2014)** attributed the Weak effect of EWL on *E. coli* due to the presence of hydrophobic outer membranes of gram-negative bacteria which generally disrupt lysozyme activity**.** While, **Cegielska-Radziejewska *et al.* (2009& 2010)** and **Derde *et al.* (2014**) stated that the inhibitory effect of lysozyme on *Escherichia coli* may be attributed to the rapid increase in the permeability of the bacterial outer membrane by forming large size pores, which considered the source of protection against the effect of antimicrobials

Table (2). Mean reduction values of *Staph. aureus* and *E. coli* using EWL and a mixture of different concentrations of EWL + SA.

Antimicrobials		Bacterial counts and reduction values (Log_{10}cfu/g)					
Group	Concentration	*Staph. aureus*			*E. coli*		
		Mean±SD	Reduction		Mean±SD	Reduction value	
			Value	%		Value	%
Control	----------	4.93^{a}±0.01	--------	-------	4.95^{a}±0.01	--------	--------
EWL	100 ppm	4.45^{b}±0.02	0.48	09.74	4.81^{b}±0.01	0.14	2.83
	150 ppm	4.4^{b}±0.01	0.53	10.75	4.58^{c}±0.01	0.37	7.48
	200 ppm	3.98^{c}±0.03	0.95	19.27	4.45^{d}±0.02	0.5	10.1
EWL + SA	100 ppm + 0.5%	4.23^{d}±0.05	0.70	14.20	4.76^{b}±0.02	0.19	3.84
	150 ppm + 1%	4.14^{e}±0.06	0.79	16.02	4.53^{c}±0.03	0.42	8.49
	200 ppm + 2%	3.89^{f}±0.01	1.04	21.10	4.2^{e}±0.08	0.75	15.15

Mean±standard deviation (n=3);
Means in the same column with different superscripted letters are significantly different ($p < 0.05$).

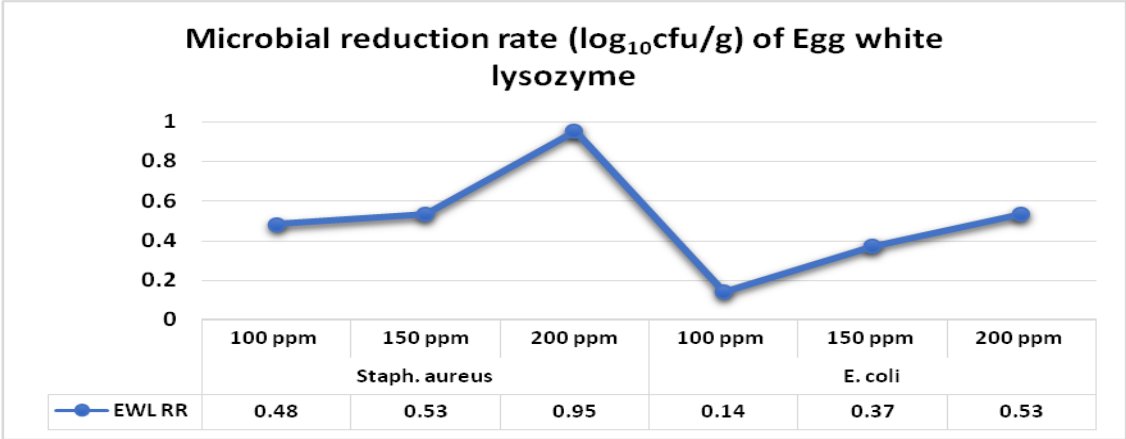

Fig. (2): Reduction rates of different EWL concentrations

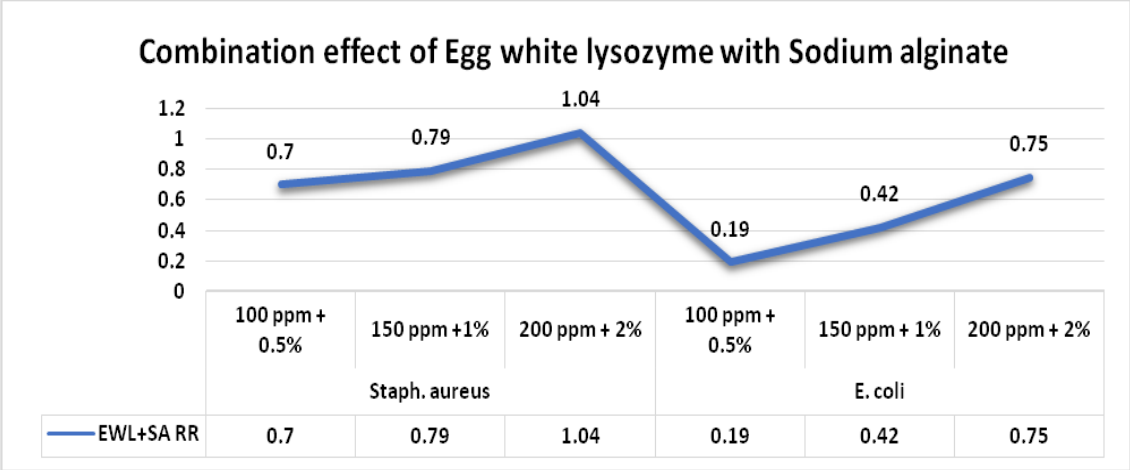

Fig. (3): Reduction rates of different concentrations of EWL + SA
EWL+SA RR: Egg white lysozyme + Sodium alginate Reduction Rate
EWL = ppm, SA= %

Numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth in foods as in recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, while the consumer trust increased with foods preserved using natural antimicrobial additives including plant extracts and their essential oils, enzymes (egg white lysozyme), peptides, bacteriocins, bacteriophages and fermented ingredients have become used as they are considered very popular and considered as safe alternatives to chemical or synthetic antimicrobials. To inhibit the growth of undesirable microorganisms in food, the antimicrobials can be directly added into the product formulation (**Aziz and Karboune, 2018**). This is what we tried to apply in the current research through addition of egg white lysozyme beside alginic acid or its salts to beef burger during its manufacturing in lab.

