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Oxidative Damage Caused by Common Foodborne Pathogenic Bacteria in Egg Yolk

Reyhaneh Afshordi,[1] Maryam Zare Jeddi,[2,3] Ali Salehi,[2] Mohammad Reza Pourmand,[4,*] Ali Akbar Saboor-Yaraghi,[5,6] Farzaneh Amin Harati,[4] and Parisa Sadighara[2,*]

[1]Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, IR Iran
[2]Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran
[3]Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, IR Iran
[4]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran
[5]Deptatment of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran
[6]Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, IR Iran

*Corresponding authors: Mohammad Reza Pourmand, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188973901, Fax: +98-2166462267, E-mail: pourmand@gmail.com; Parisa Sadighara, Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188973901, Fax: +98-2166462267, E-mail: parisasss@yahoo.com

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Abstract

Background: Bacteria in foodstuff are the most important agent of foodborne disease. Aside from their infectious effects, obligate aerobes have a respiratory metabolism with oxygen as the terminal electron acceptor. Therefore, they can produce reactive oxygen species and free radicals in contaminated food. Malondialdehyde (MDA) is a product of lipid peroxidation used as an indicator of oxidative stress.
Objectives: This study aimed to evaluate the oxidative damage produced by two common food pathogenic bacteria in foodstuff.
Materials and Methods: The egg yolks were incubated with different dilutions (10^5,10^6, and 10^7) of *Staphylococcus aureus* and *Salmonella enteritidis* at 37°C for 20 hours. The level of MDA in egg yolk was measured by fast and simple enzymatic or colorimetric methods, such as the thiobarbituric acid reactive species method.
Results: The high group (10^7) had a higher MDA level of 1.97 ± 0.11 (µg MDA/g) in *S. aureus* and 1.65 ± 0.27 (mg MDA/L) in *S. enteritidis* than the control (0.90 ± 0.13 mg MDA/L).
Conclusions: We concluded that common food pathogenic bacteria can induce oxidative damage in foodstuff aside from other common problems. Heating or sterilization methods cannot protect foodstuff from the damage caused by the presence of pathogenic bacteria.

Keywords: Bacteria, Lipid Peroxidation, Oxidative Stress, Free Radicals

1. Background

Food serves as excellent culture and protective medium for some microorganisms that induce foodborne diseases (1). Foodborne diseases are one of the common health problems in the department of public health and hygiene. About 250 different foodborne diseases have been identified, and bacteria are the causative agents of two-thirds of foodborne disease outbreaks. The pathogenesis of bacteria that cause foodborne poisoning depends on their capacity to produce toxins (exotoxin or endotoxin) after ingestion, on whether they are found in the digestive tract or before it, and on the toxins preformed in foodstuff (1). These bacteria produce reactive oxygen species (ROS) because of aerobic metabolism. Obligate aerobic bacteria have aerobic respiration and use free oxygen as a final electron acceptor, and thus they are known as obligate aerobes. O_2 and H_2O_2 are unavoidable byproducts of aerobic respiration. H_2O_2 can produce HO, a powerful oxidant and free radical that reacts with molecules in a Fenton reaction. ROS are generated in exponentially growing bacteria by the auto-oxidation of components of the respiratory chain (2).

ROS and free radicals induce oxidative stress, which is defined as a disturbance in the balance between the production of ROS and antioxidant defenses. ROS cause damage to macromolecules, such as DNA, lipids, proteins, and carbohydrates, and they cause mutation in genes in biological systems. Mounting clinical and experimental evidence indicates that free radicals play an important role in many physiological and pathological conditions. Free radicals have been attributed to arthrosclerosis, Parkinson's disease, Alzheimer's disease, ischemia, aging, diabetes mellitus type I, and pathogenesis of chronic disease such as cataract, heart failure, rheumatoid arthritis, and cancer (3).