Cha *et al.* (2002) reported that SA in combination with either acetic acid, nisin or lysozyme was exhibited the highest inhibitory effect against all the investigated Gram-positive and Gram-negative microorganisms. This agreed with the recorded results in the present study as the antimicrobial effect of combination of EWL + SA salt of all three concentrations on *Staph. aureus* and only a concentration of 200 ppm EWL + 2% SA on *E. coli* in beef-burger was increased strongly which indicates the presence of a synergistic action.

The effect of EWL did not showed a significant reduction ($P>0.05$) in *E. coli* growth while combination effect between Nicin and lysozyme had a best performance ($P<0.05$) in increasing the antibacterial activity against *E. coli* and *Staph. aureus* (**Moshtaghi *et al.*, 2018)**. This support the obtained data in the present study where it showed the use of 200 ppm of EWL had a significant effect on *Staph. aureus* while the same concentration found to have a lower activity against *E. coli.* Combination between 2% SA and 200 ppm EWL increased the Synergistic action and improve the activity strength against *E. coli* ($P<0.05$). In this respect, **Huopalahti *et al. (*2007)** mentioned also that Lysozyme is known for its antibacterial property, especially against Gram-positive bacteria.

Lower reduction values were reported by **Malinowska-Panczyk and Kotodziejska (2009**) who found that lysozyme 400 mg/l. was able to induce significant reduction ($P<0.05$) of *Staph. aureus* strains by 0.4 log cycles from (8.9±0.1 to 8.5±0.1), while, lysozyme showed non-significance log_{10}cfu/g reduction ($P>0.05$) for *E. coli* K-12 (from 8.8±0.1 to 8.8±0.1). In this regard, **Fedtke *et al.* (2004)** and **Sudagidan and Yemenicioglu (2012)** concluded that lysozyme 5 mg/ml did not show enormous effective inhibition on growth of *Staph. aureus* while at the same time, the reduction rate was significant ($P<0.05$). These results support the hypothesis that lysozyme resistance is an important virulence factor for *aureus* Furthermore **Matouskova *et al.* (2016)** mentioned that the inhibitory effect against *E. coli* was very slight and at lower concentrations of lysozyme, no effect was observed. And more antimicrobial effect induced as high concentration used (1 mg/ml was more effective than 0.5 mg/ml.). While, Higher results were recorded by **Vilcacundo *et al.* (2018):** who concluded that native HEWL showed a reduction of 1.6 log_{10}cfu of *E. coli* while heat denaturated HEWL at 120 °C and pH 6.0 (1.0 mg/mL) inhibited 78.20% of the growth of *E. coli*.

Hen egg white lysozyme (E1105)is a widely used enzyme authorized for food preservation in **EU under EC Regulation No.2008/1333**on food additives. EWL has also been accepted as an antimicrobial substance in Ready-to-eat products **(FDA, 2007 and Colak *et al.* 2015).**

Lysozyme exhibits a strong antimicrobial activity against Gram-positive bacteria and some gram-negative bacteria. It damages peptidoglycans in bacteria cell wall by catalyzing hydrolysis of β-1-3 glycosidic linkage between N-acetylmuramic acid and N-acetylglucosamine. Furthermore, **EC No. 1272/2008** proclaimed that alginic acid and its salts were classified as GRAS (generally recognized as safe) when used as a preservative in manufactured meat products. Meaning it does not constitute a public health hazard. Furthermore, World Health Organization (WHO) allow the use of lysozyme as a preservative in foods. Currently, it is being used in Chinese noodles, cheese, sushi, kimuchi pickles, and wine production

(**Abeyrathne *et al.,* 2013**)

Conclusion and Recommendation

This review of results has shown that

The natural antimicrobials have the potential to replace chemical additives in meat products to accomplish and satisfy the consumer its safety and quality.

The strong antimicrobial activities of some Enzymes like egg white lysozyme were shown to be promising natural antimicrobials due to their ability to produce antimicrobial compounds or due to their ability to disintegrate the outer membrane of some bacteria; however, their applications in food products have to be further investigated as they could able to effect on *Staph. aureus* as gram positive bacteria while, have a slightly effect on gram negative bacteria (*E. coli)*

It is required from the scientist's further exploration of these natural antimicrobials to determine their synergy and allow their more effective use in food products. Moreover, further studies are needed in order to determine the best method of incorporation of these natural additives into food**.**

Further researches on alginic acid and its salts are required, in particular incorporation with other natural antimicrobial agents, to improve its ability to eliminate or reduce the contamination to an acceptable level which be safe and does not constitutes a public health hazard.

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