In foods, ROS and free radicals attack polyunsaturated fatty acids and cause lipid peroxidation. They are a major cause of quality deterioration in food (4). Lipid peroxidation, known as rancidity, is one of known effects of ROS studied for the first time in 1820 (5). Lipid peroxidation refers to the oxidative degradation of lipids. It usually af-

fects polyunsaturated fatty acids because they contain multiple double bonds in between methylene bridges (-CH2-) that possess reactive hydrogens. Lipid peroxidation changes the type and the concentration of molecular species in food (6).

Staphylococcus aureus is a facultative microaerophilic gram-positive coccal bacterium also known as "golden staph" and Oro staphira. *S. aureus* appears as grape-like clusters when viewed through a microscope, and it has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. *S. aureus* is found on the skin, nose and throat of most people. Infected wounds and acne are rich sources (1). It can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, scalded skin syndrome, and abscesses, to life-threatening diseases, such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, sepsis, and food poisoning (7).

Foods with high protein content such as meat and poultry products are the most common food source linked to *S. aureus* infections.

Salmonella enterica serovar Enteritidis is a Gram-negative, rod-shaped, flagellated, aerobic bacterium that causes the foodborne salmonellosis pandemic in humans. Egg is one of the most common foods it infects (1). *S. enteritidis* has the unique ability to contaminate eggs without causing discernible illness in the birds infected. The infection route to humans involves colonization, survival, and multiplication of the pathogen in the hen house environment, the bird, and finally the egg (8). Eggs are the most common food source linked to *S. enteritidis* infections. *S. enteritidis* can cause fever, abdominal cramps, and diarrhea 12 to 72 hour after consuming contaminated food. *Salmonella* food poisoning is also characterized by nausea, vomiting, and chills (1).

Lipid oxidation is one of the major causes of food spoilage, particularly in fat-containing foods such as traditional fish products. It leads to the development of various off-odors generally called rancidity and discoloration, which render these foods unacceptable or reduce their shelf life. In addition, oxidative reactions can decrease the nutritional quality of food, and certain oxidation products are potentially toxic. Malondialdehyde (MDA) is one of the well-known secondary products measured by the2-thiobarbituric acid (TBA) method. The TBA test involves the reaction of TBA with MDA in edible oils to produce a chromogen that can then be determined spectrophotometrically at 532 nm – 535 nm.

2. Objectives

This study aimed to evaluate the oxidative damage produced by two common food pathogenic bacteria (*S. aureus* and *S. enteritidis*) in foodstuff.

For this purpose, the level of free radicals caused by *S. aureus* and *S. enteritidis* as aerobic bacteria in egg yolks as foodstuff was investigated using the TBA test. An egg yolk is full of unsaturated fatty acids and is capable of oxidative damage and lipid peroxidation (9). Furthermore, an egg yolk contains Fe ion, which can stimulate and improve lipid peroxidation (10).

3. Materials and Methods

A stock of egg yolk was prepared. *S. aureus* and *S. enteritidis* cultures were obtained from the Faculty of Health, Tehran University of Medical Sciences. The cultures were separated from urine samples from SINA hospital patients. Sub-cultures from the two bacteria were then prepared. Identification tests for confirming the identity of bacteria were conducted. The results of the experiments are shown in Tables 1 and 2.

Different dilutions (10^5, 10^6, and 10^7) of *S. aureus* and *S. enteritidis* were added to the stock. The samples were incubated at 37°C for 20 hours. After the growth of bacteria, the MDA level was evaluated. MDA formation was measured according to the thiobarbituric acid method (11).

Briefly, the sample solutions were mixed with 20% trichloroacetic acid. The samples were prepared using a serial dilution of one-half McFarland of each bacterium, and they were added to the stock. The samples were incubated at 37°C for 20 hours. After the growth of bacteria, MDA level was evaluated. MDA formation was measured according to the thiobarbituric acid (TBARS) method (12). TBARS was added to the supernatant, and the samples were heated in 90°C for 90 minutes. The absorbance of the supernatant was measured at 532 nm. MDA level was expressed in mg MDA/L. All tests were performed in triplicate.

The statistical analysis was conducted by one-way analysis of variance (ANOVA). P-values 0.05 were considered significant.

Table 1. Identification Tests of *S. aureus*

Identification Tests	Result
Catalase	+
Coagulase	+
Mannitol salt agar culture	+
Sensitivity to novobiocin disk	Sensitive

Table 2. Identification Tests of *S. enteritidis*

Identification Tests	Result
SF Broth culture	+
XLD	Red colony with black center
TSI	ALK/ACID
Glucose	+
Lactose	-
Sucrose	-
H2S (TSI)	+
Indole	-
MR	+
VP	-
Citrate (Simmons)	+
Urea (Agar)	-
Motility	+

4. Results

The results of the experiments are shown in Table 3. The MDA level was analyzed in terms of OD measured at wavelength with a spectrophotometer and the standard MDA formula (y = 0.3483x + 0.1533). A significant difference was found between the high group of *S. aureus* and *S. enteritidis* with the control. The ability of *S. aureus* in the production of free radicals is greater than that of *S. enteritidis* in all three doses of bacteria.

We conclude that bacteria in these doses can induce oxidative damage plus food poisoning and infection. In these doses, even if heating and sterilization methods are used, foodstuff cannot be protected from the damage caused by the presence of pathogenic bacteria. Our previous study also revealed similar results (13).

Our results indicate that *S. aureus* and *S. enteritidis* can produce high MDA concentration and cause oxidative damage in foodstuff. MDA is a product of lipid peroxidation that has been used as an indicator of oxidative stress. Consumption of MDA concentration in foodstuff has been associated with some problems. This component is highly reactive that it can deteriorate biological molecules such as DNA (12). It is mutagenic and tumorigenic (13), and it affects the function of mitochondria. MDA cross-links with valuable amino acids, which reduce the nutritive value of food (14). Moreover, lipids are the major component of food. They contribute to food flavor, aroma, color, shelf life and nutritional value. Lipid peroxidation is one of known effects of ROS in foodstuff containing fat, such as meat, milk, and egg products. Lipids are the reservoir of fat-soluble vitamins, and lipid peroxidation causes damage to fat-soluble vitamins.

In this study, significant results were observed for the high group (107) compared with the control group. The correlation between oxidative damage in food and growth of bacteria was elucidated. Therefore, using antioxidant compounds in food is necessary.

Table 3. MDA level of *S. aureus* and *S. enteritidis*

Total Count	MDA, mg MDA/L of *S. aureus*	MDA, mg MDA/L of *S. enteritidis*
Control (0)	0.90 ± 0.13	0.90 ± 0.13
10^5 /ml	1.31 ± 0.29	1.05 ± 0.23
10^6 /ml	1.77 ± 0.02	1.36 ± 0.09
10^7 /ml	1.97 ± 0.11	1.65 ± 0.27

5. Discussion

MDA is an established biomarker of lipid peroxidation in foods. The spectrophotometric measurement of the pink adduct of MDA with TBA (maximum absorbance at 532 nm - 535 nm) is possibly the most widely used method to determine MDA in foods and biological samples because of its low cost and simplicity. Common food pathogenic bacteria can induce oxidative damage and secondary oxidation (MDA) in foodstuff in addition to other common problems. Heating or the use of sterilization methods cannot protect foodstuff from the damage caused by the presence of pathogenic bacteria. Therefore, secondary oxidation products may be susceptible to cause significant deterioration in chemical, sensory, and nutritional food properties and adverse health effects.

Footnotes

Authors' Contribution: Mohammad Reza Pourmand, Parisa Sadighara, and Ali Akbar Saboor-Yaraghi designed the experiments and the first draft of the manuscript. Reyhaneh Afshordi, Farzaneh Amin Harati, and Ali Salehi conducted the experiments. Maryam Zare Jeddi edited and wrote the final draft of the manuscript.

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